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PHYTOCHEMICAL SCREENING, CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF ETHANOL, METHANOL AND CHLOROFORM EXTRACTS FROM THE LEAVES OF DIGITARIA SANGUINALIS, DIGITARIA ISCHAEMUM AND THE BARK OF CARAPA GUIANENSIS FOUND IN GUYANA, SOUTH AMERICA.

Rajendra Kumar Khelawan

PHYTOCHEMICAL SCREENING, CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF ETHANOL, METHANOL AND CHLOROFORM EXTRACTS FROM THE LEAVES *OF DIGITARIA SANGUINALIS, DIGITARIA ISCHAEMUM* AND THE BARK OF *CARAPA GUIANENSIS* FOUND IN GUYANA, SOUTH AMERICA.

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ABSTRACT

PHYTOCHEMICAL SCREENING, CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITIES OF ETHANOL, METHANOL AND CHLOROFORM EXTRACTS FROM THE LEAVES *OF DIGITARIA SANGUINALIS, DIGITARIA ISCHAEMUM* AND THE BARK OF *CARAPA GUIANENSIS* FOUND IN GUYANA, SOUTH AMERICA.

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Chloroform, Ethanol and Methanol extracts of the leaves from *DIGITARIA SANGUINALIS, DIGITARIA ISCHAEMUM* and the bark from *CARAPA GUIANENSIS* found in Guyana were obtained via maceration and reflux extraction processes. Ethanol extracts obtained from the 25-minute reflux process produced the best percentage yield and the most thin layer chromatographic spots. All further analyses were conducted on the ethanol extracts of all plant materials. The ethanolic extracts showed positive phytochemical testing results for Terpenes, Flavonoids, Steroids, Tannins, Phenolic, Proteins, Cardiac Glycoside, Reducing Sugars, and Carbohydrates. Conversely, Saponins were absent in Smooth Crabgrass and Crabwood's bark but present in Long Hairy Crabgrass. A mixture of elution solvents used in the column separation yielded 250, 128 and 188 fractions for Smooth Crabgrass, Long Hairy Crabgrass, and Crabwood's bark respectively. These were analyzed with TLC and pooled into 27 fractions for Smooth Crabgrass, 26 for Long Hairy Crabgrass and 22 for Crabwood's bark. GC – MS analyses of these fractions resulted in 682 NIST hits. Medicinal uses of the NIST compounds range from anti-inflammatory, insect repellent, skin moisturizer, antioxidant, antibacterial,

anticancer, antidiabetic, anthelmintic, expectorant, antifungal, cholesterol lowering, treatment for acne. Some of these compounds are Eucalyptol, Isolongifolan-8-ol, Limonen-6-ol, pivalate, Estra-1,3,5(10)-trien-17β-ol, Ethyl iso-allocholate, Cryptomeridiol, γ-Sitostenone, Stigmasterol, Levodopa, Glycidyl palmitate, 2-Myristynoyl-glycinamide, Androst-5-en-4-one, Cholestan-3-one, Retinol, acetate, Linoleic acid ethyl ester, Neophytadiene, Vitamin E, Geranyl isovalerate, Melibiose, and d-Mannose. Antimicrobial Assay against; *STAPHYLOCOCCUS AUREUS, STAPHYLOCOCCUS EPIDERMIS, ESCHERICHIA COLI, PSEUDOMONAS AERUGINOSA and CANDIDA ALBICANS* were conducted via Disc Diffusion and Agar Well methods. Crabwood's bark ethanol extract showed a positive indication of growth inhibition against *STAPHYLOCOCCUS AUREUS* and *STAPHYLOCOCCUS EPIDERMIS* in both antimicrobial testing methods, while Smooth crabgrass indicated inhibition for only *STAPHYLOCOCCUS EPIDERMIS* in the Disc diffusion assay.

Key words: Phytochemical, Anti – microbial, Digitaria sanguinalis, Digitaria ischaemum, Carapa guianensis, Mass Spectrometry, Gas Chromatography, Disc Diffusion method, Agar Well method, thin layer chromatography.

DEDICATION

This research is dedicated to my fallen friend who died by suicide; Vishal Hemraj, my family members, and the people of Guyana.

The late Vishal Hemraj – Physics and Mathematics lecturer at UOG

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1.0 - INTRODUCTION

Some of the usefulness of plants have been identified since the beginning of civilization. In the early days, plants were used by humanity to heal, cure, or prevent diseases. As we advance in intelligence and technologies, plants have the potential for aiding in the production of new drugs with great benefits to humankind (Brito , et al., 2016) (Henriques & Penido, 2014).

On the north – east region of South America, the Co-operative Republic of Guyana can be found occupying approximately 215,000 square kilometers. Its borders include the Northern Atlantic Ocean, Suriname to the east, Brazil to the south and Venezuela to the west (Commonwealth Secretariat, 2021). According to (Guyana Forestry Commission, 2017), approximately 75% of the total area of Guyana is covered with natural vegetation and the southern regions intersect the Amazon rain forest. Guyana's most recent survey of the richness of flora species quantifies at 8000 species including ferns, mosses etc., in which approximately 6500 of those species have been identified (Guyana Environmental Protection Agency, 2010).

The first set of people to settle in Guyana are called the Amerindians and were the first tribe to use plants a medicine in Guyana. It is their advice that fuels this research on *Digitaria sanguinalis (long hairy crabgrass), Digitaria ischaemum (smooth crabgrass)* and the bark from *Carapa guianensis (crabwood).* They indicated a handful of remedies in which these plants are used as – antipyretic, analgesic, dry skin treatment, diarrhea. As of today, numerous studies have been done on the chemical composition and antimicrobial activities of crabwood leaf and fruit extracts, one study done on *Digitaria sanguinalis* in Egypt but none on *Digitaria ischaemum*.

This type of research and findings will offer potential information about the efficacy of these extracts against the listed microorganisms, further identifying their chemical composition and in the future, chemical synthesis of possible compound candidates to combat antimicrobial resistance.

2.0 - BACKGROUND

Global health is at risk because microbes are constantly changing and adapting to treatment regimens available. (World Health Organization, 2020) coined the term "Antimicrobial resistance (AMR)" – which occurs when microorganisms; bacteria, viruses, fungi, and parasites change over time and no longer respond to medicines, making infections harder to treat and increasing the risk of disease, morbidity, and mortality rates.

A recent report published by (WHO Regional Office for Europe/European Centre for Disease Prevention and Control, 2022) pointed out that each year, more than 670,000 infections in the EU are due to bacterial-resistant to antibiotics, causing nearly 33,000 people to die consequently. A more daunting article issued by (World Health Organization, 2021) illustrated that there is a global shortage of innovative antibiotics because all 43 antibiotic drugs currently in clinical development proved to be ineffective.

The impact of antimicrobial resistance is so great that it was one of the major issues championed by the Prime Minister of Barbados, Ms. Mia Mottley during the 9th Summit of the Americas at the Los Angeles Convention Center in Los Angeles, CA. She illustrated that antimicrobial resistance is a slow onset pandemic which is already upon us, fueled by the methods used for farming, abuse of antibiotics and waste management (CMS Editor, 2022).

In most cases, nature provides answers to our most complex situations, one of which is to provide medicinal herbs for various ailments. The medicinal plants in the Amazon Rain Forest are mostly unexplored and unstudied. Compared to the number of plant species available, only a few are being used by the indigenous people in Guyana. The basis of this research stems from their experiences with the crab - oil tree, long hairy crabgrass, and smooth crabgrass. Much research has been focused on the scientific evaluation of crab – oil but not so much on long hairy crabgrass and smooth crabgrass.

In the hinterland regions of Guyana, the local people harvests the crabwood tree's fruit, leaves and stems/bark and used these as herbal remedies. The fruit is processed into an oil that is used against hacking cough and bronchial tract discomfort. This oil can also be used on dogs to control mange and ground itch (anti-fungal), while the grated nut is mixed with palm oil and used as an analgesic for muscle pains. Additionally, it is used as a natural insect repellent. According to some studies, the seed can be used to treat genital

herps (Smithsonian National Museum of Natural History), while the leaves are used to clean open wounds and cuts. It is also said to be effective against ulcers. The stem/bark can be crushed and soaked overnight in water; this mixture is then used for chicken pox or measles. The juice from the crushed bark is a remedy for eczema and fever because of its rich tannin content. Additional uses of the bark include treatment of diarrhea, malaria, burns, sores, stomach-ache, and wounds/cuts (Smithsonian National Museum of Natural History).

It is customary for the indigenous people in Guyana to use long hairy crabgrass and smooth crabgrass as treatment for inflammatory conditions, gonorrhea, as an antifungal, diarrhea, and pneumonia.

Scope

- Obtain ethanol, methanol, and chloroform extracts from *Digitaria sanguinalis (Long Hairy crabgrass), Digitaria ischaemum (Smooth crabgrass)* and the bark from *Carapa guianensis (crabwood)* by both maceration and reflux techniques.
- Determine the better method and best time duration for the extraction processes using thin – layer chromatography.
- Phytochemical screening of the best resulting TLC extracts for each plant species.
- Column chromatographic separation of the best resulting TLC extracts.
- TLC analysis of these fractions along with chemical functional group staining.
- Antimicrobial assay on the best extracts obtained.
- Gas chromatography – Mass Spectrometry analyses on the fractions.

Significance of study

With the increase in antimicrobial resistance, it has become even more essential to find alternatives and newer drugs to fight against these microbes (World Health Organization, 2021). With the forests coverage in Guyana being approximately 75%, it is possible to discover plants which may contain compounds that can be used as antimicrobial agents. The results of this research can directly impact the pharmaceutical industries by providing new compound/drug candidates to fight against microbial infections.

Objectives

- To collect samples, identify and tag the following plant species located in Guyana's hinterland; Digitaria *sanguinalis (Long Hairy crabgrass), Digitaria ischaemum (Smooth crabgrass)* and the bark from *Carapa guianensis (crabwood)*.
- To obtain ethanol, methanol, and chloroform extracts of the above-mentioned plant species by both maceration and reflux processes.
- To determine the better method of extraction as well as the optimum extraction / reflux time required to obtain the maximum number of components via TLC analyses.

- To obtain crude samples of each using a rotary evaporator and vacuum drying oven.
- Determine the yields of each crude extracts.
- To perform phytochemical analyses on the best TLC crude obtained for each plant species.
- To perform column chromatography and obtain separations (fractions) of the best crude extracts of each plant species.
- To perform TLC analysis of each fraction and use staining techniques to determine the chemical functionality of each.
- To perform antimicrobial assay on the best crude extract for each plant species.
- To perform gas chromatography - mass spectrometry and deduce the compounds present in these fractions via the NIST library.

3.0 - LITERATURE REVIEW

Description of Plant Species:

Carapa guianensis – Crabwood:

The plant is described to be semi-evergreen which is about 25 – 35 meters in height. It has a dense crown with its bark having a greyish colour and a scaling in square pattern. The young branches are thick and rufous brown in colour. The leaves alternate, paripinnate or with a vestigial terminal leaflet which are asymmetric at the base and usually arranged elliptically in 4-8 pairs. They range mostly between 20 – 40 cm long and 6 – 14 cm broad with a hairy, wine – red midrib. The flowers are unisexual with numerous cymule which are 5 – 6 mm long. Petals are white or creamy – white, pinkish outside while the staminal tubes are orange at the end. Capsule subglobose is 4 valved, 5 – 10 cm long, 6 – 8 cm broad. The seeds are angular, brown, smooth, or pitted and 4 – 5 cm in diameter.

Figure 1. - Picture of Carapa guianensis plant. (a) Leaf (b) Leaflet (C) Bark (Luz, et al., 2019), (Szulecka, 2009)

Digitaria sanguinalis - Hairy crabgrass:

The leaf blades of this type of crabgrass are said to be between 5 – 15 cm long, 3 – 12 mm wide with a green to purple colour on both sides. Silky, shiny hair are found on the leaf blades and are reddish in the center but pale at the margins. The sheath base has long blister – like hairs which can be green to reddish violet. New leaves are found to be rolled. The ligule membranous is white, truncated to 1 – 2 mm in length. Auricles are not present. The base of the stem prostrates where rooting occurs at the lower nodes, distinctly bent at the

lower nodes. Under harsh conditions, tillers and leavers with reddish tonalities can be visible (Kissman & Groth , 1993).

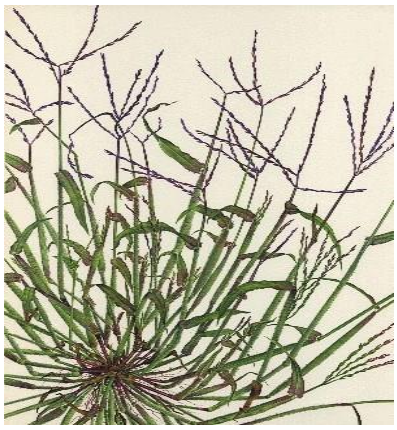


Figure 2. – Picture of Digitaria sanguinalis (CABI, 2021).

***Digitaria ischaemum* - Smooth crab grass:**

The stems of this specie grow upright and branches from the base. These stems are flattened in cross section and rolled in the bud. They have a prominent midvein which are 6 – 8 mm wide and up to 12.5 cm long. Hairs can be found only at the position of auricle, but the remainder of the leaf is hairless. Some of the bases of the leaves are reddish (Agriculture and Natural Resources, University of California, 2016).

Figure 3.- Picture of Digitaria ischaemum (UMass Amherst, 2021).

Phytochemicals:

Phytochemicals, commonly known as secondary plant metabolites are produced by plant cells through metabolic pathways derived from the utilization of primary metabolites (sugars, amino acids, etc.). Secondary metabolites are classified according to their chemical structures; these include: Phenolics, Alkaloids, Saponins, Terpenes, Lipids, Carbohydrates, Steroids, Reducing Sugars, and Flavonoids (Hussein & El-Anssary, 2018).

Phenolics compounds:

These are the largest group of the secondary metabolites with functionality as; anti-inflammatory, antihepatotoxic, and antioxidants. They consist of one or more phenol groups with one aromatic ring, to highly complex poly aromatic rings. Phenols are further classified into simple phenolics, tannins, coumarins, flavonoids, chromones and xanthones, stilbenes and lignans (Hussein & El-Anssary, 2018). Many simple phenolic compounds were found to be antimicrobial in nature.

Tannins are polyphenols which is subclassed into; hydrolysable (gallotannins and ellagitannins) and condensed (proanthocyanidins) tannins. Tannin drugs offer protection against bacterial and fungal infestation, antidiarrheals, and antidotes in heavy metals poising (Hussein & El-Anssary, 2018).

Another major class of phenolic compounds is the coumarins. These are known derivatives of benzo – α – pyrone, the lactone of O – hydroxycinnamic acid. Some known biological activities of coumarins are anti – inflammatory, anticoagulant, anticancer and anti – Alzheimer's (Hussein & El-Anssary, 2018).

Flavonoids are said to be the largest group of naturally occurring phenols. The chemical structure consists of a chroman ring bearing an aromatic ring in position 2, 3 or 4. The most common flavonoids are anthocyanins, flavones (yellow) and flavonols. Their biological activities include anti-inflammatory effects, antiallergic effects, antithrombotic, Vaso protective properties, and tumor inhibition (Hussein & El-Anssary, 2018).

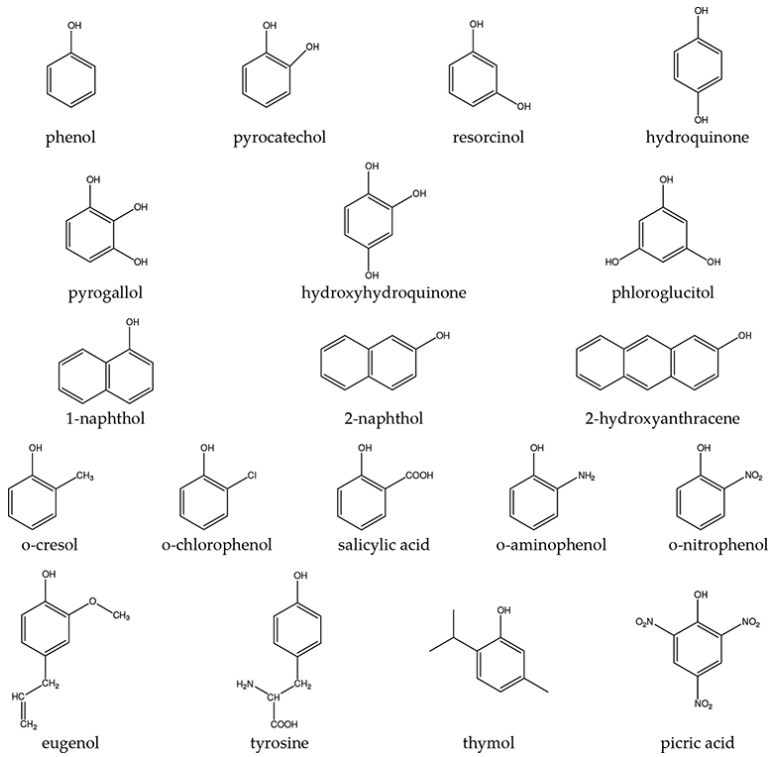


Figure 4. – Structures of some examples of phenols (Sobiesiak, 2017).

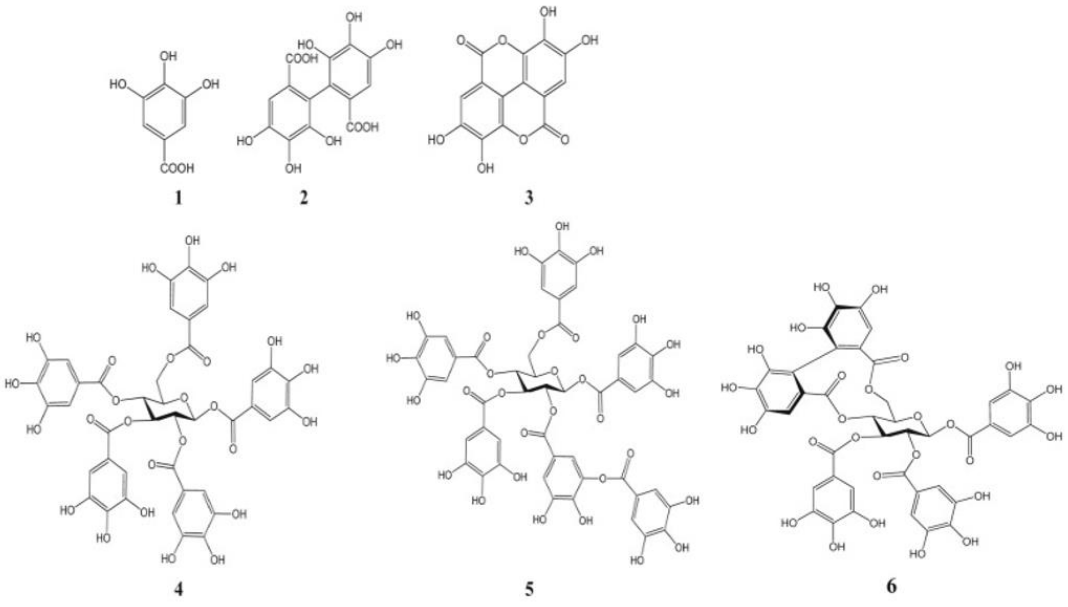


Figure 5. – Structures representative Tannins - gallic acid (1); hexahydroxydiphenic acid (2); ellagic acid (3); pentagalloylglucose (4), the basic unit of hydrolysable tannins; 2-O-digalloyl-1,3,4,6-tetra-O-galoyl-β-D-glucopyranose (5), the example of gallotan

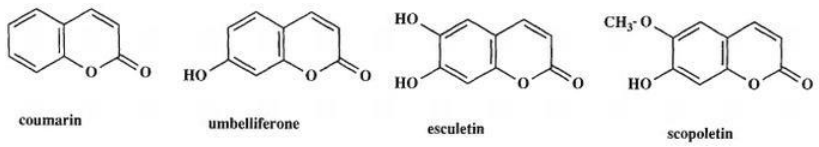


Figure 6.- Representative coumarins (Hussein & El-Anssary, 2018).

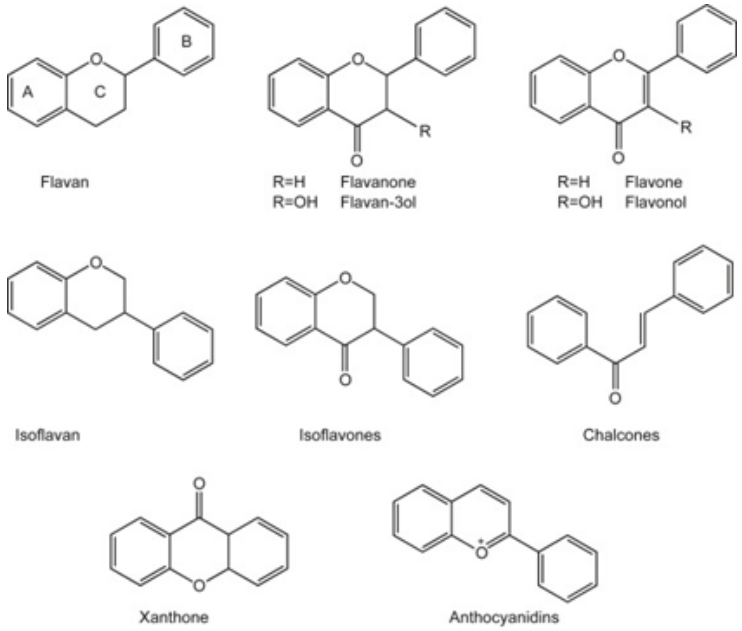


Figure 7. – Structures of representative Flavonoids (Hernández-Rodríguez, Baquero, & Larrota, 2019).

Alkaloids:

These are organic compounds which contain at least one nitrogen atom in a heterocyclic ring. Some major classes of alkaloids are imidazoles, indoles, quinolines, purines, and pyrrolidines. They demonstrate pharmacological activities such as analgesia, local anesthesia, cardiac stimulation, respiratory stimulation and relaxation, vasoconstriction, muscle relaxation and toxicity, as well as antineoplastic, hypertensive, and hypotensive properties (Hussein & El-Anssary, 2018).

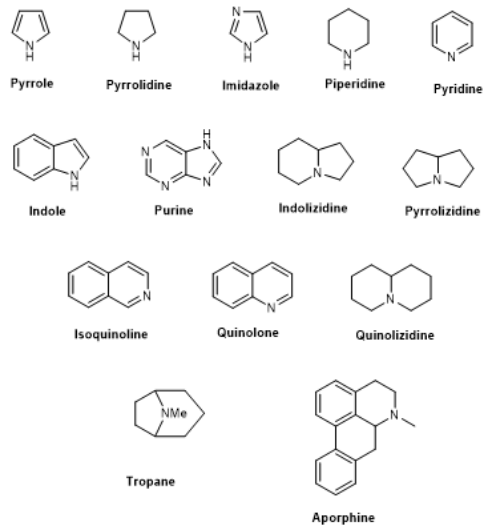


Figure 8.– Structures of alkaloids (Ghirga & Casciaro, 2020).

Saponins:

Saponins are natural bioorganic compounds having at least one glycosidic linkage (C-O sugar bond) at C3 between aglycone and a sugar chain. These are subcategorized into triterpenoid (monodesmosidic and bidesomsidic), steroid saponin and alkaloid saponin. Literature shows that saponins exhibit a biological role and medicinal properties such as hemolytic factor, anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer, cytotoxic and molluscicidal (Ashour, Aziz, & Melad, 2019).

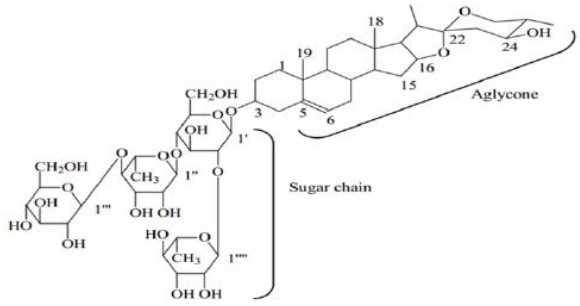


Figure 9.– Structure of a representative steroid saponin (Ashour, Aziz, & Melad, 2019).

Terpenes:

These represent another large and diverse group of plant secondary compounds. Terpenes are chemically derived from 5-carbon isoprene units. They are classified according to the number of isoprene units in the molecule; hemiterpenes (single), monoterpenes (two units),

sesquiterpenes (three units), diterpenes (four units), sesterterpenes (five units), and triterpenes (six isoprene). These are used as (monoterpenes) anti-irritants, anthelmintics, (sesquiterpenes) antibacterial, antifungal, antiprotozoal, (diterpenes) analgesic, antibacterial, antifungal, anti-inflammatory, antineoplastic and antiprotozoal activities (Hussein & El-Anssary, 2018).

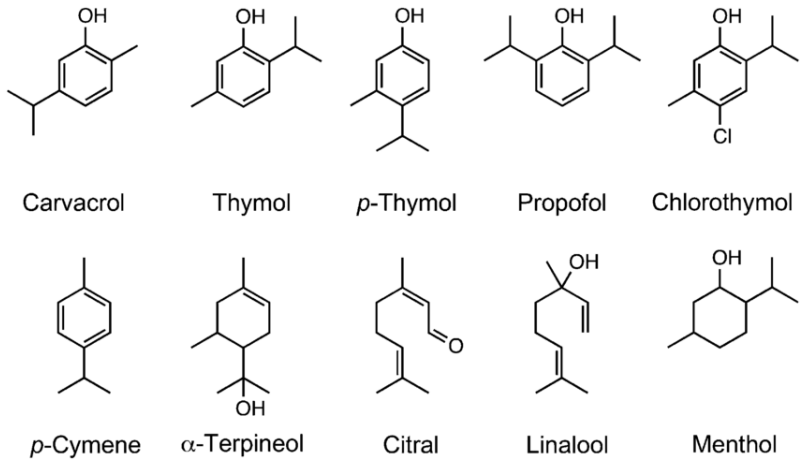


Figure 10.– Representative monoterpenes (Lansdell, et al., 2015).

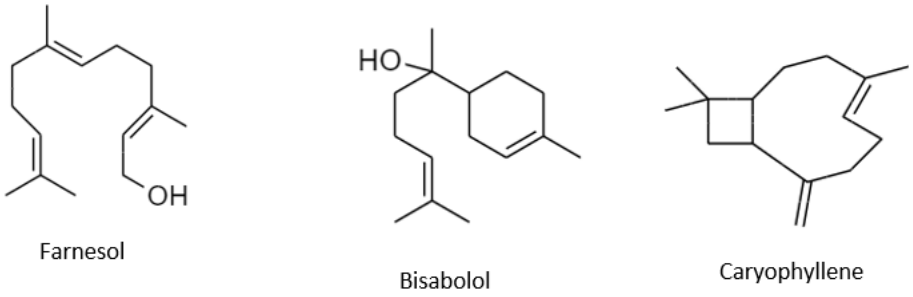


Figure 11.– Structures of sesquiterpenes (Hussein & El-Anssary, 2018).

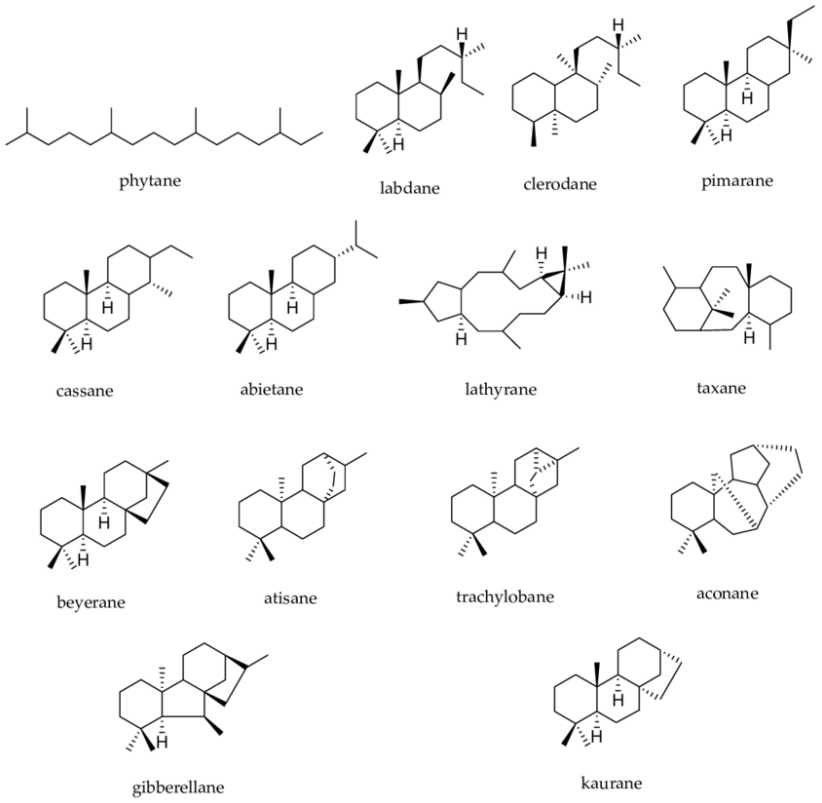


Figure 12. – Structures of diterpenes (De Sousa, Teixeira, & Furtado, 2018).

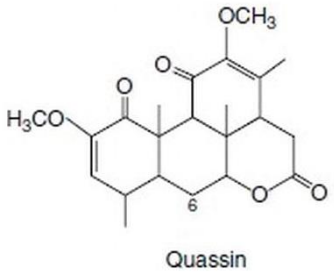


Figure 13. – Structure of a triterpene (Hussein & El-Anssary, 2018).

Lipids:

These are naturally occurring and include fixed oils (palmitic, steric, and oleic), waxes (long aliphatic chains), essential oils, sterols, fat soluble vitamins and phospholipids. Lipids function as antioxidants, anti-inflammatory, prevention of cardiovascular diseases, vehicle to enhance drug absorption, moisturizers, antiseptic, antimicrobial, analgesic, and as sedatives (Hussein & El-Anssary, 2018).

Cardiac Glycosides:

These are produced as a component of the defensive mechanism in plants. Cardiac glycosides are steroidal compounds which possess the ability to exert specific and powerful actions on the cardiac muscle. The main medicinal purpose is to treat congestive heart failure. This class of compound is made up of two main types differing in the structures of their aglycone moieties (Morsy, 2017).

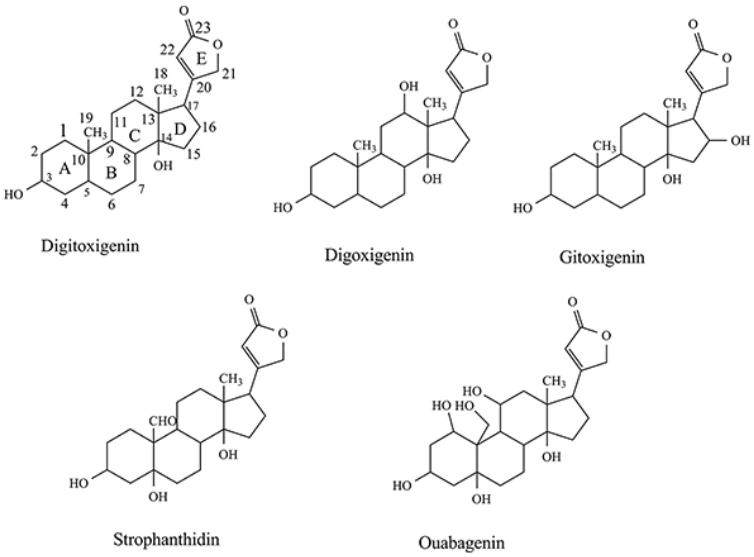


Figure 14. - Structures of some common cardiac aglycones (Morsy,2017).

Phytochemicals found in these plant species:

Crabwood:

(Luz, et al., 2019) found that the ethanolic leaf extract contained phenols, triterpenes, steroids, flavones, xanthones, flavonols, flavones and catechins. Those phytochemicals that were absent are tannins, coumarins, alkaloids, anthocyanidins and anthocyanins. They further ran thin layer chromatographic analyses which resulted in five spots. A comparison with analytical Rutin standard was done and they deduced the presence of flavonoid. Additionally, (Nayak, Kanhai, Malcolm, Pereira, & Swanston, 2011), performed phytochemical analysis on the ethanolic leaf extract and found the presence of alkaloids, essential oils, saponins and tannins. Triterpenoids and flavonoids were absent. The bark of another specie of crabwood, *Carapa procera* was studies by (Owusu, Afedzi, & Quansah, 2021). They found that a methanolic extract of the bark consisted of steroids, tannins and saponins, but no alkaloids.

Digitaria sanguinalis:

(Ibrahim, El-Hela, Dawoud, & Zhran, 2019) found that various chromatographic fractions of Long hairy crabgrass ethanol extract consist of steroids, hydroxycinnamic acids and flavone. (Kanupriya, Sharma, & Dhiman, 2021) examined the Digitaria genus and reported that some of the major phytochemicals present were terpenoid, volatile oils, alkaloids, flavonoids, phenolics, waxes and tannins.

Digitaria ischaemum:

As it relates to this crabgrass, no study was found that made mention to any of its phytochemical composition. One can suggest that similar composition to the long hairy crabgrass, as illustrated by (Kanupriya, Sharma, & Dhiman, 2021) is possible.

Thin Layer Chromatography:

Thin layer chromatography involves a thin layer of material that is either self – supporting or coated on a glass, plastic, or metal surface along with the mobile phase which moves upward through the stationary phase by capillary action. Plates are selected based on the type of separation that is required. High – performance plates provide sharper separations in shorter analysis time when compared to commercial plates (Skoog, Holler, & Crouch, 2016).

Depending on whether quantitative or qualitative results are desired, the technique of applying sample onto the plate is very critical. Usually, the sample is dissolved in a diluent and applied as a spot onto the plate, about 1 cm away from the edge. The spot is applied using a capillary tube and is approximately 5 mm in diameter. The technique involves multiple applications with drying between (Skoog, Holler, & Crouch, 2016).

The plate is developed, a process in which the sample is carried through the stationary phase by the mobile phase (elution solvent), allowing separation. This process is achieved by placing the spotted plate in a closed container saturated with vapors of the developing solvent. One end of the plate is immersed in the developing solvent, avoiding direct contact with the spot (sample) and the solvent. The developing solvent is left to navigate two thirds of the length of the plate, after which the plate is removed and allowed to dry (Skoog, Holler, & Crouch, 2016).

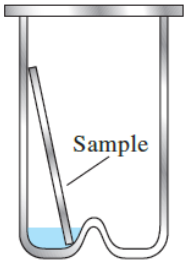


Figure 15. - The ascending flow chamber (Skoog, Holler, & Crouch, 2016).

Detection of the analyte can be done using different methods. Iodine staining and incorporating fluorescent materials into the stationary phase and examining under UV light are the two most frequently used methods. Additionally, for organic compounds, spraying with dilute sulfuric acid which yields dark products is commonly used (Skoog, Holler, & Crouch, 2016).

An important detection factor in TLC analyses is retention factor. It is defined as:

$$R_F = \frac{distance\ travelled\ by\ spot\ (d_R)}{distance\ travelled\ by\ solvent\ (d_M)}$$

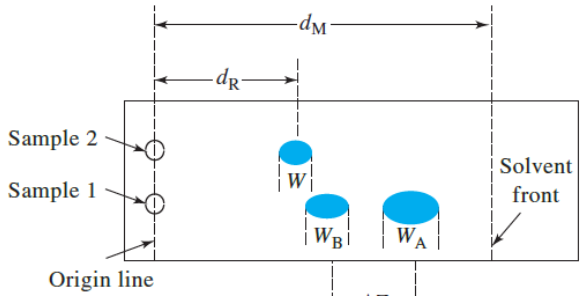


Figure 16. - Diagram of the definition of the RF value (Skoog, Holler, & Crouch, 2016).

Thin Layer Chromatography Visualizing Techniques:

Only the techniques used in this research will be discussed.

UV Light:

This is a very common, non - destructive visualization technique used for aromatics and conjugated systems. Two modes of UV lights are normally used; short – wave (254 nm)

and long – wave (365 nm). Most TLC plates available are incorporated with a fluorescent material that glows when placed under short wave UV light. When a compound absorbs 254 nm light, it will appear as a dark spot, while when placed under 365 nm, compounds glow intensely (Nichols & College, 2022).

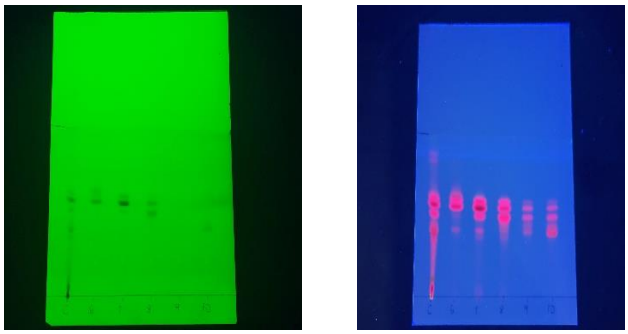


Figure 17. – Pictures of Short wavelength UV (256 nm)(Left) and long wavelength UV (365 nm) (right) visualization.

Vanillin Stain:

Vanillin stain is considered as a general-purpose staining agent which works for strong and weak nucleophiles (alcohols and amines), aldehydes and ketones. After performing TLC analysis, the plate is submerged into a vanillin + sulfuric acid mixture and mildly heated. The plate will develop into a light to dark pink colour.

This stain undergoes Aldol and acetalization reactions producing highly conjugated compounds on the TLC plates. These are highly coloured.

Some aldehyde and ketone undergo keto-enol tautomerism due to the acid conditions caused by the sulfuric acid. The enol undergoes acid catalysed nucleophilic addition to the vanillin via an aldol mechanism. Addition of heat facilitates the dehydration of the aldol product, resulting in a highly conjugated coloured compound. (Nichols & College, 2022).

Acetalization reactions occurs between vanillin and some alcohols, producing highly conjugated compounds which are colourful (Nichols & College, 2022).

Figure 18. - Picture of a TLC plate stained with Vanillin.

Permanganate Stain:

Permanganate is considered as a universal stain because it can be used to visualize alkenes, alkynes via addition reactions, and is capable of oxidizing functional groups such as aldehydes, alcohols and other oxidizable groups. It is a deep purple stain which reacts with compounds on a TLC plate, leaving a yellow spot. In most cases, some amount of heat is required to improve the contrast (Nichols & College, 2022).

Figure 19. – Picture of a TLC plate stained with potassium permanganate.

Iron (III) Chloride Stain:

Ferric chloride stain is very specific to phenols; hence it is used for the visualization of phenols and carbonyl compounds with high enol content. The chemical interaction occurs whereby Fe^{3+} ions form coloured complexes, more often blue with phenols (Nichols & College, 2022).

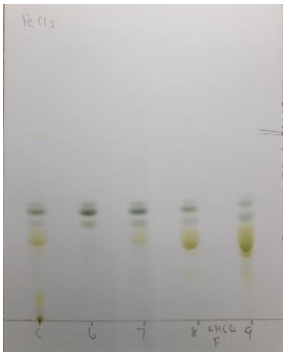


Figure 20. – Picture of a TLC plate stained with Iron (III) Chloride.

Bromocresol Green Stain:

This stain is specific for acidic compounds that produce a solution with pH value lower than 5. This works well for carboxylic acids. Application of heat after staining improves the contrasts between the spots and the TLC background. When the solution is below pH 3.8, the stained spot will be yellow and above 5.4, a blue colour will result (Nichols & College, 2022).

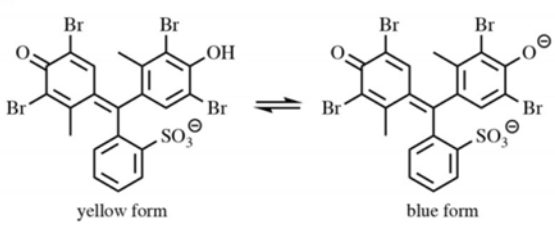


Figure 21. - Chemical structures of the yellow and blue forms of bromocresol green.

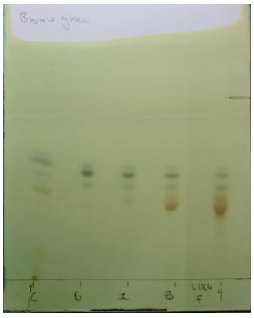


Figure 22. - Picture of a TLC plate stained with Bromocresol green.

Column Chromatography:

In column chromatography, the stationary phase is held in a narrow tube through which the mobile phase is forced under pressure. The stationary phase is a finely divided inert solid, usually silica that is tightly packed within the wall of a tube (glass column), fitted with a stopcock. The mobile phase is usually a mixture of solvents with a desirable polarity which occupies the spaces between the particles of the stationary phase (column packing). For separation of a sample composite, the sample is introduced at the head of the packed column. The component distributes themselves between the mobile phase and the stationary phase as a quantity of fresh mobile phase is continuously added and washed through the column – elution. Small amounts of elution solvents are collected for further TLC analysis (Skoog, Holler, & Crouch, 2016).

Silica used for the stationary phase is a polar absorbent. Due to the nature of its polarity, polar compounds from the sample mixture will interact with the silica more strongly than non – polar compounds. Therefore, one can expect that non – polar compounds will elute first, followed by polar compounds. When a sample is composed of analytes with similar polarities, the separation process can become challenging, whereby the mobile phase will have to be adjusted based on trial and error (Millar, 2012).

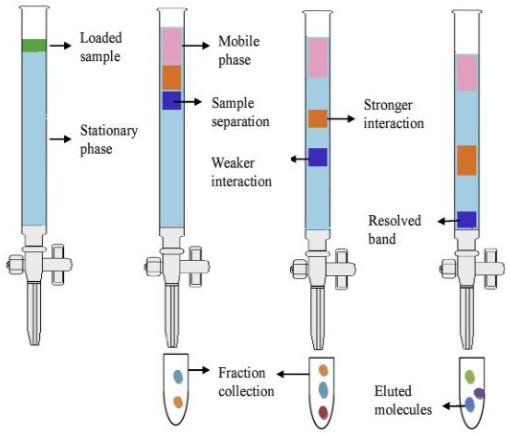


Figure 23. - The process of column chromatography (Rajeshwar p. Sinha, 2021).

Gas Chromatography – Mass Spectrometry:

In this instrument, a carrier gas is used as the mobile phase which is inert. Some of the common types of inert carrier gas used are helium, argon, nitrogen, and hydrogen. These are stored in pressured container in which the flow is controlled by a two-stage pressure regulator knob. A major precaution is that the carrier gas must be molecularly sieved to remove any moisture or impurities. Generally, an assumption is made that the flow rate of the carrier gas is constant. The carrier gas flows throughout the entire system (Skoog, Holler, & Crouch, 2016).

A sample must be injected properly into the injection port to achieve high column efficiency. It should be noted that slow injection or high injection volume causes band separation and poor resolution. Samples are injected automatedly through the septum with a micro syringe into the heated sample port located at the head of the column. The temperature of the sample port is about 500 ^{o}C above the boiling point of the least volatile solvent used. Sample injection can be done via split or splitless modes. Splitless injection offers better sensitivity (Skoog, Holler, & Crouch, 2016).

Two types of columns are used in GC; packed columns and capillary columns. The more common type used today is the capillary ones. These columns vary in length between 2 to 60 m or more. The body is constructed from stainless steel, glass, fused silica, or Teflon. Designed to be long, it is found as coils having diameters of 10 – 30 cm. Columns are usually situated within an oven. Column temperature is very important and must be controlled precisely. The column temperature (oven temperature) depends on the boiling point of the analytes and the degree of separation required. The elution time is usually between 2 to 30 minutes. Most modern systems can be temperature programmed to achieve better resolution (Skoog, Holler, & Crouch, 2016).

Mass spectrometric detector:

The feed from the GC capillary is fed directly into the ionization chamber of the mass spectrometer because of the low flow rate of the carrier gas. The sample is ionized, and the positive ions separated from electrons and molecular species by a negative voltage. They

are then accelerated and focused by a magnetic ion lens onto the entrance orifice of a quadrupole mass analyser (Skoog, Holler, & Crouch, 2016).

Quadrupole mass analyser:

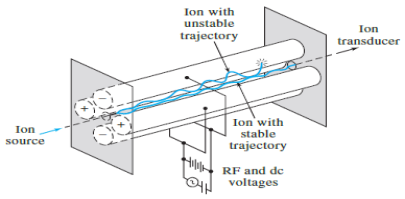


Figure 24. – Diagram of a quadrupole mass analyzer (Skoog, Holler, & Crouch, 2016).

This component is composed of four parallel cylindrical rods that are electrodes. The opposite rods are connected electronically; one pair being attached to the positive side and the other pair to a negative side of a variable DC voltage. A 180^{o} phase out radio frequency AC voltage is applied to each rod. A mass spectrum is obtained when ions are accelerated into the spaces between the rods by a potential difference of 5 to 10 V. These voltages on the rods are adjusted simultaneously while maintaining a constant ratio. All the ions except the ones having a desired mass to charge (m/z) ratio will strike the rods and covert to neutral molecules. The analytes with the desired m/z ratio will pass on to the transducer (Skoog, Holler, & Crouch, 2016).

Transducer – Electron Multipliers:

There are two configurations of this detector available.

The discrete dynode electron multiplier collects and converts positive ions into an electrical signal. Each dynode is kept at a successively higher voltage. How this function is that ions or electrons that are excited strike the Cu-Be coated surface of the cathode and the dynodes, and a burst of electrons is emitted. The electrons are attracted to the next dynode down the chain and this process is repeated until the last dynode is reached. At this point, there is a large number of electrons that appear for every ion that initially hits the cathode. This is depicted in the figure 3A below (Skoog, Holler, & Crouch, 2016).

The second configuration is known as the continuous dynode electron multiplier. This device is constructed with glass that is shaped like a cornucopia which is doped with lead. The addition of lead provides the conduction of electrons. A voltage of 1.8 to 2 kV is applied across the entire body of this transducer to facilitate a voltage gradient through both ends. An ion striking the surface at the entrance slit ejects electrons that are then attracted to a higher voltage point farther along the glass tube. As these electrons hit surfaces down the path, more and more electrons are ejected – secondary electrons. At the narrow ending of the glass tube, the electrons are quantified (Skoog, Holler, & Crouch, 2016).

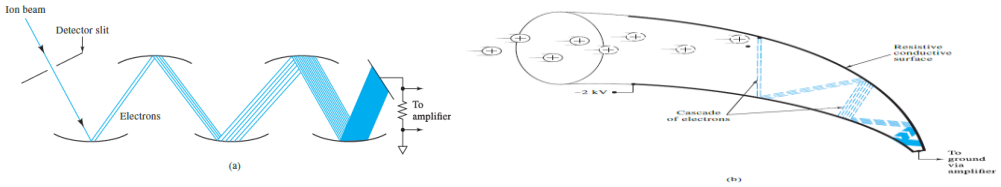


Figure 25. - Diagrams of the two different transducers A and B (Skoog, Holler, & Crouch, 2016).

Antimicrobial Resistance (AMR):

According to (WHO Regional Office for Europe/European Centre for Disease Prevention and Control, 2022) antimicrobial resistance is a major health concern which estimates more than 670, 000 infections that are due to bacterial resistant to antibiotics and approximately 33, 000 deaths have been resulted as a direct consequence in the European areas.

The report mentioned that antimicrobial resistance is common in the European nations, but its severity varies according to bacterial species, antimicrobial group, and geographical region. Surveillance showed that antimicrobial resistance continued be high and increasing with carbapenem resistance in *Escherichia coli* and *Klebsiella pneumoniae* and vancomycin resistance in *Enterococcus faecium*. Resistance to third generation cephalosporins and carbapenems in *K. pneumoniae* along with carbapenem resistant *Acinetobacter* species and *Pseudomonas aeruginosa* in several countries have been observed (WHO Regional Office for Europe/European Centre for Disease Prevention and Control, 2022).

Methods for Antimicrobial Assays:

Disc Diffusion method:

The disc diffusion technique was adopted in the 1950s by most clinical microbiology laboratories in the United States for testing susceptibility of bacteria to antimicrobials but later standardized in 1956 by W.M.M. Kirby. This method is known as Kirby – Disk Diffusion Susceptibility Test. The main purpose of this test is to determine the sensitivity or resistance of pathogenic aerobic and anaerobic bacteria to various antimicrobial compound. The way this works is that the pathogenic organism is cultivated on Muller – Hinton agar in the presence of the selected antimicrobial agent impregnated on the 6 mm filter discs. The presence or absence of growth around the discs is an indirect measure of the ability of that compound to inhibit that organism (Hudzicki, 2016).

Upon placement of the 6 mm disc impregnated with the antimicrobial agent on to the Mueller - Hinton agar plate, water is absorbed into the disc. This causes the antimicrobial agent to diffuse into the surrounding agar, hence the highest concentration of the antimicrobial agent can be found closest to the disc. A critical point to notes is that the rate of diffusion of the antimicrobial agent through the agar depends on the diffusion, solubility properties and molecular weight of the antimicrobial agent in the Mueller – Hinton agar. With these in consideration, each antimicrobial agent would have its own zone size, indicating susceptibility (Hudzicki, 2016).

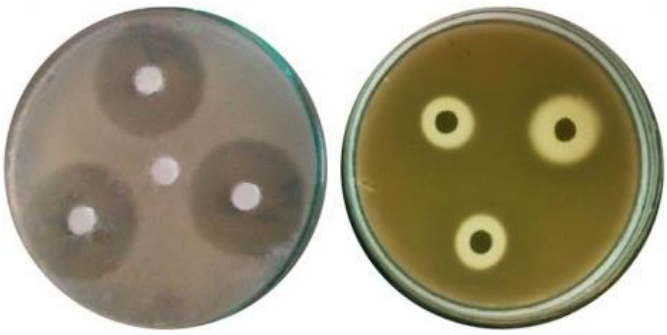


Figure 26. - Illustration of the disc diffusion method (Ibnsouda, Sadiki, & Balouiri, 2016).

Agar Well Method:

Agar well diffusion method is very similar to agar disc diffusion method. This method is used more to evaluate antimicrobial activities of plants extracts. On the Mueller – Hinton

agar plates, the surface is inoculated by spreading a quantity of the micro-organism to be tested over the entire surface. A sterile cork borer is used to punch 6 to 8 mm holes into the agar gel, after which a desired quantity of the antimicrobial agent is placed into the wells. The agars plates are then incubated at a specific temperature and time depending on the tested organism. Similarly, the antimicrobial agent diffuses into the agar medium and prevents the growth of the micro – organism (Ibnsouda, Sadiki, & Balouiri, 2016).

Figure 27. - Illustration of the agar well method (Ibnsouda, Sadiki, & Balouiri, 2016).

Antimicrobial Assays in previous studies:

(Mecciaa, et al., 2013) in their research studied antimicrobial properties of hydro distilled leaf extract of Carapa guianensis. Antimicrobial activity of the crude extract as well as the essential oil was obtained using the disc diffusion assay. The microorganisms used were *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Salmonella Typhi, Pseudomonas aeruginosa, Candida albicans and C. krusei.* The essential oil was active only against *Staphylococcus aureus* and *Enterococcus faecalis* with a MIC of 400 µg/mL for both microorganisms. The methanolic crude extract was active against *S. aureus* and *E. faecalis* showing a MIC of 50 mg/mL. The other two extracts were not active against any of the tested microorganisms.

Conversely, (Nayak, Kanhai, Malcolm, Pereira, & Swanston, 2011) performed antimicrobial analysis on the ethanolic leaves extract from Carapa guianensis which was found to be inactive against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli, Staphylococcus aureus*, and methicillin- resistant *S. aureus.*

Antimicrobial studies were performed on the bark of another specie of crabwood; *Carapa procera.* This is commonly known as African crabwood and predominantly found in West

Africa, Congos. (Owusu, Afedzi, & Quansah, 2021) carried out antimicrobial testing with methanolic extract of *Carapa procera* bark against *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyrogenes, Staphylococcus aureus* and *Candida albicans.* They used four different concentrations with the agar well diffusion method for the antimicrobial assays. All the extract concentrations exhibit antimicrobial activity on the four bacterial and the fungal strains.

Digitaria Sanguinalis L. was examined in Cario, Egypt by (Ibrahim, El-Hela, Dawoud, & Zhran, 2019). They evaluated antimicrobial activities on the alcoholic extract and isolated compounds from the aerial sections on the plant. Disc diffusion method was utilized in their studies using gram – positive bacteria, *Staphylococcus aureus* and *Bacillis subtilis,* gram – negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa,* fungal strains, *Aspergillus fumigates* and *Candida albicans.* The tested samples were prepared at a concentration of 1 mg/mL for fractions and 250 μg/mL for isolated compounds dissolved in DMSO. They found that all the tested fractions showed antimicrobial activity against all the tested microorganisms.

Chemical Composition:

(Qi, Wu, Zhang, & Luo, 2004) in their study isolated nine compounds from the ethyl alcohol extract of the twig of Carapa guianensis Aubl using numerous instrumentation techniques. These are: 1,3-di-benzene carbon amine-2-octadecylic acid-glyceride (1), hexacosanoic acid – 2,3 – dihydroxy – glyceride (2), ursolic acid (3), naringenin (4), scopoletin (5), 3,4 – dihydroxymethylbenzoate (6), 2,6 – dihydroxymethylbenzoate (7), tetratriacontanoic acid (8), triacontanoic acid (9). The researchers indicated that; compound 1 was new, compound 2 was firstly isolated from nature and compounds 3 – 9 were firstly obtained from this plant source.

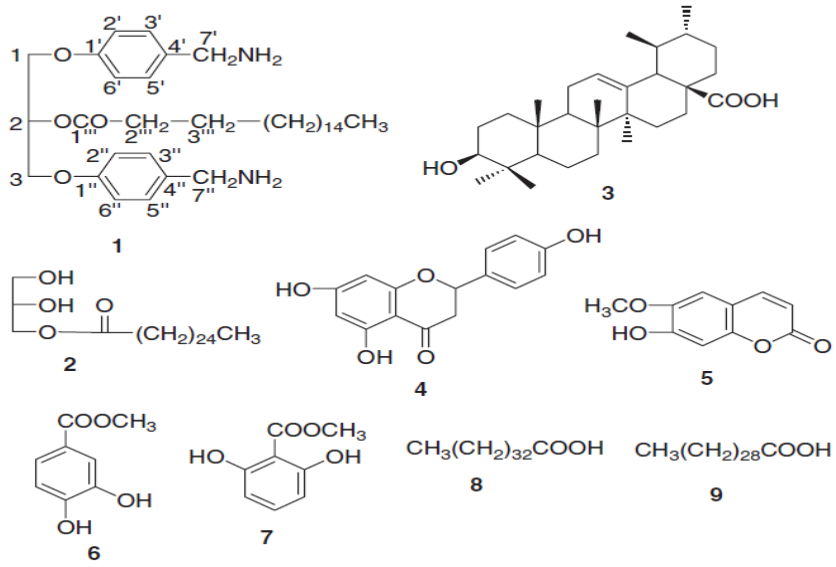


Figure 28. – Compounds isolated from the EtOH extract of Carapa guianensis (Qi, Wu, Zhang, & Luo, 2004).

(Mecciaa, et al., 2013) in their research isolated twenty-three compounds from the essential oil obtained from the leaves of Carapa guianensis Aubl via Gas Chromatography – Mass Spectrometry and Gas Chromatography – Flame Ionization Detection. These compounds represent 93.7% of the oil where the major constituents were; 28.5% bicyclogermacrene, 17.2% α – humulene, 11.9% germacrene B and 9.9% trans – β – caryophyllene. Sesquiterpenes represented 92.5% of the essential oil.

(Oliveira, et al., 2018) chemically analyzed the seed oil of C. guianensis by electrospray ionization mass spectrometry (ESI – MS). All the C. guianensis seed oil samples analyzed exhibited the same pattern of fatty acids. 7-Deacetoxy-7-hydroxygedunin, deacetyldihydrogedunin, deoxygedunin, andirobin, gedunin, 11β-hydroxygedunin, 17-glycolyldeoxygedunin, 6α-acetoxygedunin, and 6α,11β-diacetoxygedunin were identified in the limonoid-rich fractions of the oil.

(Marcelle & Mdotoo, 1975) mentioned that the seeds of Carapa guianensis yielded several tetranortriterpenoids. Upon investigation they found the heart wood consisted of 11β-acetoxygedunin, 6α 11β-diacetoxygedunin and 6α-acetoxygedunin.

(Inoue, et al., 2013) isolated two novel limonoids; guianolides A and guianolides B from the seeds of Carapa guianensis AUBLET and established their structures via spectroscopic analyses and Xray crystallography.

(Ibrahim, El-Hela, Dawoud, & Zhran, 2019) isolated seven compounds from *Digitaria Sanguinalis L* found in Cairo, Egypt with structural elucidation achieved based on spectroscopic analysis – Ultraviolet, Infrared, ^{1}H and ^{13}C Nuclear Magnetic Resonance and Mass Spectrometry. These compounds are *p* – coumaric acid (1), tricin (2), *p* – hydroxybenzoic acid (3), stigmasterol (4), β – sitosterol – 3 – O – β – D – glucoside (5), tricin – 7 – O – β – D – glucopuranoside (6) and isoorientin (7).

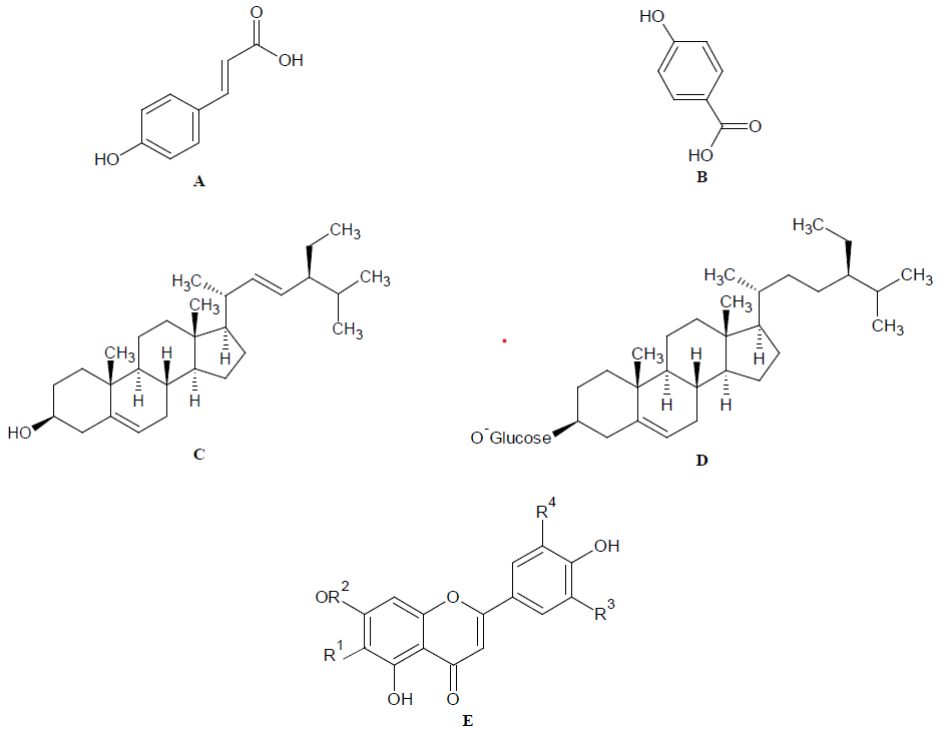


Figure 29. - Structures of the isolated compounds: (A) Compound (1); (B) compound (3); (C) compound (4); (D) compound (5); (E) compound (2): R1- H, R2- H, R3- OCH3, R4- OCH3; compound (6): R1- H, R2- glucose, R3- OCH3, R4- OCH3; compound (7): R1- g

4.0 – METHOD

Shown below is a schematic representation of the methos sequence followed in this investigation.

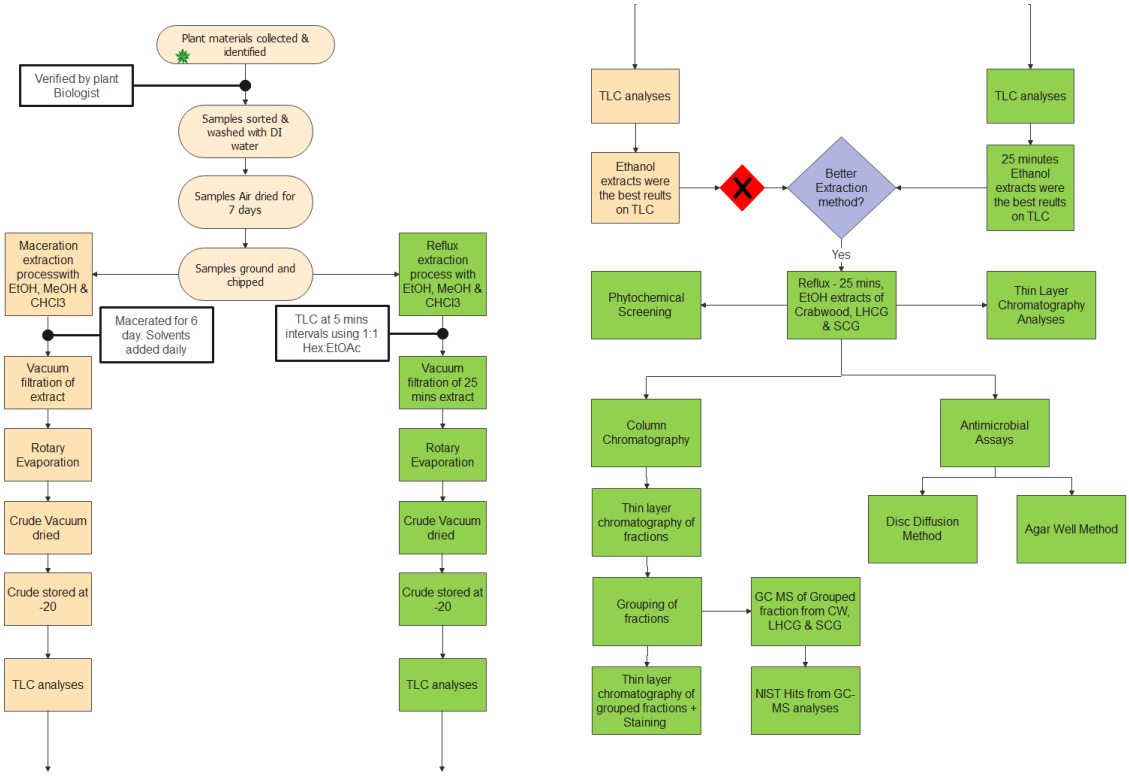


Figure 30 - Summary of the method sequence.

Collection, Identification and Crude Preparation:

Collection:

Plant materials of *Digitaria sanguinalis* and *Digitaria ischaemum* were collected from the neighborhood in Leonora, West Coast Demerara, Guyana, South America. The bark from *Carapa guianensis* was obtained from Mahase Lumber yard which is located on the Linden Highway.

These plants were verified by a plant Biologist who is affiliated with the University of Guyana where voucher specimens were tagged and deposited in the Herbarium of the University of Guyana.

The grass samples were sorted, de – leafed, washed with deionized water and air dried for 7 days. Similarly, the crabwood bark was washed with deionized water, air dried and chipped.

Two methods of extractions were used: maceration and reflux using three solvents; ethyl alcohol, methyl alcohol and chloroform. For the reflux method, the samples were further ground with an electric grinder.

Figure 31. – Picture of the cutting of the Crabwood bark on Mahase's Lumber Yard.

Figure 32.– Picture of the collected Long Hairy Crabgrass.

Figure 33.– Drying of the grass samples after washing.

Figure 34. – Drying of the chipped Crabwood bark.

Figure 35. – Picture of the dried Long Hairy Crabgrass (left), Smooth Crabgrass (centre) and Crabwood bark (right).

Figure 36.– Picture of the ground samples (top) and chipped samples (bottom) – Crabwood, Long Hairy Crabgrass and Smooth Crabgrass.

The maceration extraction process:

Various quantities of each sample were weighed, placed into 4-litre ambered glass bottles and soaked into the desired solvent (chloroform, ethanol, and methanol). Initially, 2 litres of each solvent were used but increments were added daily. These were left to brew for six days, after which were vacuumed filtered with Whatman # 4 filter paper and a Büchner filtering apparatus. All the filtrates were stored in ambered glass bottles and rotary evaporated to concentrate the extracts. The crude extracts were subsequently vacuum dried to obtained solid crystals. The yields were calculated, and all crudes and crystals were stored properly in the refrigerator, protected from light or/and in desiccators.

Figure 37. – Long Hairy Crabgrass being soaked in the respective solvents.

Figure 38.– Smooth Crabgrass being soaked in the respective solvents.

Figure 39.– Crabwood being soaked in the respective solvents.

Figure 40.– Picture of the Rotary Evaporation of the solvent being done with a Buchi R 210.

The reflux extraction process:

50.0 g of each of the ground sample was weighed and added to separate boiling flask fitted with a three-neck connector. Approximately 600 – 700 mL of ethanol was measured and added to each of the boiling flask containing the samples. Condensers were fitted onto each flask and the apparatus placed into a temperature-controlled water bath. Chilled water was allowed to flow through the condensers and the reflux process was started and timed. Sampling of the reflux mixture from each flask was conducted at 5 minutes intervals up to a total of 25 minutes using a 1 mL glass pipette. Concurrently, the sampled reflux mixtures were subjected to thin layer chromatographic analyses using 1:1, Hexane : Ethyl Acetate as the developing solvent. At the end of the reflux process, the mixture was vacuum filtered, and the filtrate stored in the refrigerator until concentrated with a rotary evaporator. The crude extracts were vacuum dried in a vacuum oven at 50 ^{o}C and subsequently stored in the freezer at – 20 ^{o}C for further analyses.

The entire process was repeated with the other two extraction solvents: Methanol and Chloroform.

The goal was to reflux all three samples with the same solvent concurrently in separate reflux apparatus. This was achieved by using a large water bath, equipped with a temperature controller. Adequate cooling for the condensers was required since low temperature solvents were used in the reflux. For this, each condenser was connected parallelly to a chiller which lowered the coolant's temperature to approximately 4 – 5ºC.

Figure 41.– Picture of the samples in their reflux flasks.

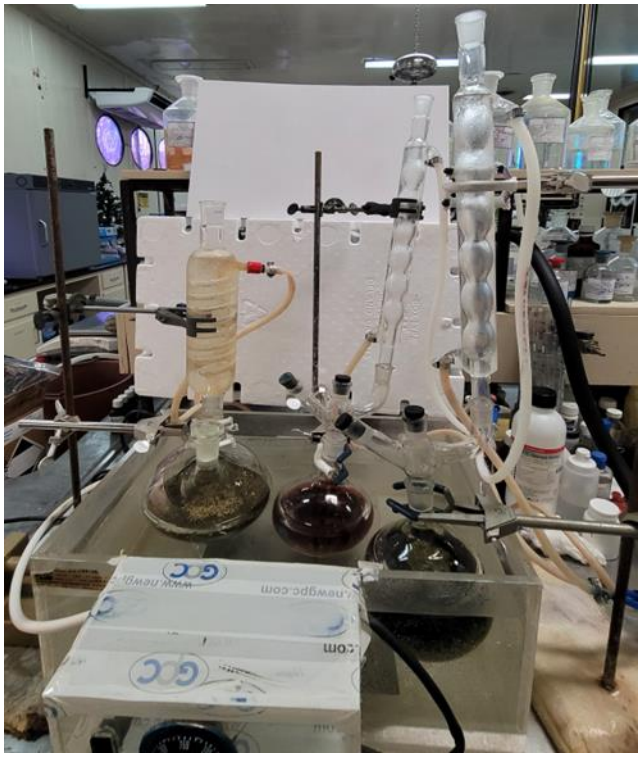


Figure 42.– Picture of the reflux setup – water bath, flasks, sample, and condensers (cold).

Thin Layer Chromatography (TLC) of the Crudes:

TLC of the Maceration extracted crudes:

The TLC developing chamber was washed with deionized water, dried, and rinsed with acetone. It was then placed under the fume hood and allowed to evaporate to dryness. TLC plates were cut with a utility knife and a ruler to dimensions of 10 inches by 8 inches. A pencil and a ruler were used to inscribe a line, 1 cm from the bottom of the plate, indicating the spotting line. A small amount of each extract was dissolved into their respective solvent of extraction; for example, the ethanol extract was dissolved into a small amount of ethanol. The elution solvent (mobile phase) used for the crudes were Hexane : Ethyl acetate (1:1) and Hexane : Ethyl acetate (5:1). This solvent was added to the TLC developing chamber, covered, and allowed to remain rested for approximately 15 minutes for the saturation of the vapor to occur. A micropipette was used to carefully load the crude extracts onto the TLC plate at the starting spot. The loaded TLC plate was then placed into the developing chamber, paying keen attention to avoiding the loaded spots getting submerged into the

elution solvent. The development was then monitored until solvent was approximately 1 inch front the top of the TLC plate, after which, a pencil was used to inscribe the solvent front. The plate was allowed to evaporate and viewed under short and long UV wavelengths where pictures were taken and spots from the separation were identified. Other visualization technique used was iodine staining.

Maceration Extraction			
Sample	***Solvent***		
Long Hairy Crabgrass	Ethanol	Methanol	Chloroform
Smooth Crabgrass	Ethanol	Methanol	Chloroform
Crabwood's Bark	Ethanol	Methanol	Chloroform

Table 1- The maceration extraction process and the solvent used.

TLC of the Reflux extracted crudes:

Similarly, The TLC developing chamber was washed with deionized water, dried, and rinsed with acetone. It was then placed under the fume hood and allowed to evaporate to dryness. TLC plates were cut with a utility knife and a ruler to dimensions of 10 inches by 8 inches. A pencil and a ruler were used to inscribe a line, 1 cm from the bottom of the plate, indicating the spotting line. The elution solvent used was Hexane : Ethyl Acetate (1:1). This solvent was added to the TLC developing chamber, covered, and allowed to remain rested for approximately 15 minutes for the saturation of the vapor to occur. During the reflux process, 1 mL of sample was withdrawn at 5 minutes intervals up to 25 minutes and stored in 2 mL amber vials. This was achieved by using separate 1 mL glass pipettes. After allowed to cool, these samples were loaded at the start line onto the TLC plate by using micropipettes. The loaded TLC plate was then placed into the developing chamber, paying keen attention to, not letting the loaded spots submerged into the elution solvent. The development was then monitored until solvent was approximately 1 inch front the top of the TLC plate, after which, a pencil was used to inscribe the solvent front. The plate was allowed to evaporate and viewed under short and long UV wavelengths where pictures

were taken and spots from the separation were identified. Other visualization technique used was iodine staining.

Reflux Extraction				
Sample	***Solvent***			***Interval time***
Long Hairy Crabgrass	Ethanol	Methanol	Chloroform	5 mins, 10 mins, 15 mins, 20 mins, 25 mins.
Smooth Crabgrass	Ethanol	Methanol	Chloroform	5 mins, 10 mins, 15 mins, 20 mins, 25 mins.
Crabwood's Bark	Ethanol	Methanol	Chloroform	5 mins, 10 mins, 15 mins, 20 mins, 25 mins.

Table 2- The reflux extraction process, its solvent used and the interval times.

Based on the TLC results obtained from the ethanol, methanol and chloroform crude extracts of long hairy crabgrass, smooth crabgrass, and crabwood's bark, from both extraction processes; maceration and reflux, showed that the 25 minutes ethanol solvent extracts from the reflux extraction process contained the most compounds.

Phytochemical Screening:

Since the 25 minutes ethanol solvent reflux extraction process showed to have the most spots with the separation, only those were subjected to phytochemical screening.

Reflux Extraction		
Sample	***Solvent***	***Interval time***
Long Hairy Crabgrass	Ethanol	25 minutes
Smooth Crabgrass	Ethanol	25 minutes
Crabwood's Bark	Ethanol	25 minutes

Table 3- The best extraction method, solvent, and interval time.

These studies were guided by “General Techniques Involved in Phytochemical Analysis” written by K. Sahira Banu and Dr. L. Cathrine, published in the International Journal of Advance Research in Chemical Science (IJARCS) along with (Ismail , et al., 2014)

Sample preparation – 300 mg of ethanol crude extract of each sample was weighed. This quantity of crude extract was dissolved in 40 mL of 100% ethyl alcohol with gentle heating. A quantity of this was diluted with nano pure water at a ratio of 1:1.
For the tests, blanks were analyzed using 1:1 ethanol: water mixture.

Salkowski’s Test - Testing for terpenoids: To 5 ml of the extract 2 ml of chloroform was added and subsequently 3 ml of concentrated sulphuric acid; formation of a reddish-brown ring confirms the presence of terpenes. This reddish – brown ring is formed because sulfuric acid is highly hygroscopic which leads to the dehydration of terpenoids. This mechanism removes two water molecules from the terpenoids, forming new double bonds. With this being the case, two terpenoids bind together whereby a diterpenoid is formed. The reddish – brown colour is primarily due to the presence of bi-sulfonic acid (product of sulfuric acid sulfonation).

Alkaline Reagent Test – Testing for Flavonoids: To 5 mL of extract, an amount of 10% sodium hydroxide solution was added. This produced a yellow colour which disappears upon the addition of dilute hydrochloric acid. This shows the presence of flavonoids.
Flavonoids are generally yellow compounds which are soluble in alkaline conditions. Since they contain conjugated aromatic systems, they show intense absorption bands in the ultra-violet and visible region of the spectrum. When dissolved in alkaline solutions, the intensity of their yellow colour increases with the number of hydroxyl groups (OH). Therefore, as the pH increases, the intensity of the yellow colour also increases. This phenomenon becomes colourless upon the addition of an acid to the solution (Mohammed, 1996).

Test for saponins: The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicated the presence of saponins.

Saponins foam in water because the molecules align themselves vertically on the surface with their hydrophobic ends oriented away from the water. This causes the water tension of water to reduce, hence foaming (Zhang, et al., 2017).

Liebermann-Burchard's Test – Testing for Steroids and Phytosterols: The extract (50 mg) was dissolved in of 2 ml acetic anhydride. To this, 2 drops of concentrated sulphuric acid are added slowly along the sides of the test tube. Change of colour from violet to blue confirmed the presence of steroids.

Sulfuric acid and acetic anhydride aids in the removal of an OH group on the steroidal molecule while sulfonation occurs on the same aromatic ring. This causes the aromatic molecule to have a lambda max of approximately 410 nm (Chih Hsu, Bai Zhou, Huan, Dutkiewicz, & Hua Li, 2019).

Keller – Kiliani's Test – Testing for Cardiac Glycosides: 50 mg of extract is treated with 2 mL of glacial acetic acid containing one drop of 5% ferric chloride solution. This is followed by the addition of 1 mL concentrated sulphuric acid. A brown ring at the interface indicates cardenolide deoxy sugar, while a violet ring below the brown ring and greenish ring in the acetic acid layer indicates the presence of cardiac glycoside.

Cardiac glycosides have two moieties; a sugar and a non-sugar. The addition of glacial acetic acid causes acid hydrolysis of the deoxy – sugars to occur. This is transformed into aglycone and a sugar residue (Maldonado Rodriguez, 2016).

Biurets Test – Testing for Proteins: 50 mg of extract is diluted with distilled water and treated with Biuret's reagent. Add 1 mL of ethanol (95%). A pink/purple layer indicates the presence of proteins.

Proteins contain peptide bonds. The biuret's reagent is made with a base, sodium hydroxide, hydrated copper (ii) sulphate, and a chelating agent, potassium sodium tartrate. When the solution is basic, copper (ii) sulphate forms complexes with peptide bonds, utilizing the unpaired electrons on the nitrogen atoms. Four nitrogen atoms donate lone pairs of electrons to form coordinated covalent bonds with the cupric ion, hence resulting in a chelating complex which absorb light at 540 nm (Aryal, 2021).

Benedict's Test – Testing for Carbohydrates: To 1 ml of sample solution, add 2 ml of Benedict's reagent. The mixture is heated on a boiling water bath for 2 minutes. A characteristic-coloured precipitate indicates the presence of sugar. Green/yellow shows traces, orange shows moderate and red indicates largely present.

Benedict's reagent is composed of anhydrous sodium carbonate, trisodium citrate dihydrate and copper (ii) sulphate. Sodium carbonate makes the sample solution basic (high pH) which causes the tautomerization of the reducing sugars to enediols. Copper 2+ ions are reduced to copper 1+ due to the presence of enediols (strong reducing agent). Copper 1+ is now in the form as insoluble copper (I) oxide or cuprous oxide (Cu_2O) which is red in colour (Dahal, 2022).

Fehling's Test – Testing for Reducing Sugar: 15 mL of Fehling's A and B were mixed. 2 mL of this mixture was added to a test tube and 3 drops of plant material added. This mixture was then place in a water bath at 60 ^oC. A positive test for reducing sugars includes colour changes to yellow, green, red and brick red.

Fehling's A is made up with copper sulphate and concentrated sulphuric acid while Fehling's B is composed of sodium potassium tartrate and sodium hydroxide. A similar mechanism as Benedict's test is followed. Heating of the sample along with the Fehling's mixture, the bis-tar-taro-cuprate (ii) complex oxidizes the aldose functionality to the corresponding carboxylic acid. When this occurs, copper (ii) ions of the complex are reduced to insoluble copper (i) oxide (red). Sodium potassium tartrate is important since it chelates the copper and prevents the formation of insoluble copper hydroxide. These complex releases copper (II) ions slowly, thus inhibiting the formation of black copper oxide (Sapkota, 2020).

Ferric Chloride Test – Testing for Tannins and Phenolic Compounds: The extract (50 mg) was dissolved in 5 ml of distilled water. To this, a few drops of neutral 5% ferric chloride solution were added. A dark green colour indicates the presence of phenolic compound.

Phenols react with the ferric chloride solution to form complexes and hydrochloric acid.

$$6\ C_6H_5OH + FeCl_3 \rightarrow [Fe(C_6H_5O)_6]_3^- + 3HCl + 3H^+$$

Wagner's Test – Testing for Alkaloids: 50 mg of extract is stirred with a few mL of dilute hydrochloric acid and filtered. A few drops of Wagner's reagent are added at the side of the test tube – the formation of a reddish-brown precipitate shows the presence of Alkaloids.

Wagner's reagent is made with iodine crystals and potassium iodide. Iodine in this mixture reacts with the iodide ion (I^-) from the potassium iodide producing I^{3-}, resulting in a brown solution. The metal ion K^+ binds through covalent coordinate bonding with nitrogen of the alkaloid producing a complex precipitate of potassium – alkaloid which is reddish brown (Adella, Nurman, Gunawan, Prestica, & Parbuntari, 2018).

Column Chromatography:

Column chromatography was performed on the ethanol crude extracts of long hairy crabgrass, smooth crabgrass and crabwood's bark obtained from the 25 minutes reflux extraction process.

Making up of the solvents:

The solvents used were: Absolute Ethanol, Hexanes, Ethyl Acetate, Methanol and Dichloromethane (DCM). Before using these solvents, they were dried over anhydrous magnesium sulfate for 48 hours and subsequently filtered and stored in airtight containers. The different ratios of solvents (varying polarity) were made up using cleaned and dried measure cylinders prior to the column separation analyses.

Column preparation:

A 28 mm internal diameter glass column, fitted with a stop cock was selected to be used for the column chromatography. This glass column was thoroughly washed with de ionized water, subsequently acetone washed and left to dry under the fume hood. A ball of glass wool was secured in the narrowest part of the column with the assistance of a long-cleaned

glass rod. The column was then placed onto the retort stand and clamped securely. An ideal amount of cleaned sand was added until the layer reached just above the neck, onto the main body of the column. The reason for this is that it provided an even base for the stationary phase and prevented concentration and streaking of the bands as they are eluted off the column. An amount of hexane was carefully added along the sides of the column to prevent the layer of the sand from being disrupted. This serves many purposes; it washes the sand and the glass wool, it allows the sand to settle, it helps the sand to be compacted, it removes air bubbles, and it prevents the sand layer to be disrupted when adding the stationary phase into the column.

Column packing:

The slurry method was used to pack the column with stationary phase. VWR High Purity Silica Gel 60 A, irregular, 70 -90 um was used as the stationary phase. Since the TLC resulted in a difficult separation, 105 g of silica was weighed into a large beaker and hexane was added. Using a glass rod as a stirrer and a funnel, this slurry mixture was slowly and carefully added to the column, while the stop cock was open. The silica in the column was allowed to settle over night with hexane above its layer.

Loading the column with crude extract:

0.5 g of the ethanol crude extract of long hairy crabgrass was weighed and dissolved in a small amount of absolute ethanol (approximately 2 – 3 mL). A plastic pipette was used to load the dissolved crude onto the stationary phase (silica) in the column. The first elution solvent (mobile phase) was carefully added along the walls of the column. This elution solvent ranged from non-polar (hexane) to polar (combination of hexane, ethyl acetate, methanol and DCM). The stop cock was open to allow drops of elution solvents to pass which were collected in 5 mL fractions. These fractions were stored in tightly capped ambered bottles and at -20^{o}C.

Similar experimental steps were performed with the smooth crabgrass and crabwood extracts. The only difference was the combination of the elution solvents which are listed in the tables below:

Smooth Crabgrass	
Elution solvent	**Fractions collected**
Hexane : Ethyl Acetate (3:1)	1 to 80
Hexane : Ethyl Acetate (1:1)	81 to 150
Ethyl Acetate (100%)	151 to 195
Ethyl Acetate : Methanol	196 to 220
Methanol (100%)	221 to 250

Table 4- The elution solvent mixtures along with the resulting fractions obtained for Smooth Crabgrass ethanol extract.

Long Hairy Crabgrass	
Elution solvent	**Fractions collected**
Hexane : Ethyl Acetate (3:1)	1 to 10
Hexane : Ethyl Acetate (1:1)	10 to 40
Hexane : Ethyl Acetate (1:3)	41 to 76
Ethyl Acetate (100%)	77 to 87
Ethyl Acetate : Methanol (3:1)	88 to 116
Methanol (100%)	118 to 128

Table 5- The elution solvent mixtures along with the resulting fractions obtained for Long Hairy Crabgrass ethanol extract.

Crabwood's Bark	
Elution solvent	**Fractions collected**
Hexane : Ethyl Acetate (1:3)	1 to 15
Ethyl Acetate (100%)	16 to 34
Ethyl Acetate : Methanol (5:1)	35 to 98
Ethyl Acetate : Methanol (3:1)	99 to 130
Ethyl Acetate : Methanol (1:1)	131 to 160
DCM : Methanol (1:1)	161 to 188

Table 6- The elution solvent mixtures along with the resulting fractions obtained for Crabwood ethanol extract.

Thin Layer Chromatography (TLC) of the Fractions

Fractions collected from the column separation run of the ethanol crude extracts from long hairy crabgrass, smooth crabgrass and crabwood's bark were analyzed by using thin layer chromatography. A similar procedure that was mentioned in the Thin Layer Chromatography (TLC) of the Crudes section was used. The elution solvents were; Hexane : Ethyl Acetate (3:1) and Hexane : Ethyl Acetate (1:1), while fluorescence TLC plates were used. Visualization techniques were done by using; normal view, UV light (254nm and 365nm), and staining techniques. After every TLC analysis, photographs were taken with a camera. This data was subsequently sorted, and similar fractions were grouped and rotary evaporated to dryness.

Thin Layer Chromatography Staining Techniques:

Four staining methods were used: Vanillin, Potassium permanganate, Iron (iii) chloride and Bromocresol green. These staining solutions were made up and placed into large beakers. Each fraction was subjected to TLC four times since there are four staining techniques. After the TLC analyses, the plates were allowed to dry and then submerged into the beaker for 30 seconds. Heat was applied with a heat gun for the spot development with Vanillin and Potassium permanganate stains while Iron (iii) chloride and Bromocresol green were left to develop at room temperature.

Vanillin Stain:

Make up – 30.0 g of vanillin was weighed and dissolved into 500 mL of absolute ethanol. 5.0 mL of concentrated sulfuric acid was added to the mixture. This solution was protected from light and stored in the refrigerator.

Potassium Permanganate stain:

Make up – 3.75 g of potassium permanganate and 25.0 g of potassium carbonate were weighed and dissolved in 300 mL nano pure water. 3.1 mL of 10% sodium hydroxide was added to this mixture and then diluted to 500 mL with nano pure water.

Iron (iii) Chloride:

Make up – 500 mL of water : methanol (1:1) was made up and use to make a 1% iron (iii) chloride solution.

Bromocresol Green:

Make up – 0.2 g of bromocresol green was weighed and dissolved in 500 mL absolute ethanol. 0.10 M sodium hydroxide was added dropwise, and the solution stirred until it changed its colour from yellow green to blue.

Chemical determination via Gas Chromatography – Mass Spectrometry:

Gas chromatography – mass spectrometry was selected for the chemical composition of the fractions from ethanol extracts of long hairy crabgrass, smooth crabgrass, and crabwood's bark. These were obtained using the Agilent Technologies 7820A GC system instrument. This analysis technique was selected because it was available at the time of study, sample preparation was very easy, and a method development was not required as in the case of HPLC – MS/PDA.

Hexane: Ethyl acetate 1:1 mixture was added to the dried fractions and a small amount was pipetted into an GC – MS vial. This was further diluted with the 1:1 hexane: ethyl acetate mixture. These vials were place into the 7693 Agilent Technologies autosampler.

Compressed Helium at a flow rate of 1 mL/min and pressure of 7.6522 psi was used as the carrier gas which was allowed to flow through Agilent gas clean filter to help with moisture interferences. The inlet's temperature was set at 250 ^{o}C at a flow of 20 mL/min along with split injection ratio of 10 with 10 mL/min split flow. Sample injection volume was set at 2 µL. The separation was done using Agilent Technologies HP – 5MS UI, 30 m x 0.250 mm, 0.25-micron column (-60 to 325/350^{o}C, SN: USN557623H). Oven parameters were initially at a temperature of 50^{o}C with a hold time of 5 minutes, then ramp 1; temperature increase at a rate of 10^{o}C/min to 150^{o}C, holding for 5 minutes, then a further increase in ramp 2 at a rate of 10^{o}C/min to 280^{o}C, holding for 8 minutes. Mass spectrums were collected with Agilent Technologies 5977B MSD. The total run time for one analysis was 41 minutes.

The spectra were collected in Agilent Mass Hunter software and analyzed with NIST 14 library to determine possible hits for the chemical composition of each fraction.

Sample	Number of fractions analyzed	Number of compounds found	Approximate analyses time
Crabwood	22	210	16 hours
Long Hairy Crabgrass	26	225	18 hours
Smooth Crabgrass	27	247	19 hours

Table 7– The compounds found in the pooled fractions of each plant material and the analyses time.

Antimicrobial Assay:

Ethanol extracts from Long hairy crabgrass, Smooth crabgrass and Crabwood's bark were assayed against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Candida albicans.* Two methods were used: Disc Diffusion Method and Agar Well Method.

Preparation of the Mueller Hinton Agar Plates:

Mueller Hinton Agar was prepared as per 38.04 g per liter of deionized water. This was sterilized in an autoclave. It was poured into the sterile petri dishes, allowed to cool, and solidified in the clean room. All the plates were appropriately labelled.

McFarland's Standard:

This standard is a suspension of barium sulfate that allows visual comparison of bacterial density. A 0.5 McFarland standard is equivalent to a bacterial suspension containing between 1 x 10^8 and 2 x 10^8 CFU/ml of E. coli.

Preparation – A 1% solution of anhydrous barium chloride and a 1% solution of sulfuric acid were made. 0.05 mL of 1% barium chloride was added to 9.95 mL of 1 % sulfuric acid, combined and completely mixed. The turbidity of this mixture was verified by measuring the absorbance using a spectrophotometer with a 1 – cm light path and matched cuvette. The absorbance at 625 nm was between 0.08 to 0.13. Once the absorbance was within the required range, the solution was stored in foil covered and tightly sealed tubes

at room temperature. Before using this standard, it was vortexed to remove all clumps of barium sulfate.

Live Organisms used:

The following live organisms were used:

1.	Staphylococcus aureus	ATCC # 25923
2.	Staphylococcus epidermidis	In House isolation
3.	Pseudomonas aeruginosa	ATCC # 27853
4.	Escherichia coli	ATCC # 25922
5.	Candida albicans	ATCC # 24058

Preparation of the inoculum:

A sterile inoculating loop was used to suspend the organism in 2 mL of sterile saline after which the solution was vortexed. The inoculum was then compared to the 0.5 McFarland's standard. This was done by holding both the standard and the inoculum tube side by side, not more than an inch from the face of the Wickerham card and comparing the appearance of the lines through both suspensions. The turbidity of the inoculum was adjusted by adding more organism if the suspension is too light or diluting with sterile saline if the suspension is too heavy. This entire process was repeated for each organisms listed above.

Inoculation of the Mueller Hinton Agar Plates:

A sterile swab was dipped into the inoculum tube and rotated against the sides of the tube to remove any excess fluid. The dried surface of the Mueller Hinton Agar Plates was inoculated by streaking the swab three times over the entire agar surface. The plate was rotated approximately 60 degrees each time to ensure an even distribution of the inoculum. The plate was then rimmed with the swab to remove any excess liquid.

Sample preparation:

Three concentrations of Long Hairy Crabgrass, Smooth Crabgrass and Crabwood Ethanol extracts were made up at 10 mg/mL, 50 mg/mL, and 100 mg/mL.

1. 1.00g (1000 mg) of crude extract was dissolved in 10 mL of 96% ethyl alcohol at 40^{o}C - (100 mg/mL).
2. 1 mL of 100 mg/mL stock was pipetted into a 10 mL volumetric flask and built to mark using sterilized deionized water – (10 mg/mL).
3. 5 mL of 100 mg/mL stock was pipetted into a 10 mL volumetric flask and built to make using sterilized deionized water – (50 mg/mL).

Positive controls:

The following positive controls were made with sterile distilled water:

1. Ampicillin 50 mg/mL for Staphylococcus aureus.
2. Vancomycin 100 mg/mL for Staphylococcus epidermidis and Pseudomonas aeruginosa.
3. Gentamycin 40 mg/mL for Escherichia coli.
4. Fluconazole 2 mg/mL for Candida albicans.

Negative controls:

Sterile distilled water was used as the negative control.

Disc Diffusion Method:

Disc preparation:

A quantity of the above extract concentrations was placed into sterile bottles and several of the 6 mm disc were placed into the solutions. 15 minutes before plating of the disc, they were place into sterile petri dishes under the laminar flow hood which allows the evaporation of any alcohol residue. A sterile forceps was used to place the impregnated discs onto the agar. After placement, the disc was gently pressed down onto the agar. Between each placement, the forceps was sterilized with alcohol and then ignited. The plates were then placed into the incubator at 35^{o}C and incubated for 3 days. After incubation period, the zones of inhibition were measured to the nearest millimeter using a ruler including the diameter of the disc in the measurement.

Agar Well Method:

For this analyses, the 100 mg/mL of Crabwood extract was exhausted hence it was not tested. The only difference with this test, is that instead of using the discs, 6 mm wells were dug into the inoculated Muller Hinton plates.

Plate preparation:

After the organisms were inoculated onto the Muller Hinton plates, a sterile cork borer was used to make 6 mm holes in the solidified agar and 100 uL of sample and controls were placed into their respective agar holes. The plates were then left in the laminar flow hood which allows the evaporation of any alcohol residue. Similarly, the plates were then placed into the incubator at 35^{o}C and incubated for 3 days. After incubation period, the zones of inhibition were measured to the nearest millimeter using a ruler including the diameter of the disc in the measurement.

5.0 - RESULTS AND DISCUSSION

Determination of the better extraction method, the best extraction solvent, and the best reflux time.

The examination of the thin layer chromatographic plates from the extracts of the maceration process and the reflux process showed that the reflux process yielded more compounds. This was inferenced primarily due to the excess number of TLC spots produced by the reflux extract as compared to the maceration extract. Evidence of this can be seen when comparing table 8 to 13. With this being the outcome, the reflux process is said to be the better extraction method. This however is on par with the method of extraction used by the Indigenous people of Guyana. Their technique of brewing involves some amount of heat; for example, simmering the leaves of the crabgrasses and the bark of crabwood in water before consuming the concoction.

Ethyl alcohol, methanol and chloroform were used as extraction solvents. One of the objectives of this study was to determine which of these solvents extracted the most compounds, hence producing the most TLC spots. Commonly, extraction of plant compounds is usually done with solvents that are, polar or non-polar in nature. The idea is to consider the general polarity of the components in a way that 'like dissolves like'. Expounding on this really means that polar compounds found in the plant leaves or bark will be extracted (dissolved) by the more polar solvents, methanol, and ethanol. On the other hand, non-polar compounds will be extracted with chloroform. A point to note is that the preparation of the medicinal concoction by the Natives, involves water as the solvent. This is understood because water is readily available in their environment and even less toxic than the solvents used in this research. Water was not used as an extraction solvent in this research because it has its own difficulty when it comes to evaporation under vacuum due to its high boiling point. This means that highly efficient vacuum is required to reduce the pressure. Additionally, water is considered a polar solvent because of its unevenly distributed electrons. Since the Natives used water as a solvent and the extract has some amount of medicinal properties, then it would be more advisable to consider the polar solvents, ethanol, or methanol for extraction. With this being the case, then chloroform was eliminated as a solvent. From the TLC results, ethanol crude extracts

showed more spots than methanol crude extracts, indicating that ethanol is the better solvent of the two. There was a clear indication that the reflux time influenced the number of compounds extracted. Five minutes showed the least, while twenty-five minutes showed the most. In summary, the ethanol extracts from the 25 minutes reflux extraction process showed to be the best outcome. Therefore, all subsequent analyses were done on those extracts only.

Reflux method: Chromatograms from TLC analyses

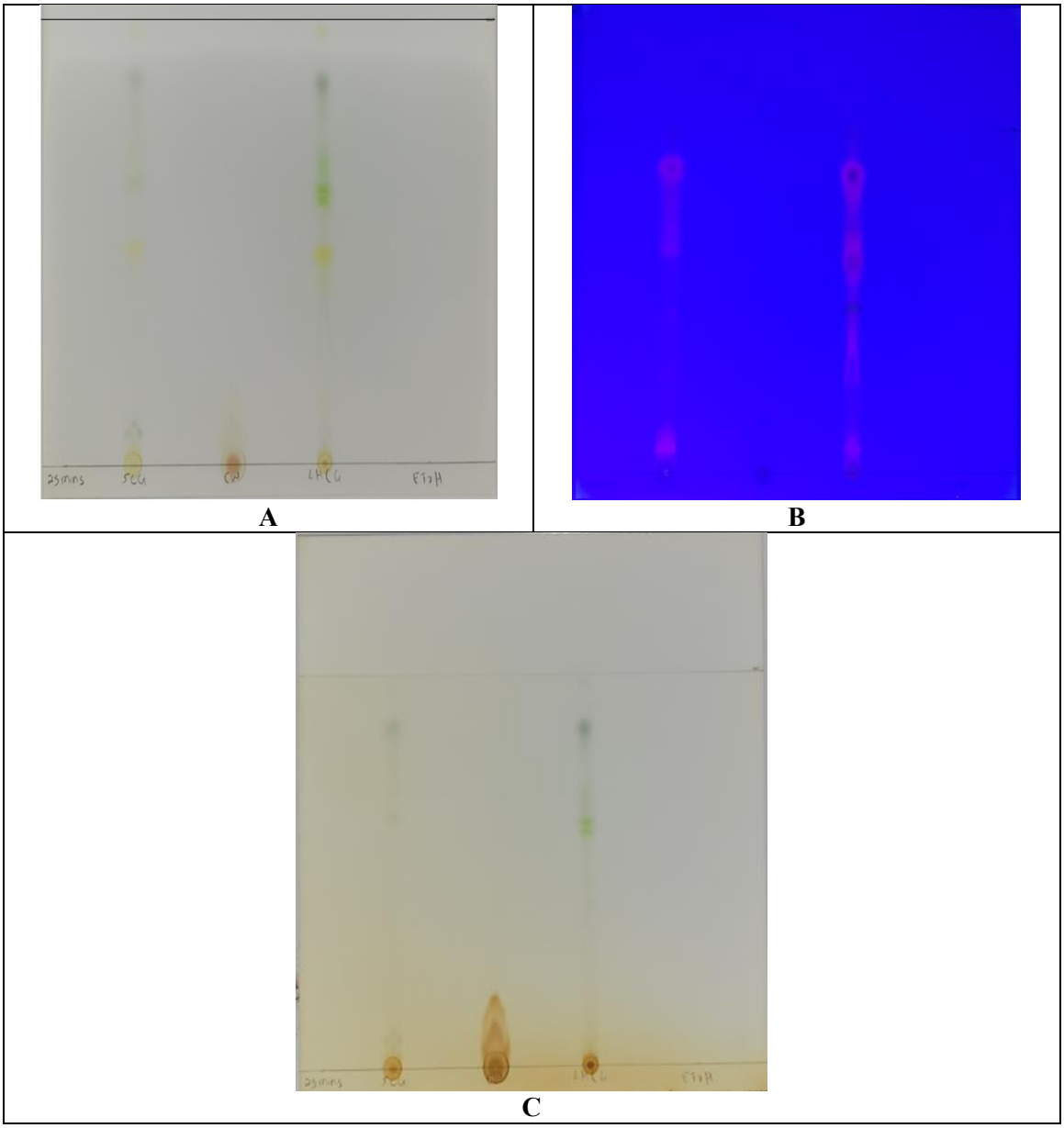


Figure 43. - Pictures of the TLC results for 25 minutes Reflux extraction process in Ethanol extraction – A – Normal view, B – UV fluorescence and C – Iodine stained.

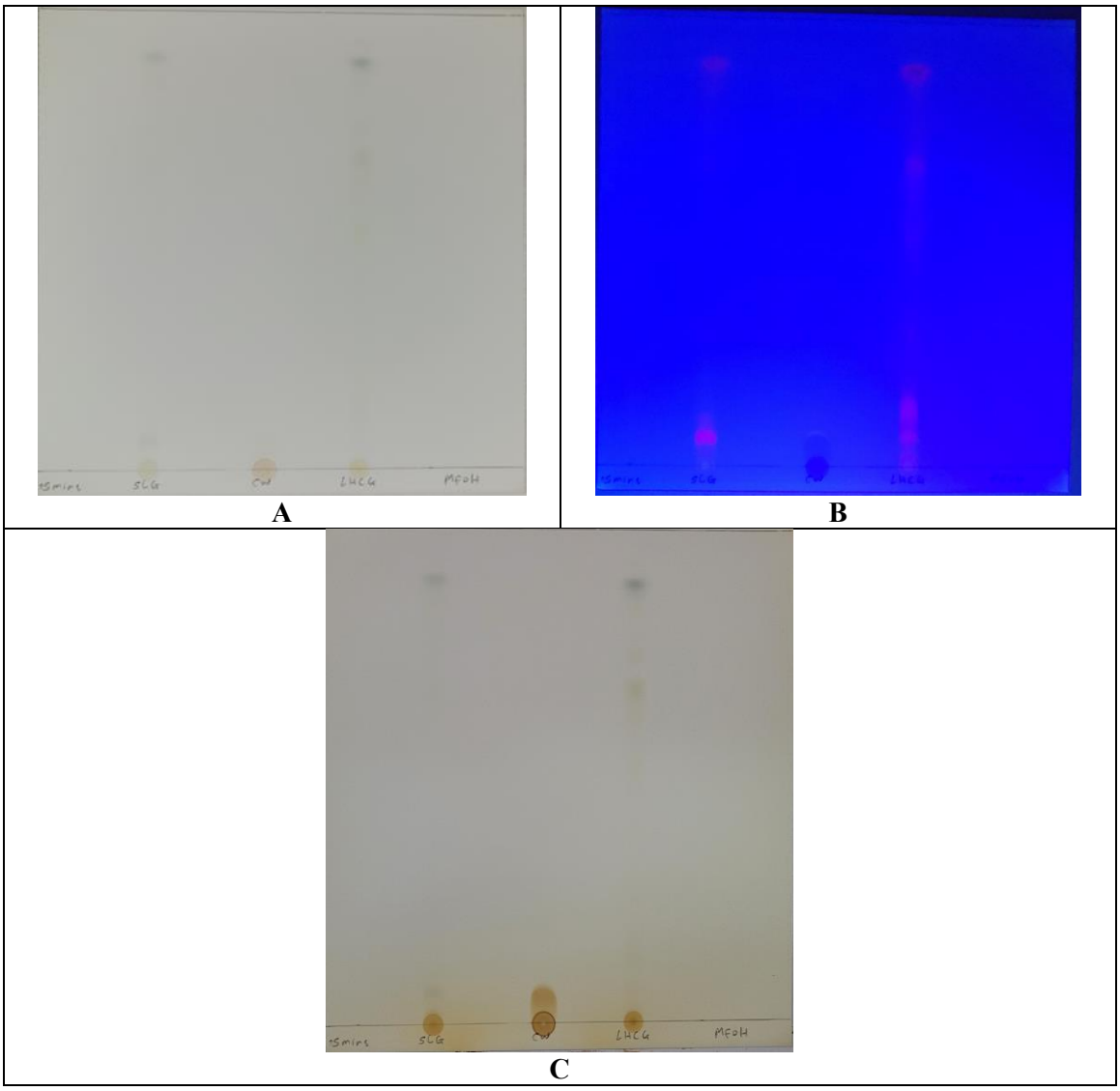


Figure 44. - Pictures of the TLC results for 25 minutes Reflux extraction process in Methanol extraction – A – Normal view, B – UV fluorescence and C – Iodine stained.

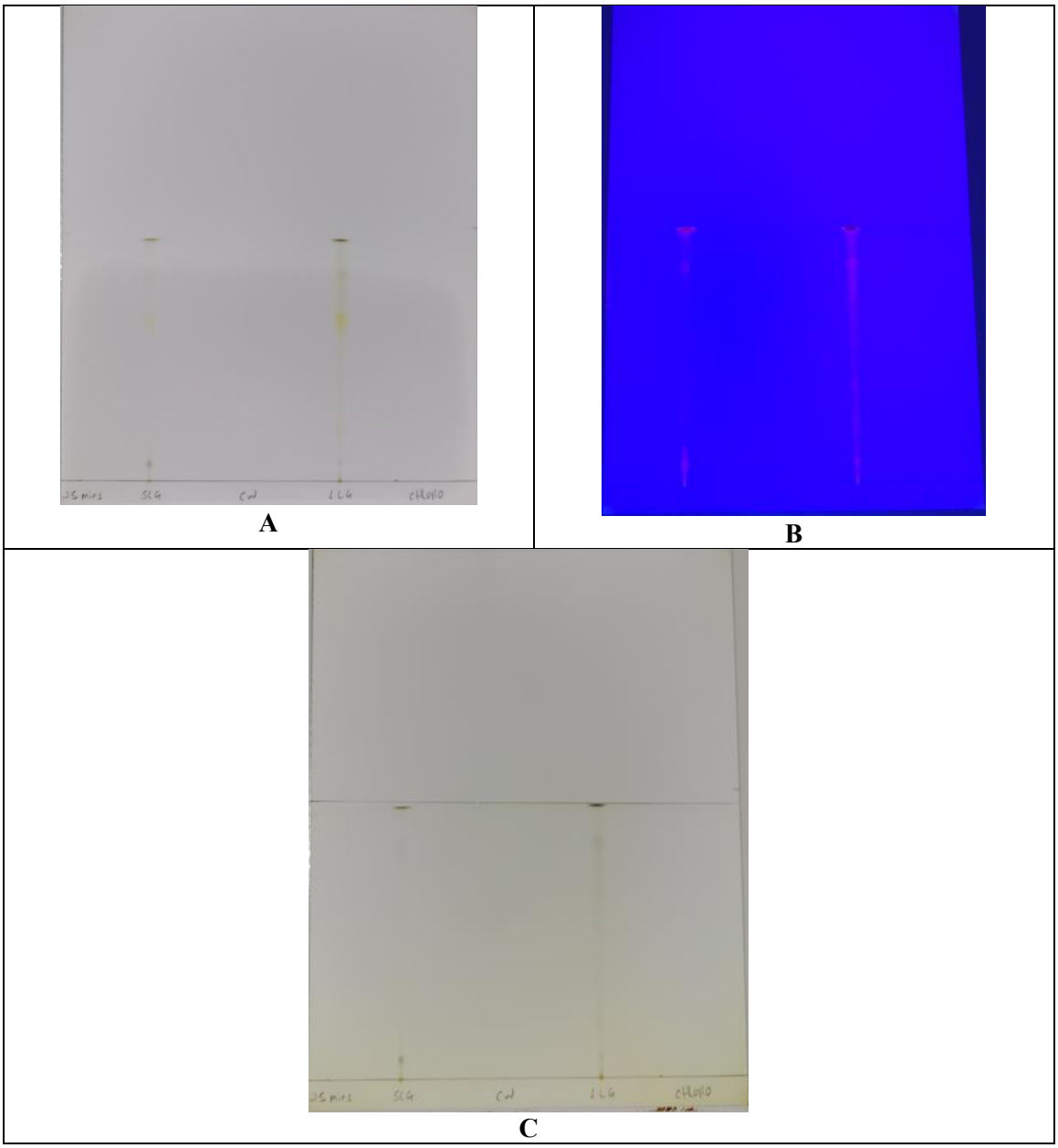


Figure 45. - Pictures of the TLC results for 25 minutes Reflux extraction process in Chloroform extraction – A – Normal view, B – UV fluorescence and C – Iodine stained.

Maceration method: Chromatograms from TLC analyses

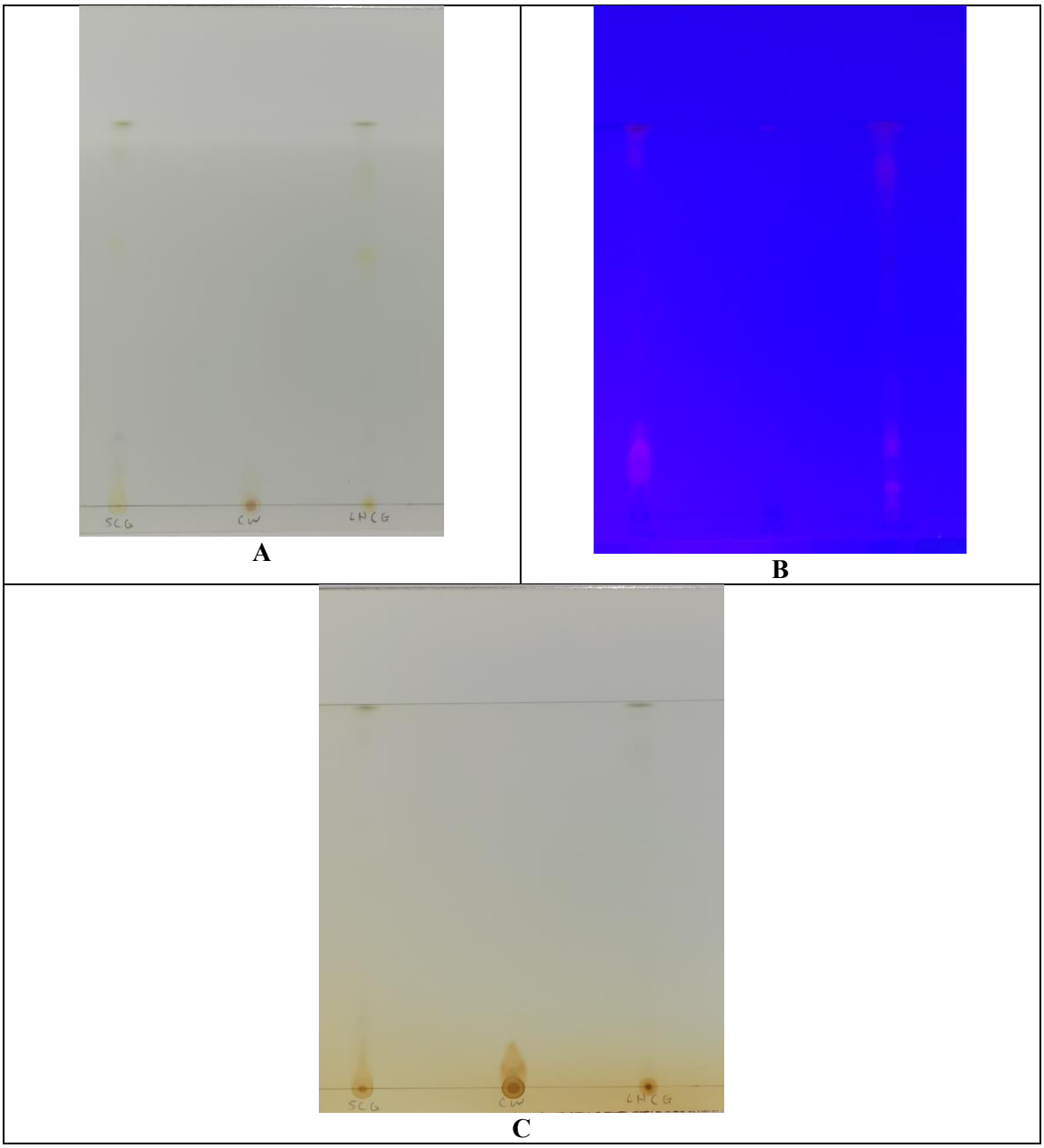


Figure 46. - Pictures of the TLC results for Maceration extraction process in Ethanol extraction – A – Normal view, B – UV fluorescence and C – Iodine stained.

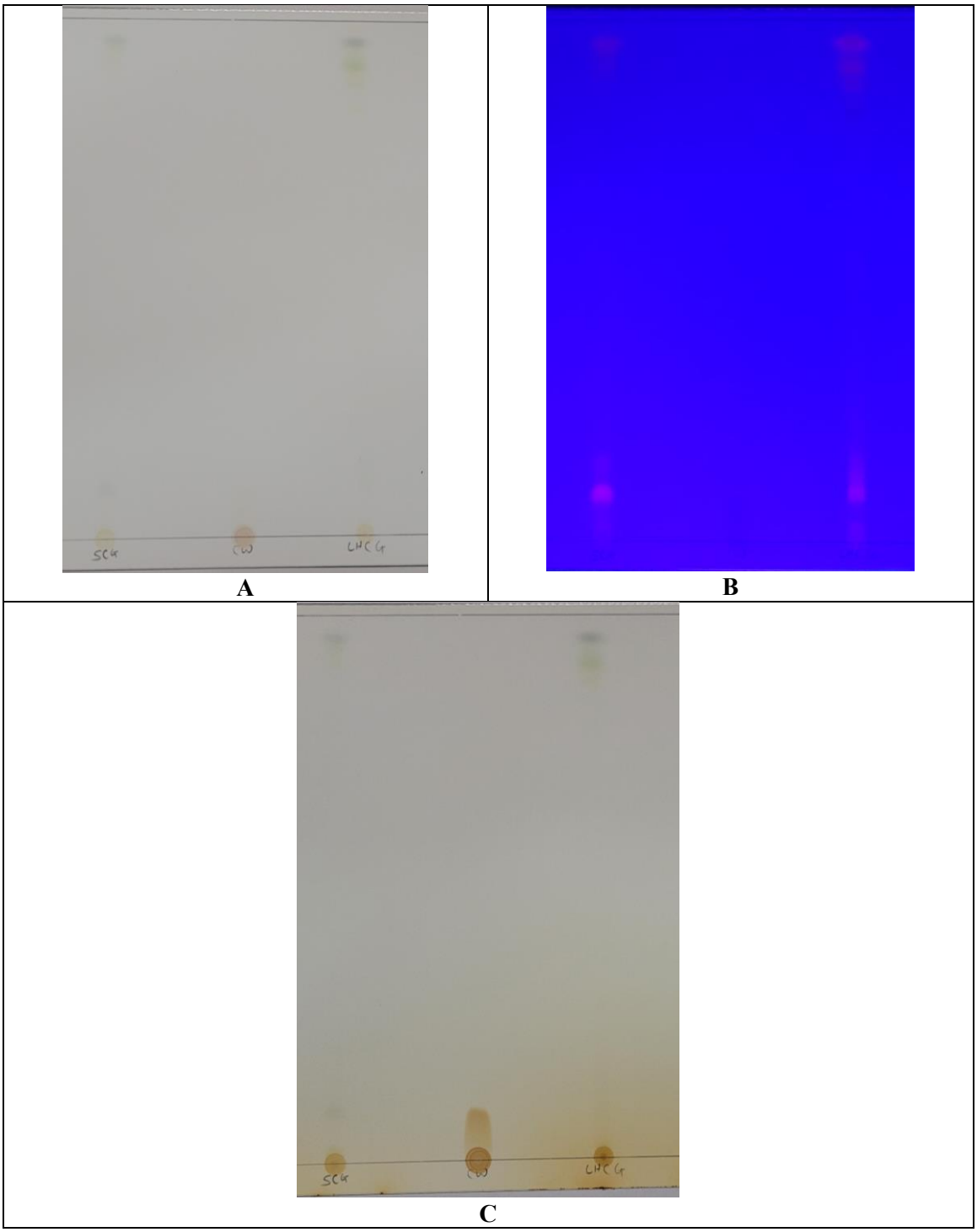


Figure 47.- Pictures of the TLC results for Maceration extraction process in Methanol extraction – A – Normal view, B – UV fluorescence and C – Iodine stained.

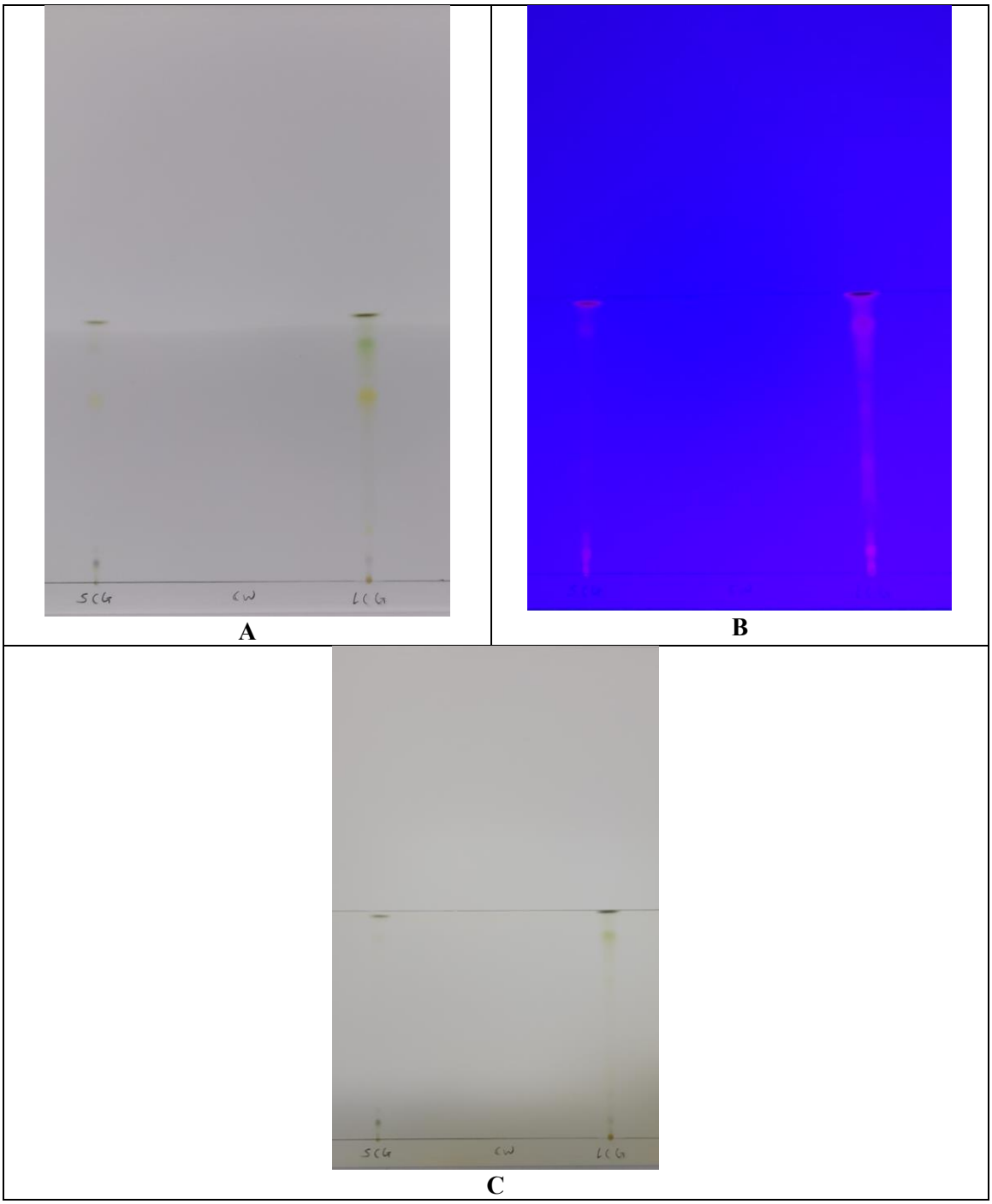


Figure 48.- Pictures of the TLC results for Maceration extraction process in Chloroform extraction – A – Normal view, B – UV fluorescence and C – Iodine stained.

Determination of the crude extract yields:

It is evident that the solvent type and extraction process affected the yields of the crude extracts. Generally, the polar solvents; ethanol and methanol extracted the larger amounts of crude materials. Considering both; the extraction methods and the plant materials, of the two solvents, methanol showed to have extracted more components than ethanol. Chloroform on the other hand extracted the least amount of crude material being less than 4% in all cases.

Considering the yields from the maceration extraction process, smooth crabgrass produced the highest yields, followed by long hairy crabgrass and then crabwood. A similar trend was observed with the crudes from the reflux process. With the exception of chloroform extracts, all the yields resulted from the reflux process exceeded the ones from the maceration process. With this being the case, the reflux extraction is more efficient than maceration process and requires less extraction time.

Low yields with the chloroform extraction may be because of the low boiling point of chloroform. With the application of heat, most of the chloroform may constantly undergoes a phase change (from liquid to gas) which limits the amount of contact time with the plant material. The higher yields resulted in the long hairy and smooth crabgrasses crude extracts were attributed to chlorophyll since their leaves were studied. On the other hand, with crabwood, a cross section of the bark was used to prepare the crude extracts.

Even though methanol resulted in the highest yields in both extraction processes, ethanol was chosen to be the best extract simply because it produced the greatest number of spots on the thin layer chromatographic analyses.

Maceration Process				
Sample	**Solvent**	**Wt of Sample (g)**	**Wt of Extract (g)**	**% Yield**
Long Crabgrass	Ethanol	190.00	15.67	8.25%
	Methanol	190.00	15.64	8.23%
	Chloroform	150.00	5.66	3.77%
Smooth Crabgrass	Ethanol	85.00	8.02	9.43%
	Methanol	85.00	15.09	17.76%
	Chloroform	85.00	3.03	3.56%
Crabwood	Ethanol	150.00	2.94	1.96%
	Methanol	150.00	6.17	4.11%
	Chloroform	150.00	1.30	0.87%

Table 8- Yields of the crude extracts obtained via the Maceration Process.

Reflux Process				
Sample	**Solvent**	**Wt of Sample (g)**	**Wt of Extract (g)**	**% Yield**
Long Crabgrass	Ethanol	50.00	5.6931	11.39%
	Methanol	50.00	6.6901	13.38%
	Chloroform	50.00	1.4189	2.84%
Smooth Crabgrass	Ethanol	50.00	8.1202	16.24%
	Methanol	50.00	10.0503	20.10%
	Chloroform	50.00	1.8543	3.71%
Crabwood	Ethanol	50.00	1.5389	3.08%
	Methanol	50.00	1.7601	3.52%
	Chloroform	50.00	0.2967	0.59%

Table 9- Yields of the crude extracts obtained via the Reflux Process.

Phytochemical analysis of the Ethanol crude extracts:

The thin layer chromatographic results indicated that the ethanol crude extracts from long hairy crabgrass, smooth crabgrass, and crabwood's bark produced the most spots, therefore were selected to be the best crudes. Phytochemical analyses were done on the ethanolic crude extracts.

Test	**Observations and Inferences**		
	Smooth Crabgrass EtOH crude	**Crabwood Bark EtOH crude**	**Long Hairy Crabgrass EtOH crude**
Terpenes/Terpenoids	+	+	+
Flavonoids	+	+	+
Saponins	- (1.5 cm layer observed)	- (1.0 cm layer observed)	+
Steroids/Polysterols	+	+	+
Alkaloids	+	+	+
Tannins and Phenolic compounds	+ Blue black colour	+ Blue black colour	+ Blue black colour
Proteins	+	+	+
Cardiac Glycoside	+ Yellow brown colour and green ring below	+ No green colour, brown and violet rings	+ Brown, violet, and green rings
Reducing Sugar	+ Blue green colour	+ Dark red colour	+ Blue green colour
Carbohydrates	+ Emerald green colour	+ Black - red colour	+ Emerald green colour

Table 10 - Phytochemical testing results for Smooth Crabgrass, Long Hairy Crabgrass and Crabwood's bark.

Ten phytochemical examinations were performed on the ethanol extracts. These tests were selected because they were the most common phytochemical examinations done on medicinal plant extracts.

From the results, all tests were positive for the ethanol long hairy crabgrass extract. Saponins was the only phytochemical absent in smooth crabgrass and crabwood's bark. These phytochemical tests provided an indication of the type of chemical compounds that are found in these extracts. Since most of these phytochemical tests are positive, then it indicates that the ethanol solvent is effective to isolate biological compounds due to its high polarity. Images of the test results are depicted in the sections that follow.

Terpenes/Terpenoids:

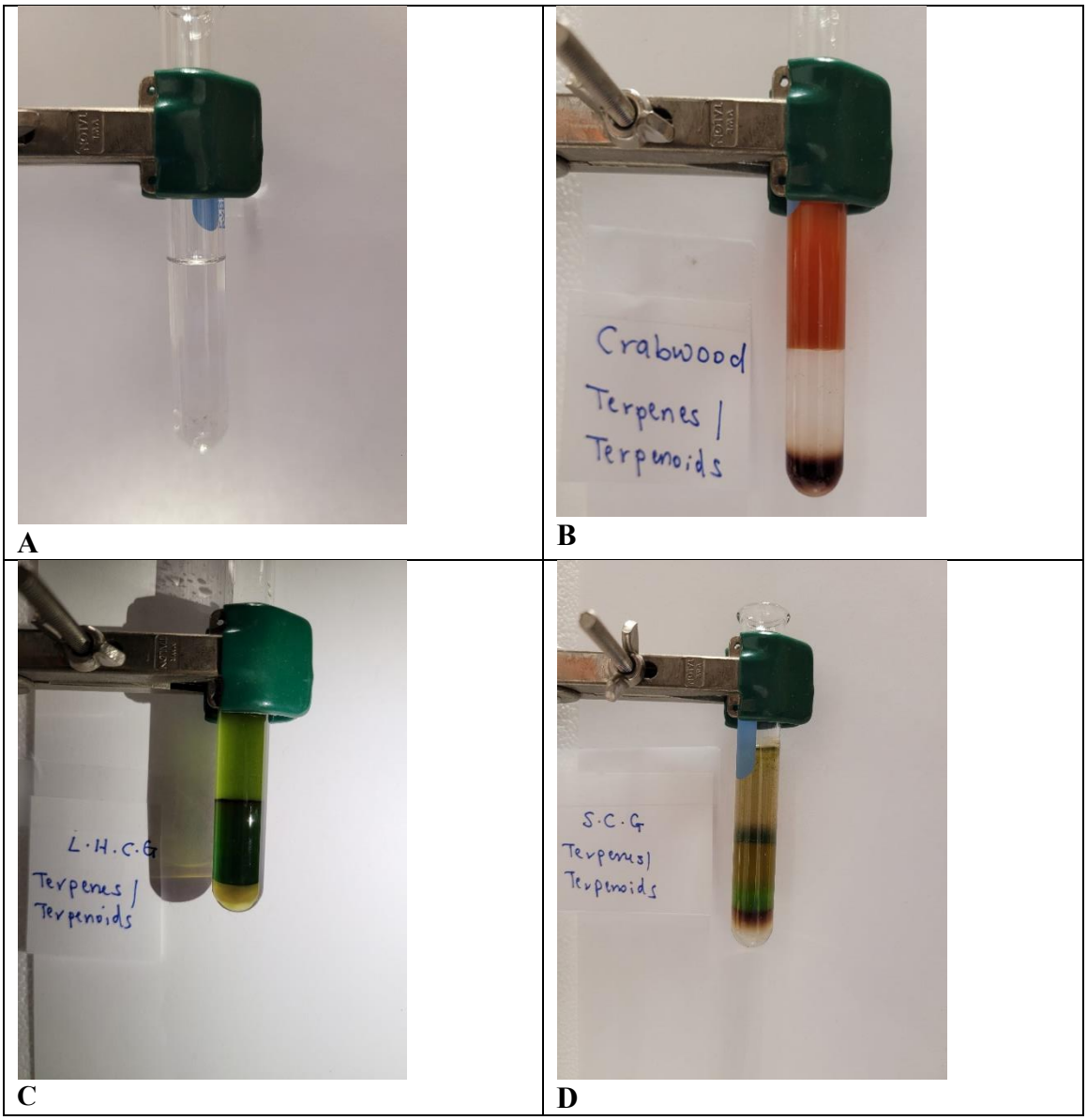


Figure 49.- Pictures of the results for the Terpenes/Terpenoids phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

The three ethanol crude extracts showed that they consist of a chemical class of compounds known as terpenes/terpenoids. The reddish-brown ring indicates the presence of terpenes which is formed from the sulfonation process of sulfuric acid binding with two terpenoids. This class of compound have antibiotics, insecticidal, anthelmintic, and antiseptic properties.

Flavonoids:

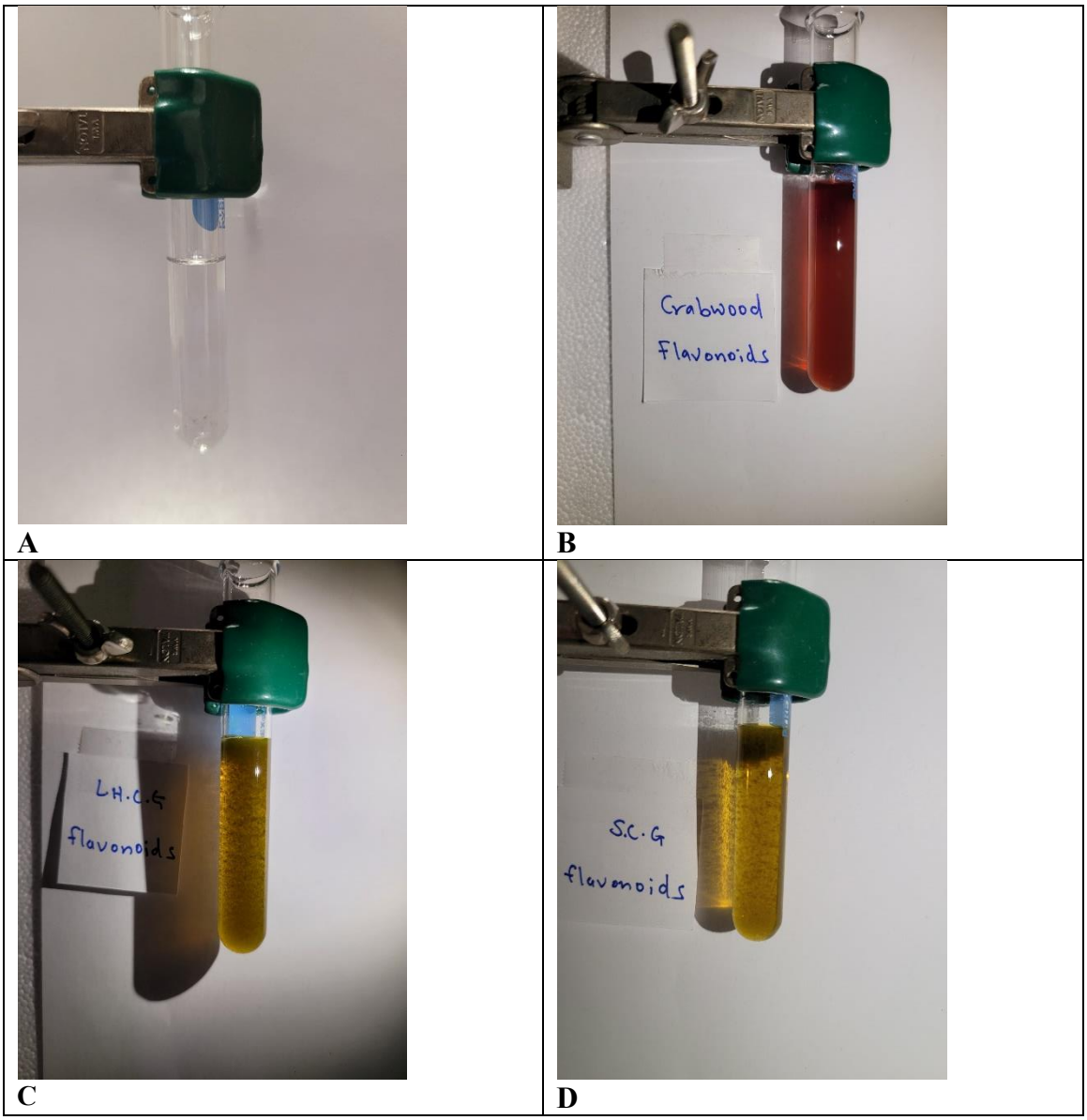


Figure 50.- Pictures of the results for the flavonoids phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

Ethanol crude extracts of long hairy and smooth crabgrasses showed a high intensity of yellow colour which indicated that the flavonoid content is high. On the other hand, crabwood had a minimal intensity of yellow colour which suggested that they are all composed of varying amounts of flavonoids. The yellow colour is due to the solubility of flavonoids in alkaline solution. Anti-inflammatory, anti – allergic effects, anti –

thrombotic, Vaso protective and tumor inhibition biological properties are some of its usefulness.

Saponins:

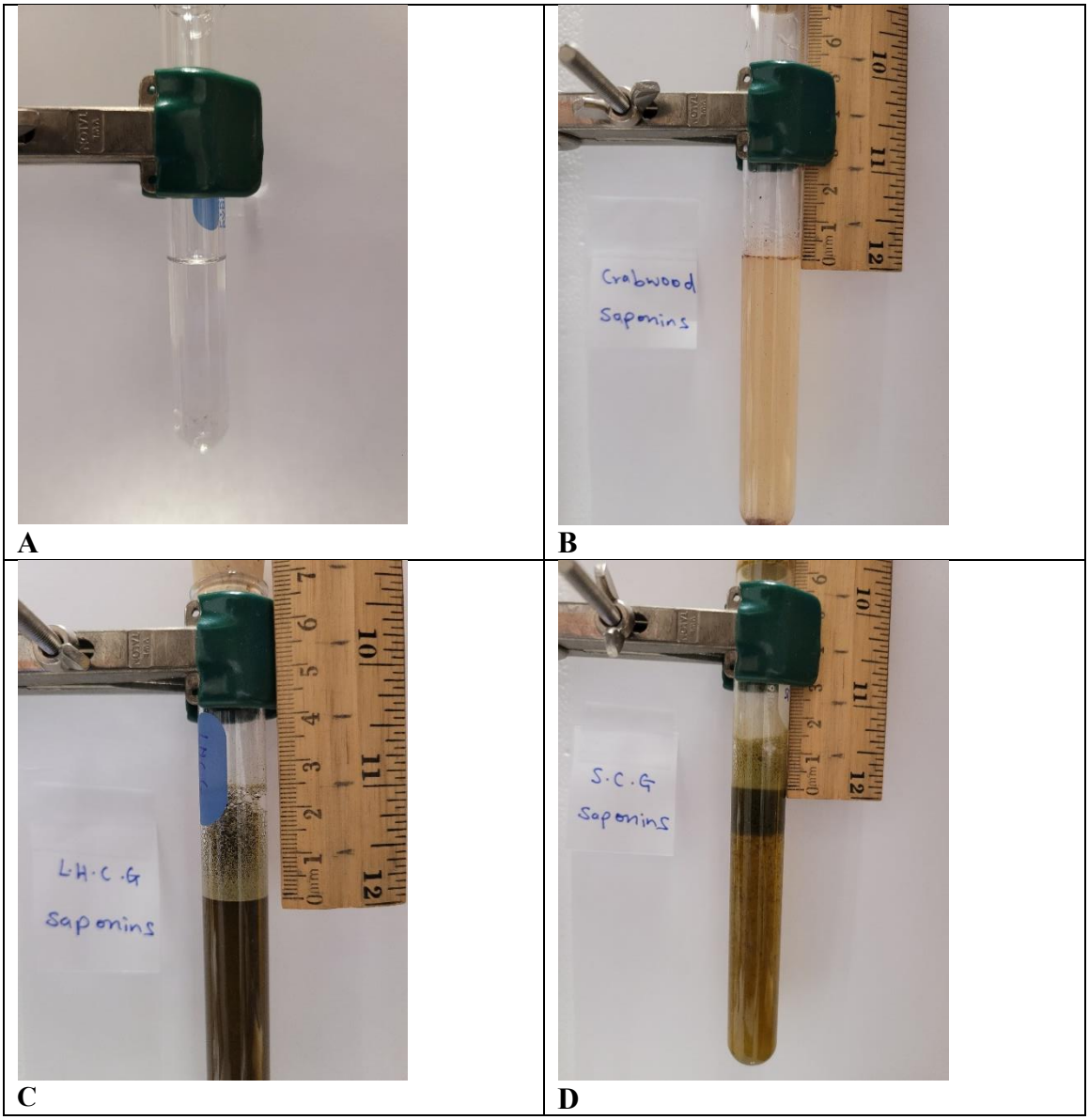


Figure 51.- Pictures of the results for the saponins phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

Saponins phytochemical test is known as the froth test which includes the addition of water to the sample and the sample shaken for 20 minutes. If the froth produced at the top of the mixture is more than 2 cm, then it shows a positive test for saponins. Only long hairy crabgrass showed a positive test for saponins with a froth layer over 2 cm. Crabwood and

smooth crabgrass showed some amount of froth layer but not above 2 cm. Medicinal benefits of saponins includes wound healing, lower cancer risks and lowering of blood glucose levels.

Steroids/Polysterols:

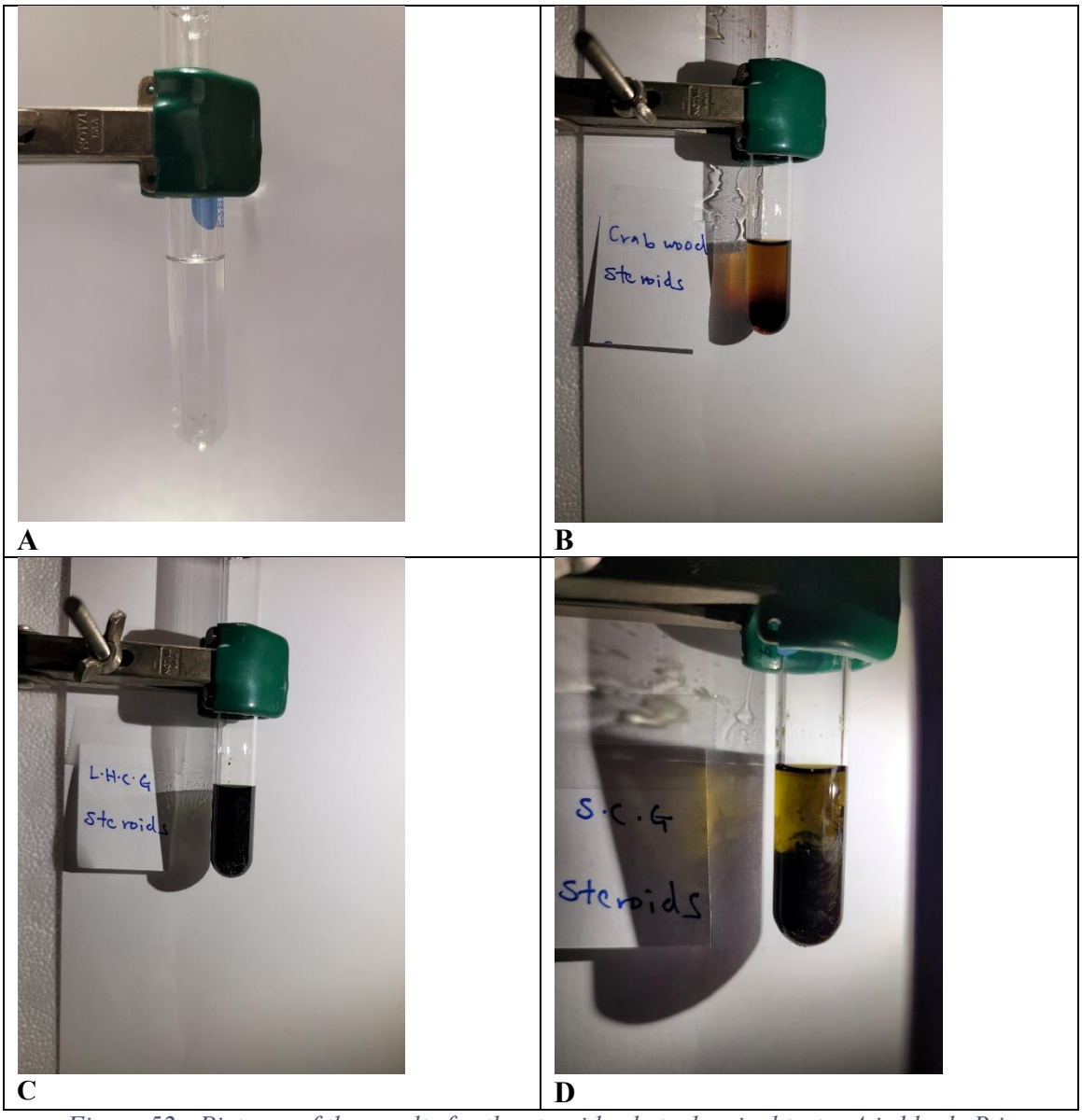


Figure 52.- Pictures of the results for the steroids phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

Steroidal compounds are known for their anti – inflammatory activities. All the ethanolic crude extracts showed that they contained steroidal compounds. These classes of compounds were also found in the crabwood tree leaves when studied by (Luz, et al., 2019).

A variety of colours show a positive test because sulfonation occurs on the steroidal molecule with the addition of sulfuric acid and acetic anhydride.

Alkaloids:

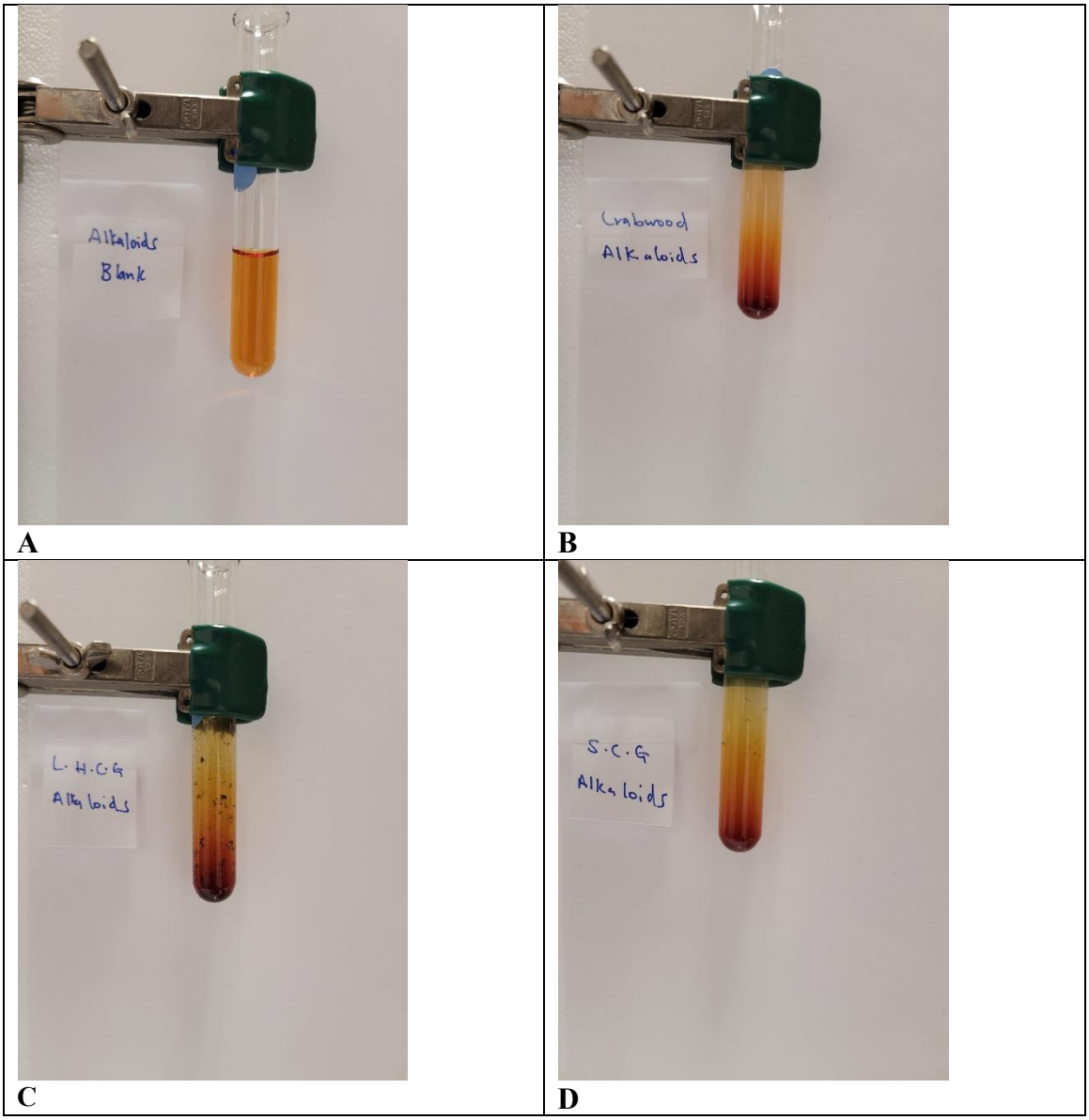


Figure 53.- Pictures of the results for the alkaloids phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

All the ethanol extracts showed to contain alkaloidal compounds. Alkaloids have been reported to possess analgesic, antispasmodic, bactericidal, antimalarial, and analgesic activities. As it relates to crabwood, a key difference seen was that alkaloids were absent in the tree leaves (Luz, et al., 2019) but present in the bark as examined in this study. This also contrast the study that was performed on the bark of Carapa procera, a different species

of Carapa guianensis. In this study, (Owusu, Afedzi, & Quansah, 2021) found that the bark of Carapa procera did not contain any alkaloids. The absence of alkaloids in that study maybe the consequence of different geographical locations in which soil minerals and environmental factors have great influence on phytochemical content of the species.

Phenolic/Tannins:

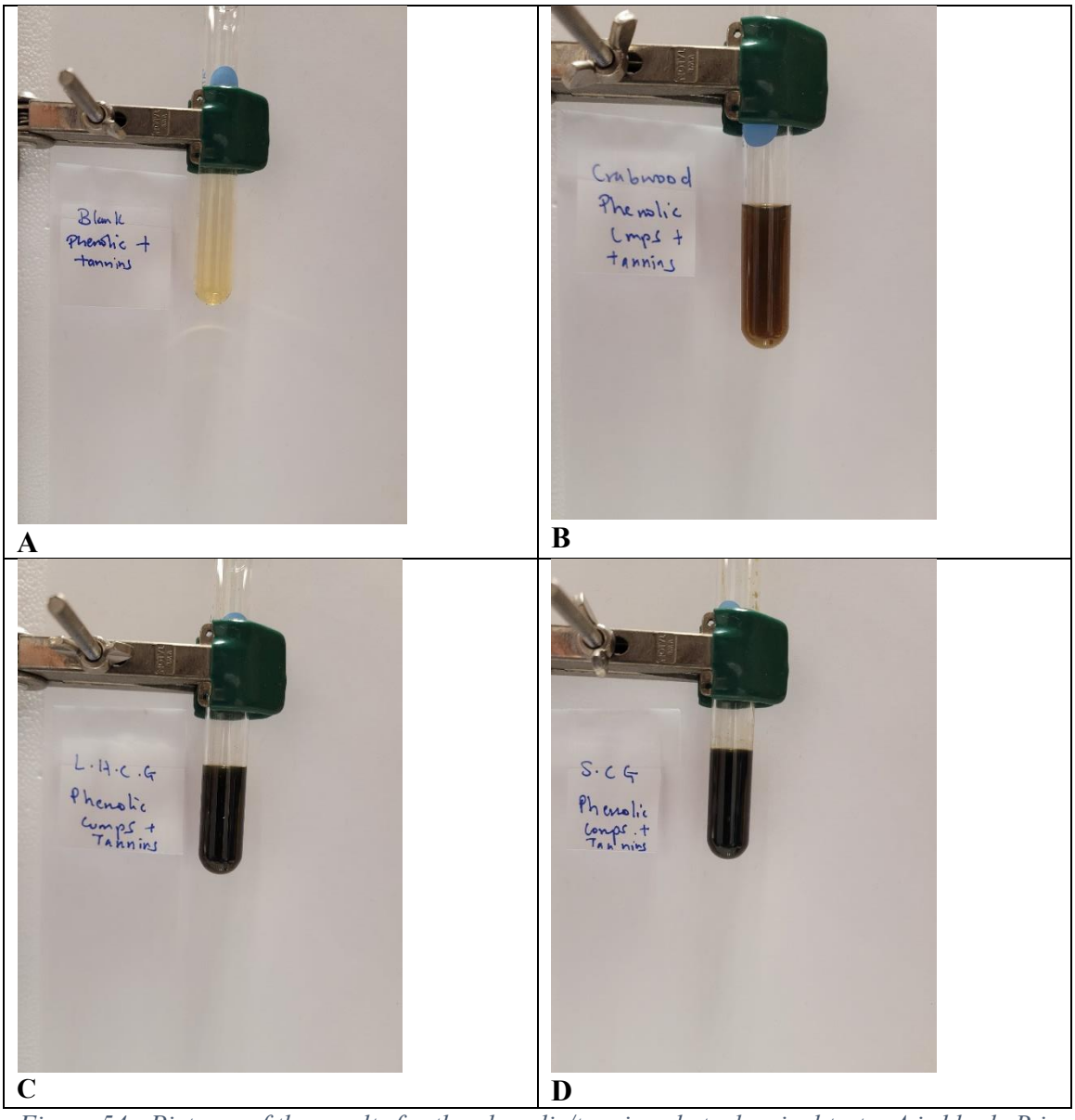


Figure 54.- Pictures of the results for the phenolic/tannins phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

Since a dark green to black colour was observed, all the ethanolic extracts were positive for phenolic compounds. This class of compound includes tannins, coumarins, flavonoids,

chromones and xanthones, stilbenes and lignans. These compounds are the largest group of secondary metabolites which is found in almost all plants. Phenols' biological properties are anti – inflammatory, anti – hepatotoxic and antioxidant. In crabwood leaves, phenols were found to be present by (Luz, et al., 2019). They were also present in the methanolic extract from the Carapa procera studied by (Owusu, Afedzi, & Quansah, 2021).

The research paper by (Ibrahim, El-Hela, Dawoud, & Zhran, 2019) done in Egypt on Digitaria sanguinalis isolated two phenolic compounds; para – coumaric acid and para – hydroxybenzoic acid. Our phytochemical results yielded a positive phenolic test for Digitaria sanguinalis which is on parr with (Ibrahim, El-Hela, Dawoud, & Zhran, 2019) even though the geographical locations were different.

Proteins:

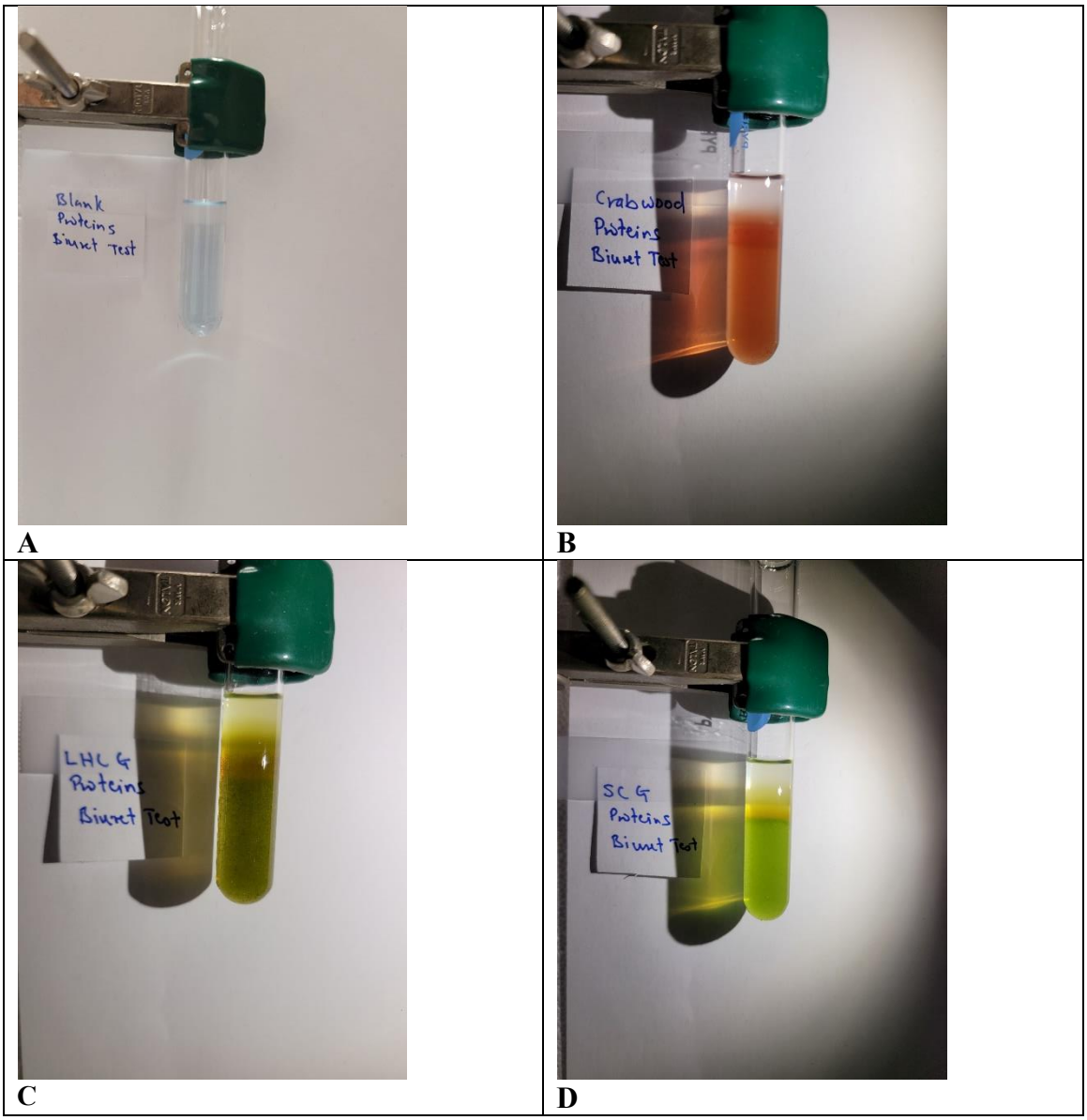


Figure 55.- Pictures of the results for the proteins phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

Proteins were found in all the ethanolic crude extracts. The pink layer was evident in all the samples. This class of phytochemical are large macromolecules which functions as antibiotic and antimicrobial agents. The way this works is that plants defend themselves against microbial pathogens by various defense responses including production of antimicrobial proteins which are small molecular mass antimicrobial peptides (Khanam, Wen, & Bhat, 2014).

Cardiac Glycosides:

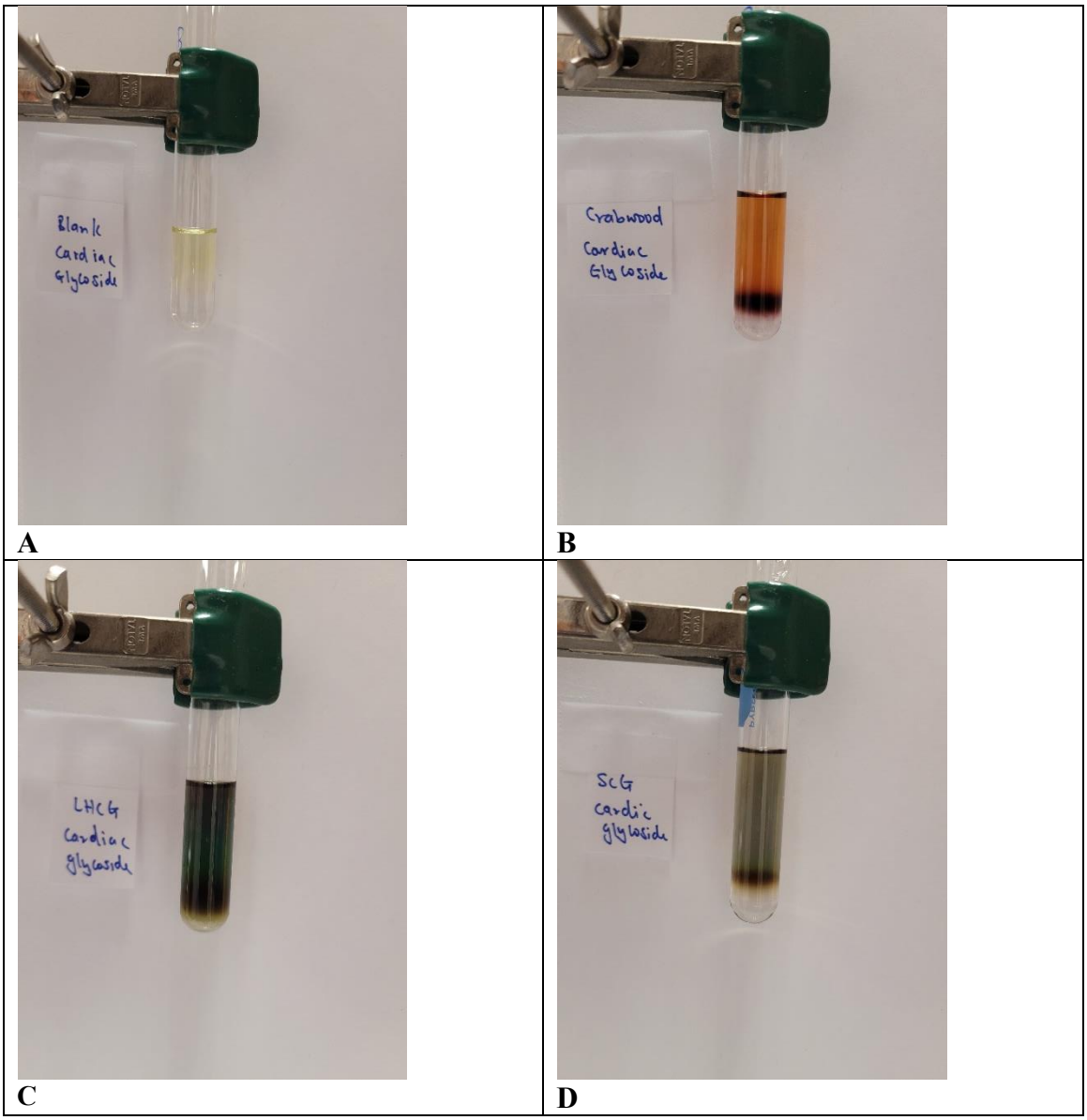


Figure 56.- Pictures of the results for the Cardiac glycoside phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

Cardiac glycosides were found in all three of the samples. This phytochemical is commonly used to treat congestive heart failure and cardiac arrhythmia (Khanam, Wen, & Bhat, 2014). These are considered to be steroids that have the ability to exert specific action on the cardiac muscles. Plants manufacture this secondary metabolite as a defense mechanism which means, at high concentrations, it can be lethal.

Reducing Sugars:

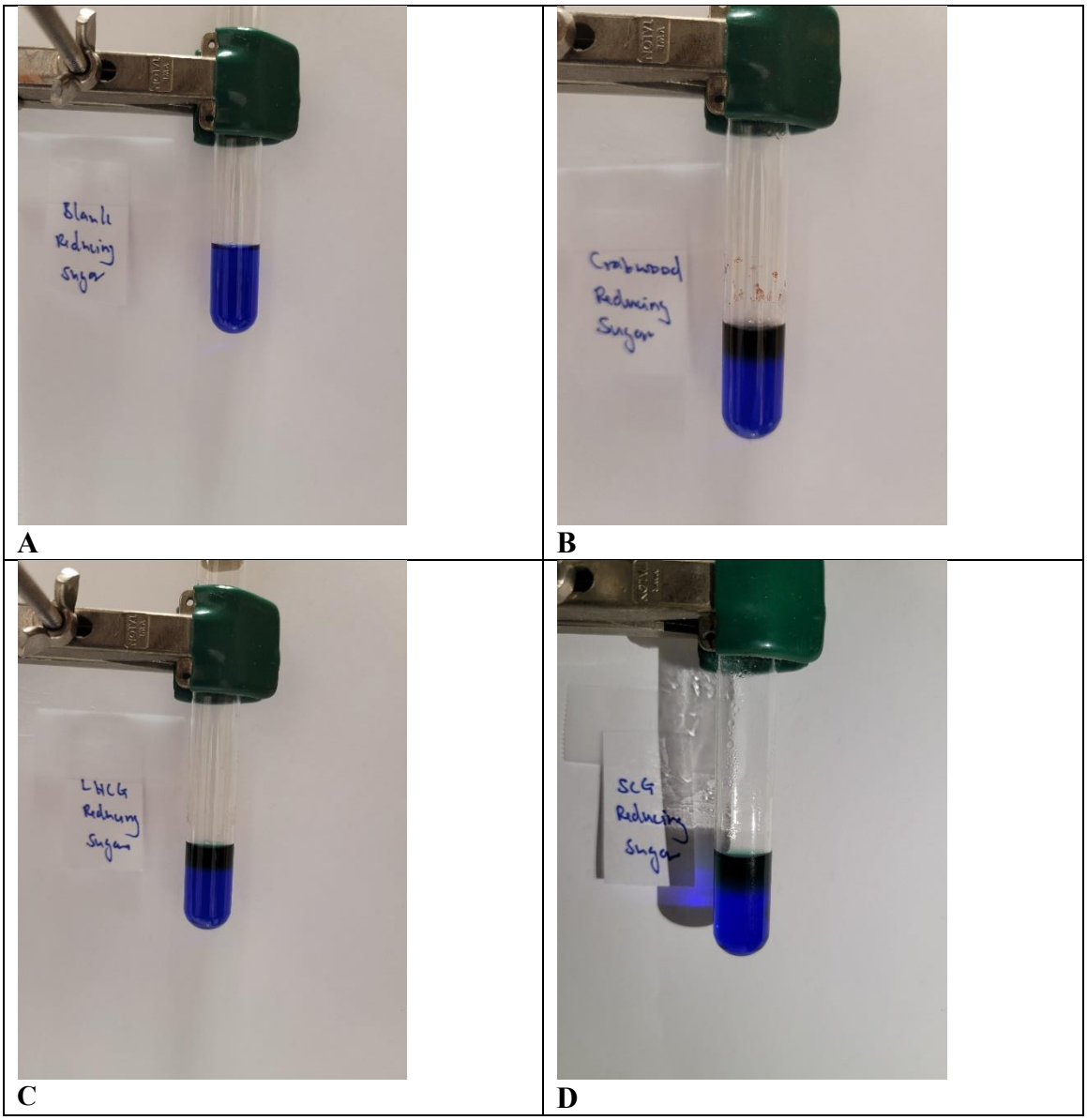


Figure 57.- Pictures of the results for the Reducing sugar phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

All the samples were positive for reducing sugars.

Carbohydrates:

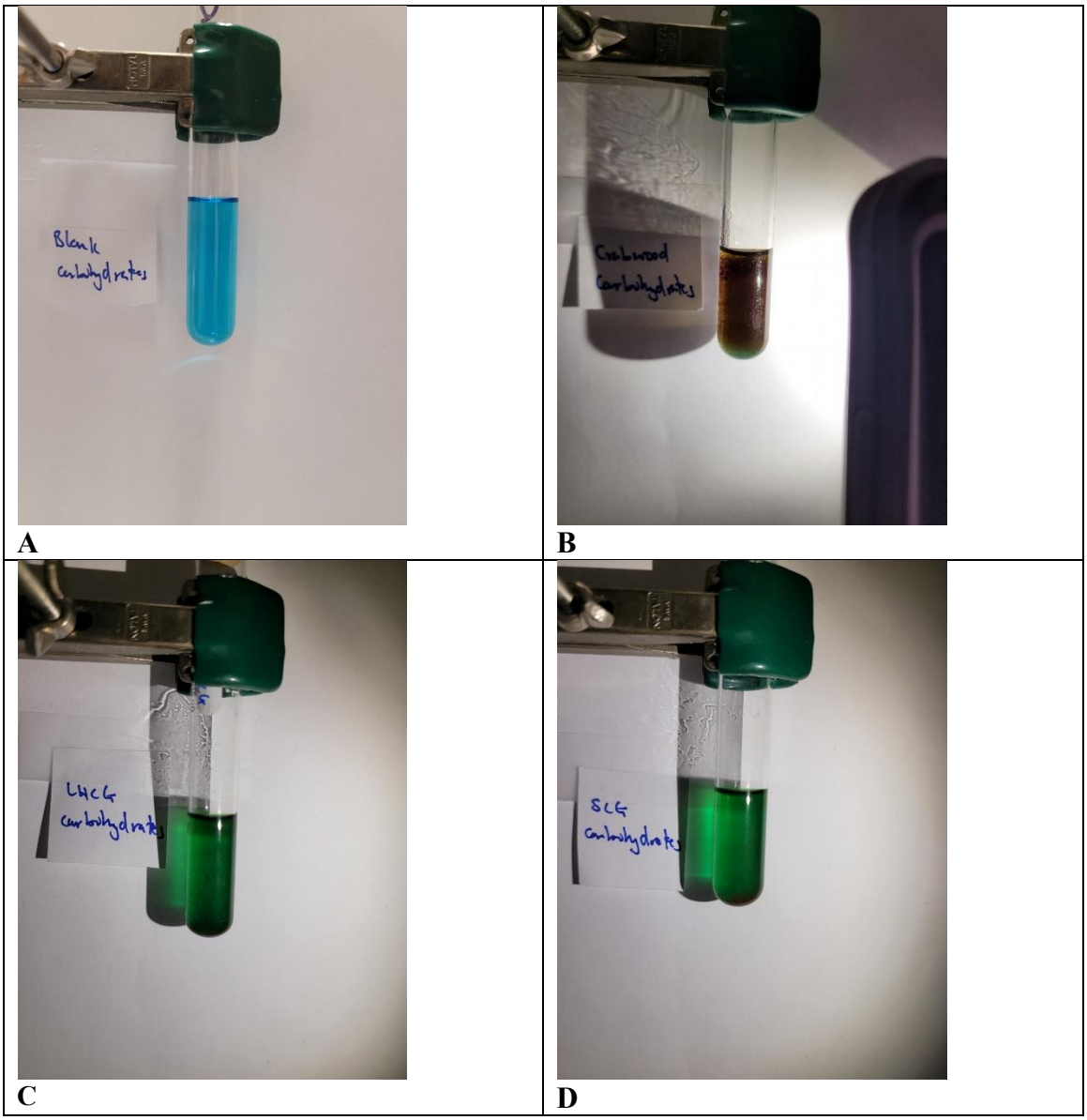


Figure 58.- Pictures of the results for the Carbohydrates phytochemical test – A is blank, B is Crabwood, C is Long hairy crabgrass and D is Smooth crabgrass.

All the samples tested positive for carbohydrates.

Column Chromatography of the best crude extracts.

Column chromatography was performed on the ethanol crude extracts of long hairy crabgrass, smooth crabgrass and crabwood's bark obtained from the 25 minutes reflux extraction process.

The number of 5 mL fractions collected were:

1. Smooth crabgrass – 250 fractions
2. Long hairy crabgrass – 128 fractions
3. Crabwood's bark – 188 fractions

Each of these fractions were analyzed via TLC using elution solvents; Hexane: Ethyl Acetate (3:1) and Hexane : Ethyl Acetate (1:1). The TLC results were then examined, grouped and rotary evaporated. After grouping, the following were obtained:

1. Smooth crabgrass – 27 fractions
2. Long hairy crabgrass – 26 fractions
3. Crabwood's bark – 22 fractions

Separation pictures are shown below:

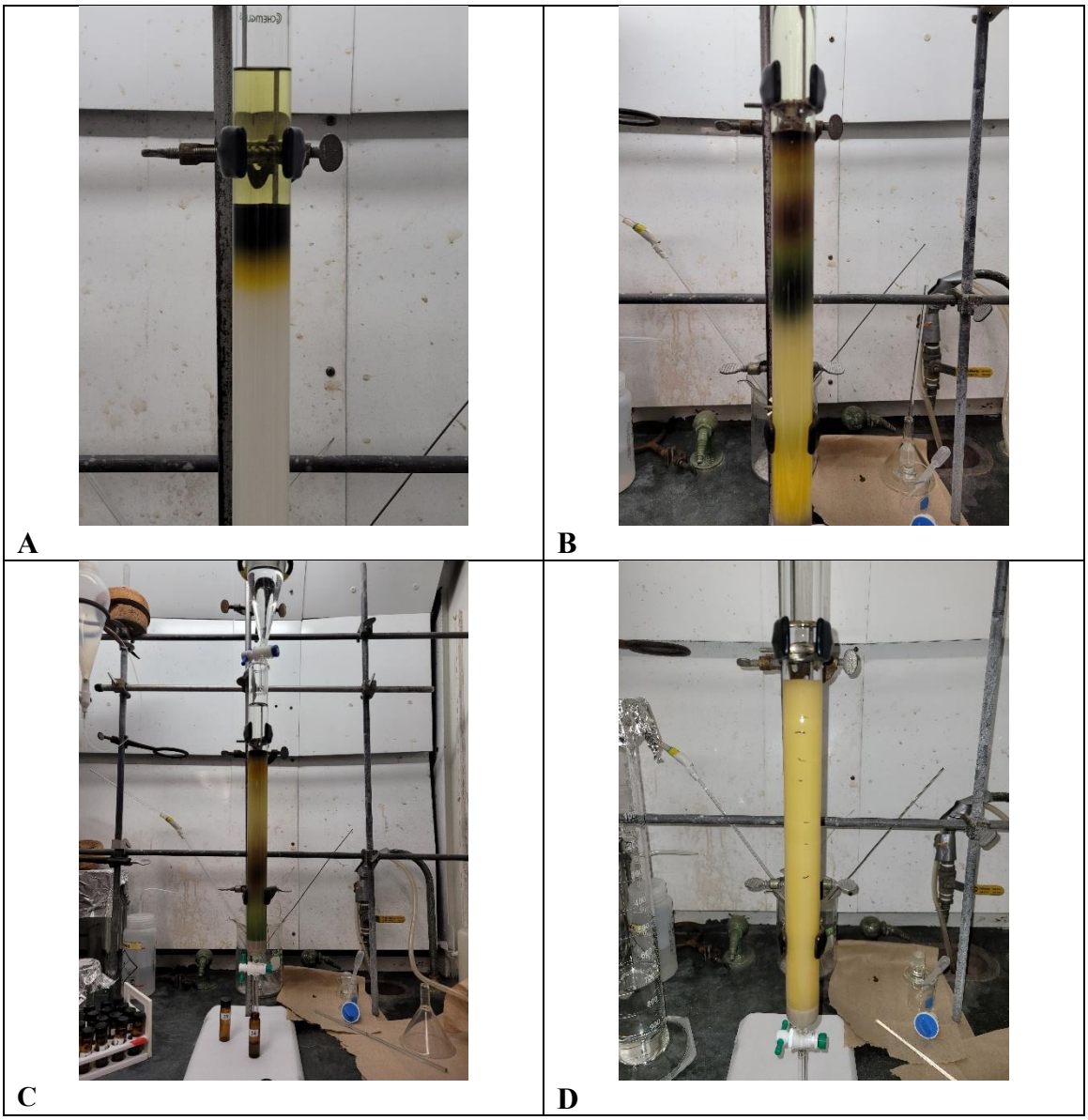


Figure 59.- Pictures of the column chromatography process of smooth crabgrass ethanol extract.

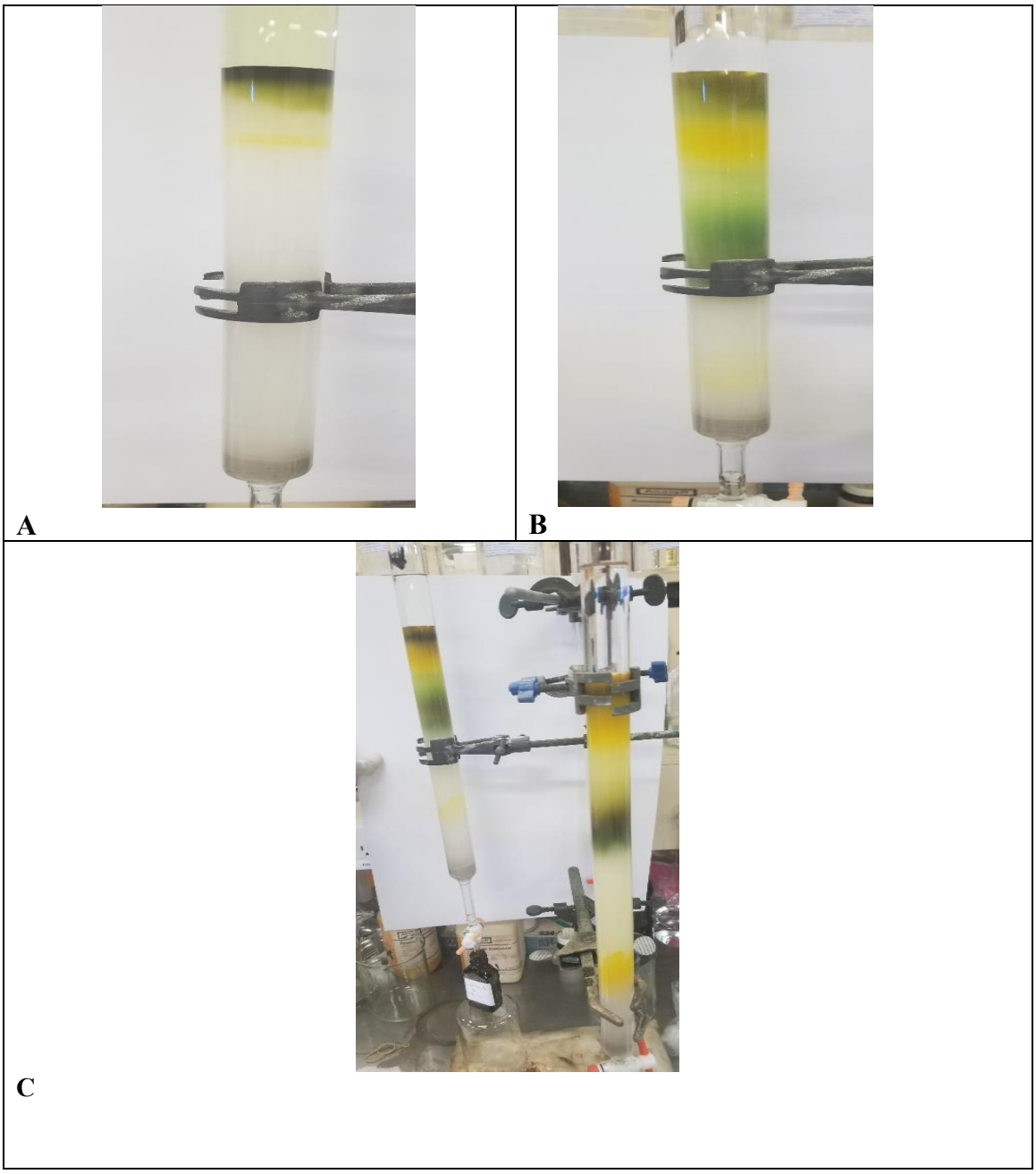


Figure 60.- Pictures of the column chromatography process of long hairy crabgrass ethanol extract.

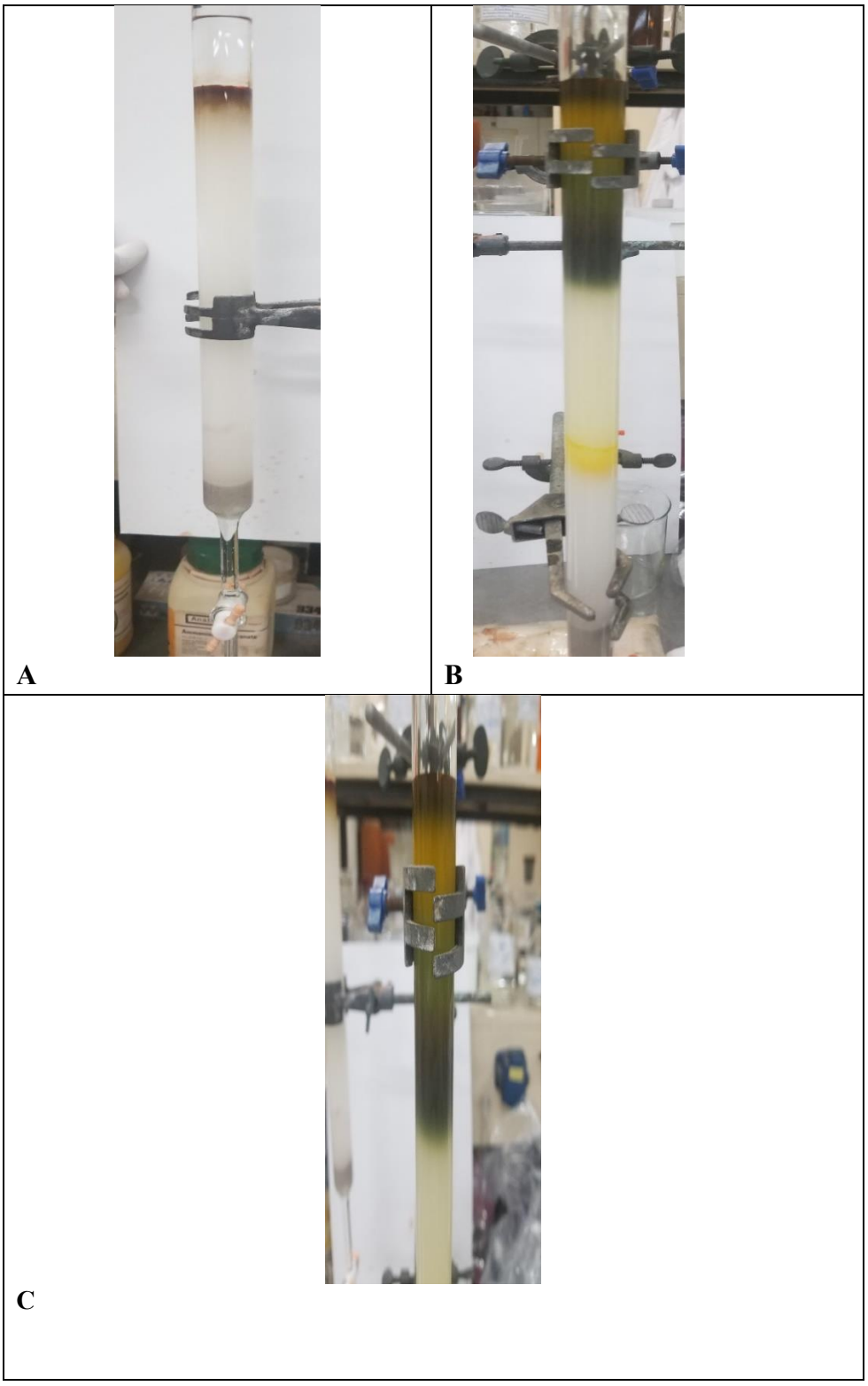


Figure 61.- Pictures of the column chromatography process of crabwood's bark ethanol extract.

TLC analyses of each fraction and staining technique to determine chemical functionality.

For the Smooth Crabgrass, twenty-seven (27) fractions resulted from the grouping of two hundred and fifty-five (255) 5 ml fractions collected during the column separation of the ethanol crude extract. One hundred and twenty-eight (128) fractions from Long Hairy Crabgrass were pooled into twenty-six (26) fractions, while one hundred and eighty-eight (188) fractions were pooled into twenty two (22) fractions for Crabwood.

These fractions were subjected to thin layer chromatography in which the resulted spots were subjected to chemical staining examinations. These staining techniques were:

1. Ferric Chloride test – used for determination of phenols.
2. Potassium Permanganate test – used for alkene, alkynes, and aldehydes (yellow colour)
3. Vanillin + Sulfuric acid test – used for strong and weak nucleophiles such as -OH, -RNH, -COOH, ROR.
4. Bromocresol Green test – used for Acid compounds (yellow colour).

Smooth Crabgrass Ethanol Extract Staining Results:

SCGF	Ferric Chloride - Specific for Phenols	Potassium Permanganate - Specific for alkenes, alkynes, aldehydes (yellow)	Vanillin + sulphuric acid (Strong and weak Nu:, -OH, -RNH, -COOH, ROR.	Bromocresol Green - Acidic compounds (yellow)	356 nm UV
1	-	+ (1 yellow spot)	+ (1 green spot)	+ (1 yellow spot)	-
2	-	+ (1 yellow spot)	+ (2 green spots)	-	+ (1 spot)
3	+ (1 Pink colour)	+ (1 yellow spot)	+ (2 green spots)	-	+ (2 spots)
4	-	+ (2 yellow spots)	+ (1 green spot, 3 dark green spots)	+ (1 yellow spot)	+ (2 spots)
5	-	-	+ (one purple drag)	-	+ (2 spots)
6	-	+ (2 yellow spots)	+ (2 green spots, 2 purple spots,)	-	+ (5 spots)
7	-	+ (4 yellow spots)	+ (3 green spots, 2 dark spots, 1 red spot)	-	+ (6 spots)
8	-	+ (2 yellow spots)	+ (3 green spots, 1 red spot, 1 purple spot)	+ (1 yellow spot)	+ (6 spots)
9	-	-	+ (1 yellow spot, 1 purple spot)	+ (1 yellow spot)	+ (5 spots)
10	-	+ (1 bleached spot, 1 dark spot)	+ (1 green spot, 1 purple spot)	+ (1 yellow spot)	+ (5 spots)

11	-	+ (1 yellow spot, 1 bleached spot, 1 dark spot)	+ (1 dark spot, 2 green spots, 2 purple spots)	+ (1 yellow spot)	+ (5 spots)
12	-	+ (1 bleached spot)	+ (2 green spots, 1 purple spot)	-	+ (6 spots)
13	-	+ (1 yellow spot, 1 bleached spot)	+ (3 green spots, 1 dark spot, 1 purple spot)	-	+ (5 spots)
14	-	+ (1 yellow spot, 1 bleached spot)	+ (1 green spot, 1 yellow spot, 1 purple spot)	+ (3 yellow spots)	+ (5 spots)
15	-	+ (2 yellow spots)	+ (1 blue spot, 1 pink spot)	+ (1 yellow spot)	+ (2 spots)
16	+ (1 faint pink spot)	+ (1 bleached spot, 1 dark spot, 1 yellow spot)	+ (2 green spots, 1 blue spot, pink spread)	+ (1 yellow spot)	+ (2 spots)
17	+ (1 blue spot, 1 faint pink spot)	+ (3 yellow spot, 1 bleached spot)	+ (2 green spots, 2 blue spots, 1 green spot, 1 pink spot)	-	+ (3 spots)
18	-	+ (1 yellow spot)	+ (1 green spot, 2 blue spots, 1 dark spot)	-	+ (3 spots)
19	-	+ (2 yellow spots)	+ (6 dark spots, 1 purple spot)	-	+ (2 spots)
20	-	+ (6 yellow spots)	+ (7 dark spots, 1 purple spot)	+ (1 yellow spot)	+ (4 spots)
21	-	-	+ (6 dark spots)	-	+ (4 spots)

22	-	-	+ (8 dark spots)	-	+ (4 spots)
23	-	+ (1 yellow spot at base)	+ (1 dark spot at the base)	-	+ (3 spots)
24	-	-	+ (1 dark spot at the base)	+ (1 blue spot at base)	+ (2 spots)
25	+ (pink spot at base)	+ (1 yellow spot at base)	+ (1 dark spot at the base)	+ (1 blue spot at base)	+ (2 spots)
26	+ (pink spot)	+ (1 yellow spot at base)	+ (1 dark spot at the base, 1 green spot)	+ (1 blue spot at base)	+ (4 spots)
27	-	-	+ (1 faint ring)	-	+ (2 spots)

Table 11- TLC staining results for crabwood's bark ethanol extract.

Smooth Crabgrass staining discussion:

Five of the combined fractions from the ethanol extract of smooth crabgrass tested positive for phenols. These were 3, 16, 17, 25 and 26. Both blue and pink colours were observed because the Fe^{3+} ion forms various complexes. A positive test here indicates that those fractions have phenol functionality or may consists of carbonyl compounds with high enol content. This is true when compared to the phytochemical studies done on smooth crabgrass; it tested positive for phenolic compounds.

Twenty – one of the combined fractions from ethanol extract from smooth crabgrass tested positive for the potassium permanganate test. Combined fraction 5, 9, 21, 22, 24, 27 test negative for this test. A yellow spot which indicates aldehyde functionality was common. In some cases, more than one spot for the same fraction on the TLC showed a yellow colour. Unsaturation was evident due to a characteristic bleached spot, inferencing the presence of alkenes and alkynes.

Vanillin and Sulfuric tests were done to determine the presence of nucleophiles. The colour of the spot provided an indication of the type of compound present. The yellow to green colour showed unsaturation and aromatic functionalities in seventeen fractions, while purple spots are indicative of terpenoid as seen in eleven of the combined fractions. Red and pink spots are linked to phenols, while red only are linked to steroids. Fractions 7 and 8 have steroidal properties while fractions 15, 16 and 17 are composed of phenols. Fractions 15, 16, 17 and 18 had blue spots which are linked to the presence of coumarins. Twelve of the combined fractions had dark spots which indicates organic compounds, with the possibility of saponins and essential oils being present.

Bromocresol green staining technique determined whether a spot is acidic or basic. A yellow colour indicates acidity, found in ten of the combined fractions. On the contrary, a blue spot reflects basicity as seen in three of the combined fractions.

Long Hairy Crabgrass Ethanol Extract Staining Results:

LHCGF	Ferric Chloride - Specific for Phenols	Potassium Permanganate - Specific for alkenes, alkynes, aldehydes (yellow)	Vanillin + sulphuric acid (Strong and weak Nu:, -OH, -RNH, -COOH, ROR.	Bromocresol Green - Acidic compounds (yellow)	356 nm UV
1	-	+ (1 yellow spot)	+ (4 blue spots)	+ (1 yellow spot)	+ (2 spots)
2	-	+ (1 yellow spot)	+ (3 blue spots, 1 purple spot, 1 green spot)	+ (1 yellow spot)	+ (3 spots)
3	-	+ (1 yellow spot)	+ (3 blue spots, 1 green spot)	-	+ (4 spots)
4	-	-	+ (1 blue spot, 2 green spots)	-	+ (5 spots)
5	-	+ (2 yellow spots)	+ (2 purple spots, 4 green spots)	-	+ (7 spots)
6	-	+ (1 yellow spot, 1 bleached spot)	+ (3 blue spots, 1 green spot)	-	+ (5 spots)
7	-	+ (1 yellow spot, 2 bleached spots)	+ (2 blue spots, 1 dark green spot, 1 green spot)	+ (1 yellow spot)	+ (5 spots)
8	-	+ (5 bleached spots)	+ (2 dark green spots, 1 green spot, 1 blue spot)	-	+ (7 spots)
9	-	+ (3 yellow spots)	+ (1 pink spot, 2 dark green spots, 1 blue spot, 2 green spots)	-	+ (5 spots)
10	-	+ (1 yellow spot)	+ (3 green spots)	+ (2 yellow spots)	+ (7 spots)
11	-	+ (2 yellow spots)	+ (2 purple spots, 2 green spots)	+ (2 yellow spots)	+ (5 spots)
12	-	+ (2 yellow spots)	+ (1 purple spot, 2 dark spots)	-	+ (6 spots)
13	-	+ (2 bleached spots)	+ (2 purple spots)	-	+ (6 spots)

14	-	+ (1 yellow spot, 2 bleached spots)	+ (3 purple spots, 1 green spot, 1 pink spots)	-	+ (7 spots)
15	-	+ (2 bleached spots)	+ (2 blue spots, 2 purple spots, 1 green spot, 1 dark spot)	+ (1 yellow spot)	+ (7 spots)
16	-	+ (3 bleached spots)	+ (2 blue spots, 2 purple spots, 1 green spot, 1 dark spot)	+ (1 yellow spot)	+ (6 spots)
17	+	+ (2 yellow spots)	+ (1 blue spot, 3 blue spots)	+ (1 yellow spot)	+ (3 spots)
18	-	+ (3 yellow spots)	+ (2 blue spots, 1 green spots)	+ (1 yellow spot)	+ (6 spots)
19	-	+ (1 yellow spot, 1 bleached spot)	+ (1 dark spot, 1 green spot)	+ (1 yellow spot)	+ (5 spots)
20	+ (3 spots)	+ (1 yellow spot)	+ (2 dark spots)	-	+ (4 spots)
21	-	+ (3 yellow spots)	+ (2 dark spots)	+ (1 yellow spot)	+ (5 spots)
22	-	+ (1 bleached spot at baseline)	+ (4 dark spots)	-	+ (3 spots)
23	+	+ (1 yellow spot at baseline)	+ (1 spot, 1 spot at baseline)	-	+ (4 spots)
24	+	+ (1 yellow spot at baseline)	+ (1 spot, 1 spot at baseline)	+ (1 yellow spot)	+ (3 spots)
25	+	+ (1 yellow spot at baseline)	+ (2 dark spots, 1 spot at baseline)	+ (1 blue spot)	+ (3 spots)
26	+	+ (1 yellow spot at baseline)	+ (1 dark spot at baseline)	+ (1 blue spot)	+ (5 spots)

Table 12- TLC staining results for long hairy crabgrass ethanol extract.

Long Hairy Crabgrass staining discussion:

Phenols were found in six of the ethanol fractions of long hairy crabgrass. Most of the positive tests came from the latter fractions from the column chromatography separation. This is consistent with the theory with thin layer chromatography where polar compounds have more interaction with the silica and hence lastly eluted. A point to note is that fraction 20 resulted in 3 separated spots. Similarly, to smooth crabgrass, long hairy crabgrass showed a positive test for phenols in the phytochemical assay.

Twenty – five of the ethanol extracts from long hairy crabgrass showed a positive test for potassium permanganate test. The negative test was seen with fraction 4. The yellow spots are indictive of aldehydes while the bleached spots are attributed to unsaturation. There were incidents where one fraction showed multiple TLC spots which when stained, showed to contain both yellow and bleached spots.

As it relates to the Vanillin and sulfuric acid staining, blue spots were seen in twelve fractions. In many of these cases, there were multiple blue spots, indicating the presence of coumarins. Terpenoids were positive in eight fractions, indicated by the purple staining. The phytochemical testing also tested positive for this class of compound. Aromaticity and unsaturation are evident in fifteen fractions due to the yellow to green colour. A dark colour was seen in twelve of the fractions. The dark spots indicate organic compounds, with the possibility of saponins and essential oils being present. Phenols were seen in two fractions: 9 and 14.

Bromocresol green staining technique determined whether a spot is acidic or basic. A yellow colour indicates acidity, found in twelve of the combined fractions. On the contrary, a blue spot reflects basicity as seen in two of the latter combined fractions.

Crabwood Ethanol Extract Staining Results:

CW -F	Ferric Chloride - Specific for Phenols	Potassium Permanganate - Specific for alkenes, alkynes, aldehydes (yellow)	Vanillin + sulphuric acid (Strong and weak Nu:, -OH, -RNH, -COOH, ROR.	Bromocresol Green - Acidic compounds (yellow)	356 nm UV
1	-	+ (1 yellow spot)	+ (2 blue spots)	-	+ (1 spot)
2	-	+ (1 yellow spot)	+ (1 blue spot)	-	+ (1 spot)
3	-	+ (1 yellow spot)	+ (1 blue spot, 1 purple spot)	-	+ (1 spot)
4	-	+ (1 yellow spot)	+ (1 blue spot, 1 purple spot, 1 green spot)	-	+ (1 spot)
5	+ (1 green spot)	+ (1 yellow spot)	+ (1 blue spot, 1 yellow spot, 1 red spot)	+ (1 yellow spot)	-
6	+ (1 green spot)	+ (1 yellow spot)	+ (3 pink spots, 1 yellow spot, 1 red spot)	+ (1 yellow spot)	+ (1 spot)
7	+ (2 green spots)	+ (2 yellow spots)	+ (2 dark spots, 2 pink spots, 1 yellow spot, 1 red spot)	+ (1 dark spot)	+ (1 spot)
8	+ (2 green spots)	+ (2 yellow spots)	+ (1 yellow spot, 1 pink spot, 1 red spot)	+ (2 yellow spots, 1 at baseline)	+ (1 spot)
9	+ (1 pink spot at base line)	+ (1 yellow spot at baseline)	+ (1 pink spot, 1 red spot at baseline)	+ (1 blue spot)	-
10 - B1	-	-	-	-	-
11 - B2	-	+ (2 yellow spots, 2 bleached spots)	+ (10 dark spots, 1 pink spot)	-	+ (1 spot)
12 - B	-	-	+ (1 pink spot)	-	-
13	+ (2 green spots)	+ (2 yellow spots)	+ (1 pink spot, 1 red spot at baseline)	+ (1 blue spot)	+ (1 spot)

14	+ (2 green spots)	+ (2 yellow spots)	+ (1 pink spot, 1 red spot at baseline)	+ (1 blue spot)	+ (1 spot)
15	+ (2 dark green spots)	+ (1 bleached spot)	+ (1 dark spot, 1 pink spot, 1 red spot at baseline)	+ (1 blue spot)	+ (1 spot)
16	+ (1 browish spot)	+ (1 yellow spot)	+ (1 red spot at baseline)	+ (1 blue spot)	+ (1 spot)
17	+ (2 dark spots)	+ (1 yellow spot)	+ (1 dark spot, 1 pink spot, 1 red spot at baseline)	+ (1 blue spot)	+ (1 spot)
18					
19	+(1 browish spot)	+ (1 yellow spot)	+ (1 dark spot, 1 pink spot at baseline)	+ (1 blue spot)	+ (1 spot)
20	+(1 browish spot)	+ (1 yellow spot at baseline)	+ (1 dark spot, 1 red spot at baseline)	+ (1 blue spot)	+ (1 spot)
21	+(1 browish spot)	+ (1 yellow spot at baseline)	+ (1 dark spot, 1 bleached spot at baseline)	+ (1 blue spot)	+ (1 spot)
22	+(1 browish spot)	+ (1 yellow spot at baseline)	+ (1 dark spot, 1 dark spot at baseline)	+ (1 blue spot)	+ (2 spots)

Table 13- TLC staining results for crabwood's bark ethanol extract.

Crabwood staining discussion:

Fourteen thin layer chromatography results tested positive for phenols in the ethanol fractions of crabwood. This is classical because not only pink and blue colour were observed but also green and red-brown were seen. These variations of colours were seen because Fe^{3+} ions form various complexes. A colour variation was expected in the different fractions because this crude extract showed a positive result for the phytochemical assay.

Twenty one of the twenty-two fractions showed a positive test with potassium permanganate. Fraction 12 showed no reaction with potassium permanganate. A majority of the fractions indicated that aldehydes are present since yellow spots were observed. One bleached single spot was seen in fraction 15, suggesting an unsaturated configuration.

With the vanillin and sulfuric acid staining, coumarins were present in fractions one to five since blue spots were observed. Terpenoids were present in fractions three and four only due to the purple spot indication. A point to note here is that the phytochemical test showed a positive result for terpenoids also. Aromaticity and unsaturation are evident in five fractions due to the yellow to green colour. Eight fractions showed dark spots indicating that the separated compounds are organic in nature. Red and pink spots were observed in fifteen fractions. This indicated the present of phenols. Those fractions which exhibited the pink and red spots were the same that showed a positive test for the ferric chloride staining test.

Acidic compounds were presented in three of the early fractions as indicated by the bromocresol green staining test. Basic compounds were observed in ten of the latter fractions due to the observed blue colour.

Antimicrobial Activities of the Ethanol Crude Extracts:

Disc Diffusion Results:

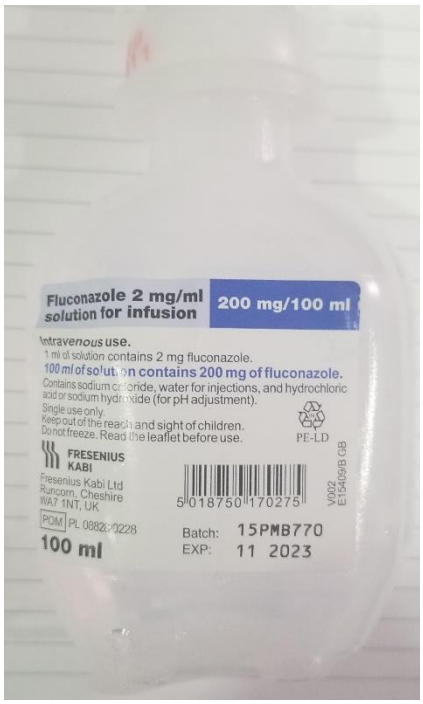
	Inhibition zone (mm)				
Solution	**E. coli**	**S. aureus**	**S. epidermidis**	**P. aeruginosa**	**C. albicans**
Controls:					
Vancomycin	22	0	12	14	0
Ampicillin	33	28	0	0	0
Gentamycin	35	24	19	44	0
Fluconazole	0	0	0	0	0
Negative	0	0	0	0	0
CW 10mg/mL	0	9	8	0	0
CW 50mg/mL	0	11	11	0	0
CW 100mg/mL	0	13	11	0	0
SCG 10mg/mL	0	0	7	0	0
SCG 50mg/mL	0	0	7	0	0
SCG 100mg/mL	0	0	8	0	0
LHCG 10mg/mL	0	0	0	0	0
LHCG 50mg/mL	0	0	0	0	0
LHCG 100mg/mL	0	0	0	0	0

Table 14- Zones of inhibition for different concentrations of the ethanol crude extracts in the disc diffusion antimicrobial assay.

Agar Well Diffusion Results:

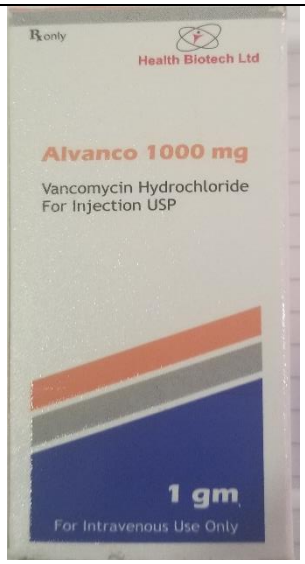
	Inhibition zone (mm)				
Solution	**E. coli**	**S. aureus**	**S. epidermidis**	**P. aeruginosa**	**C. albicans**
Controls:					
Vancomycin	24	0	13	11	0
Ampicillin	32	21	0	12	0
Gentamycin	40	18	22	50	0
Fluconazole	0	0	0	0	0
Negative	0	0	0	0	0
CW 10mg/mL	0	11	10	0	0
CW 50mg/mL	0	12	12	0	0
CW 100mg/mL	-	-	-	-	-
SCG 10mg/mL	0	0	0	0	0
SCG 50mg/mL	0	0	0	0	0
SCG 100mg/mL	0	0	0	0	0
LHCG 10mg/mL	0	0	0	0	0
LHCG 50mg/mL	0	0	0	0	0
LHCG 100mg/mL	0	0	0	0	0

Table 15- Zones of inhibition for different concentrations of the ethanol crude extracts in the agar well antimicrobial assay.

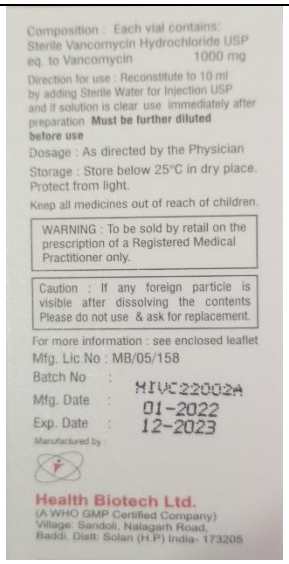


A

B



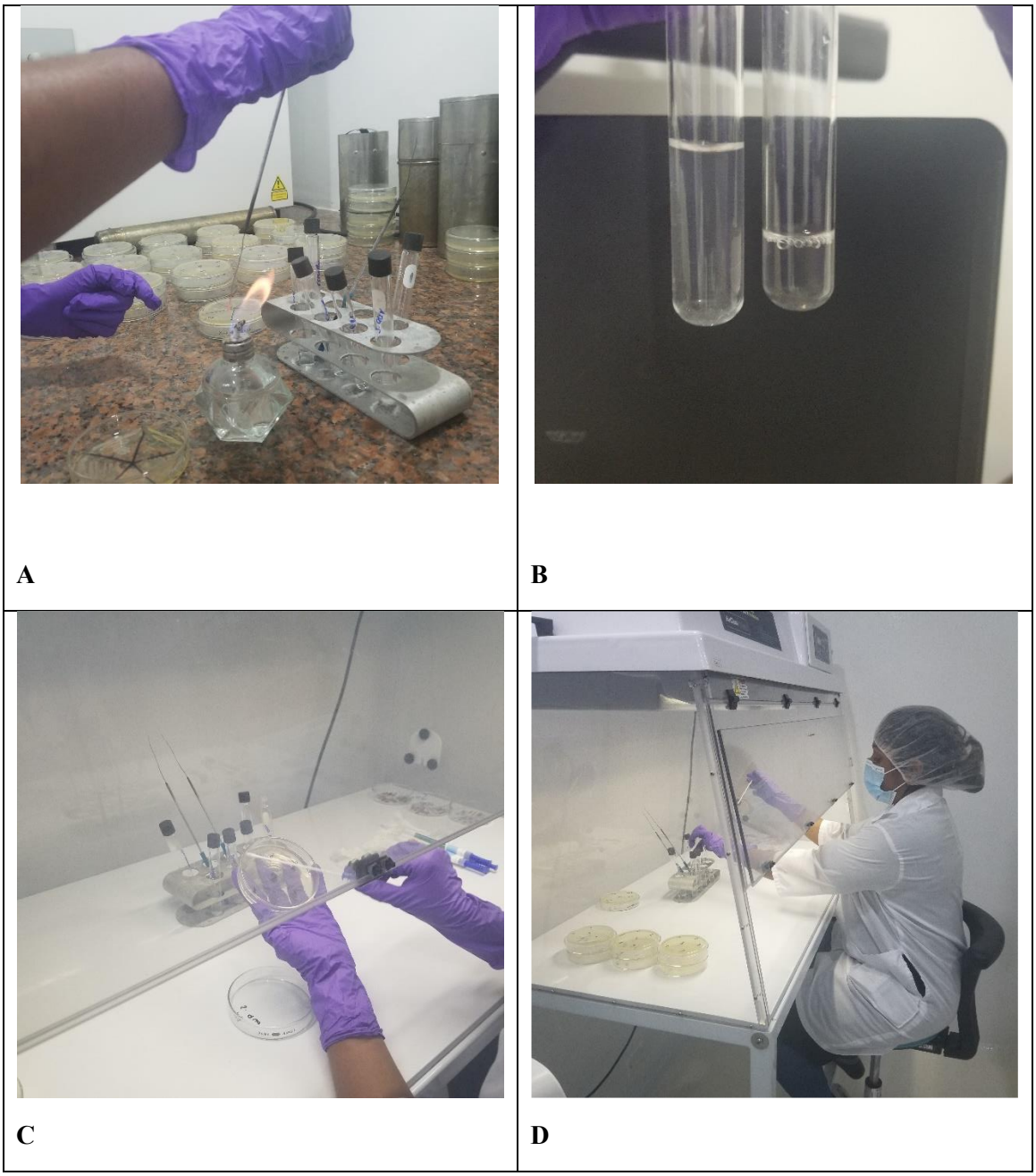
C



D

Figure 62.- Pictures of the actual Positive controls used in the antimicrobial assays – Fluconazole 2mg/ml (A), Gentamicin 40mg/mL (B), Vancomycin HCl 1g (C&D).

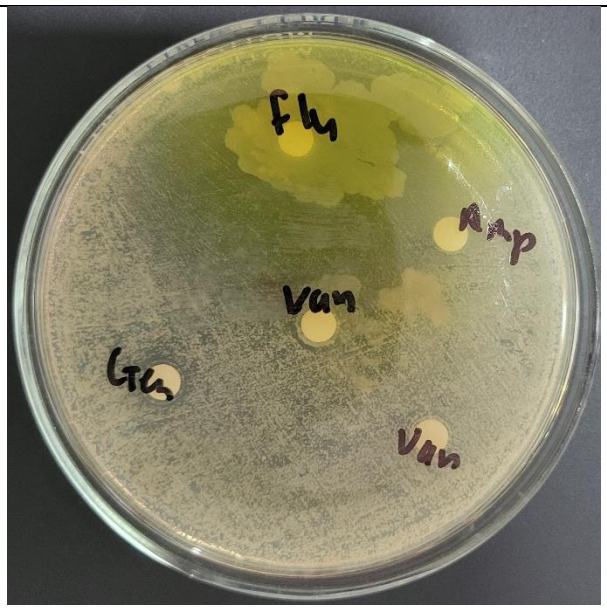
Figure 63.- Pictures of the actual Positive controls used in the antimicrobial assays – Ampicillin 500mg (A&B) and Fluconazole 150mg (C&D).



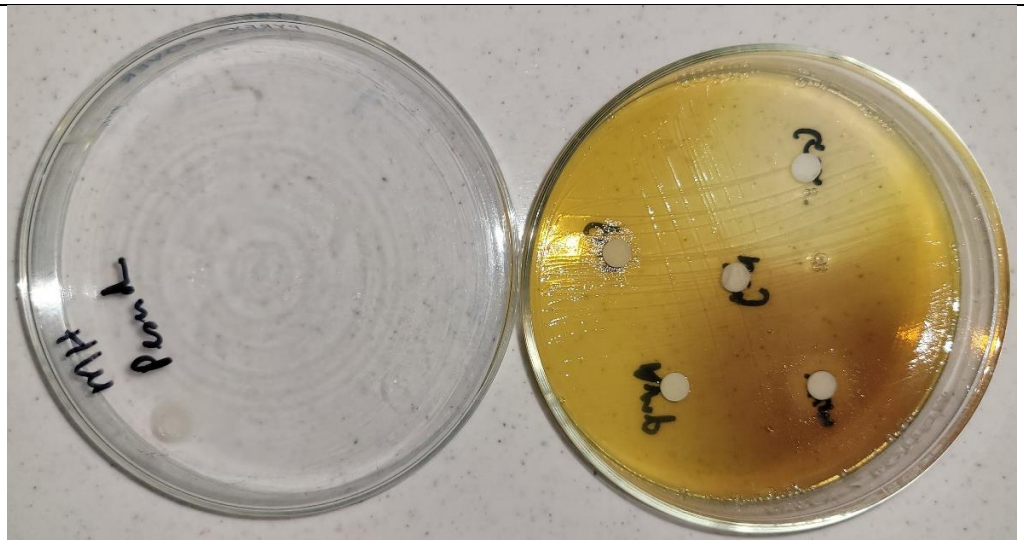
A **B**

C **D**

Figure 64.- Preparation of the agar plates before the antimicrobial assay – Sterilization of inoculum loop (A), comparison to the Mc Faraday's standard (B), inoculation of the plates with the microorganism (C), and the microbiologist (D).



A



B

Figure 65.- Pictures of the positive controls for the disc antimicrobial assay.

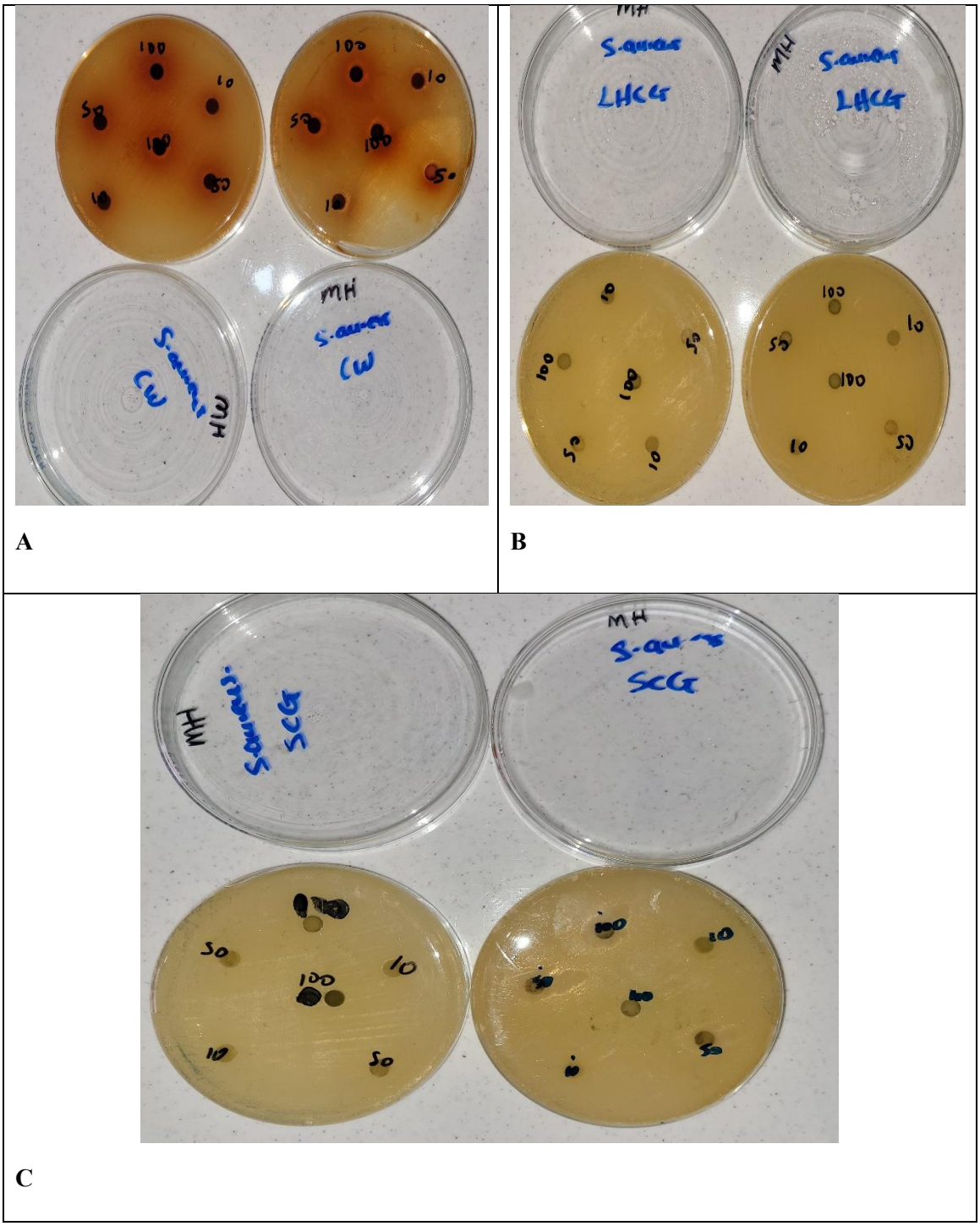


Figure 66. - Pictures of the results of the disc antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Staphylococcus aureus.

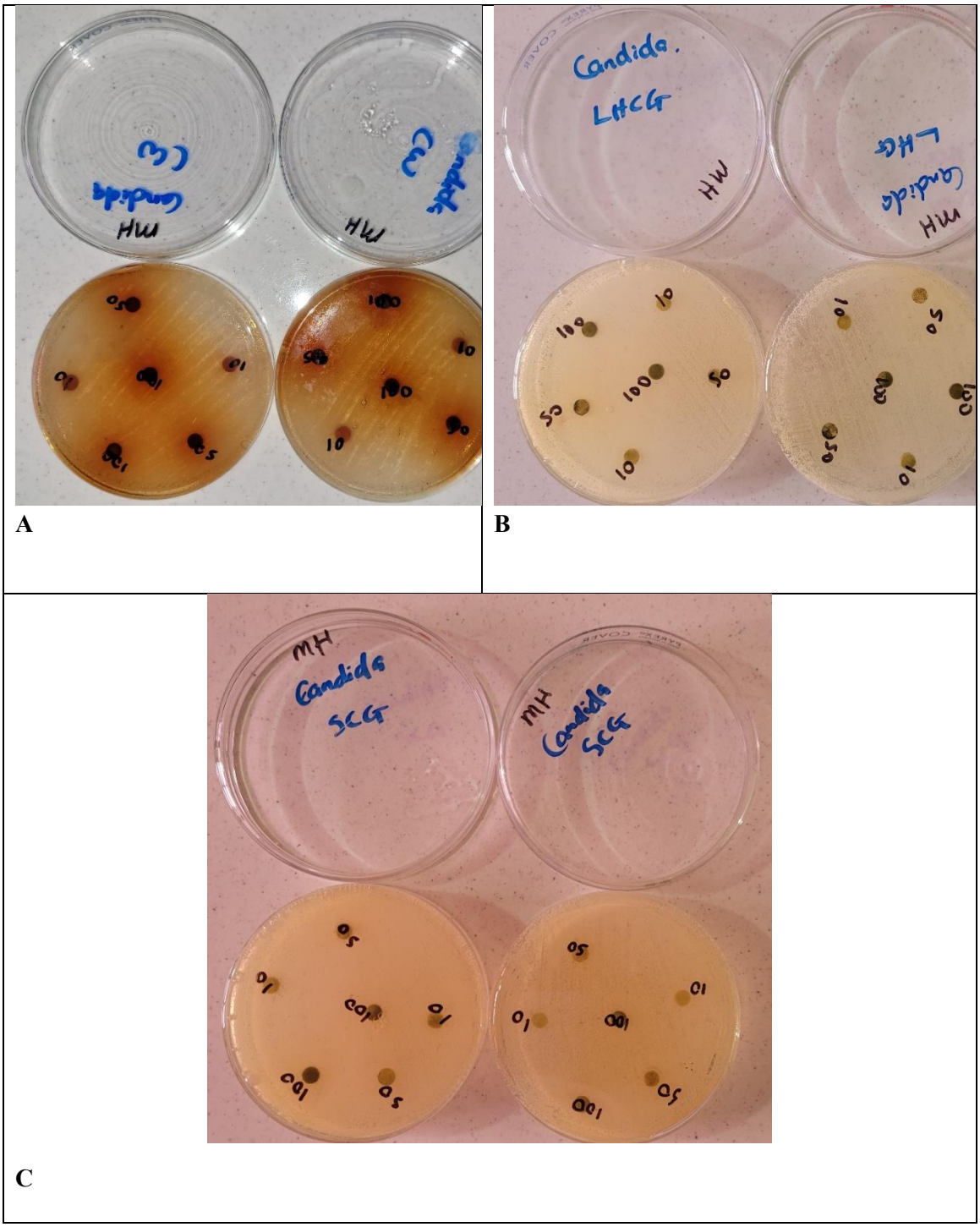
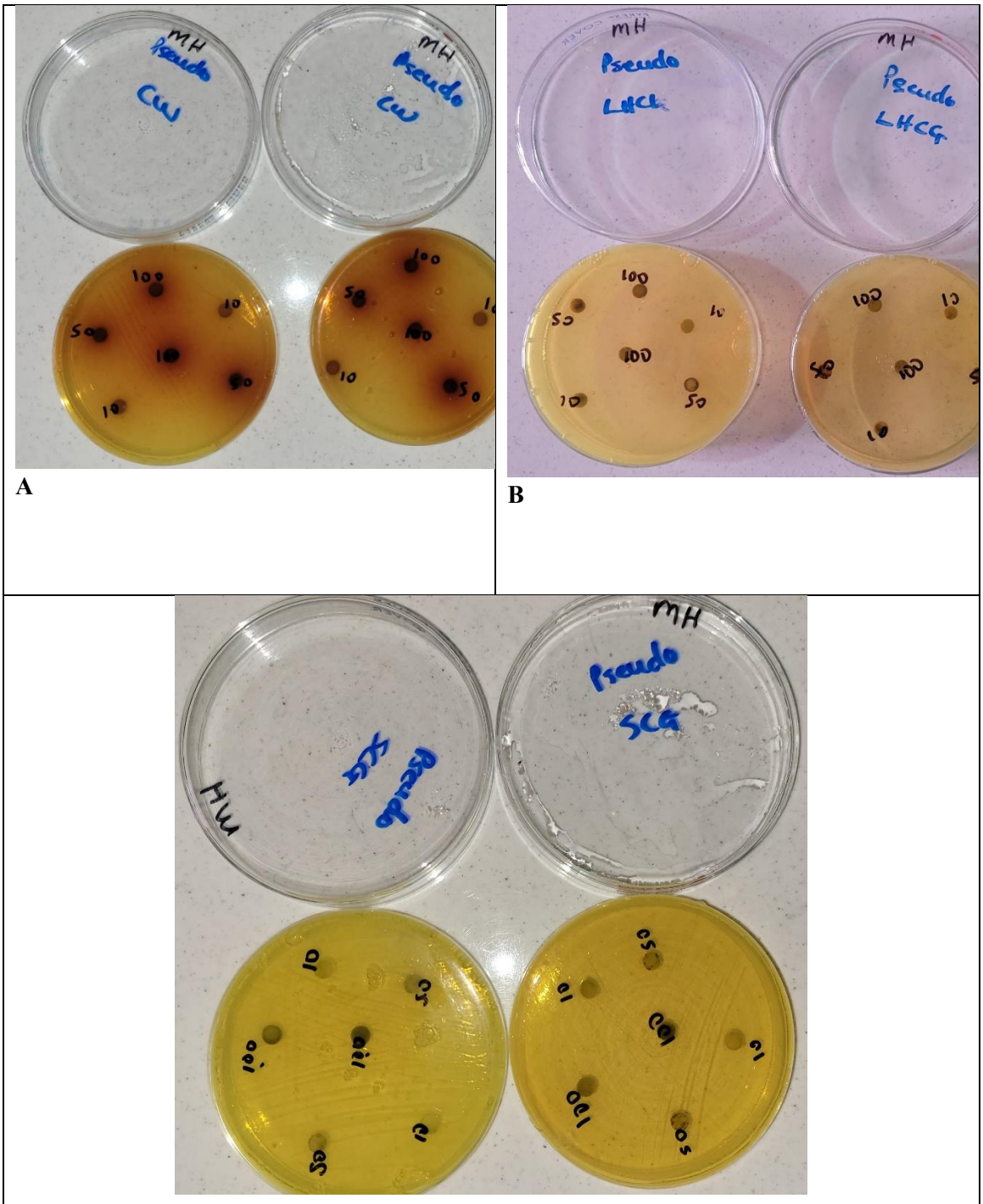


Figure 67.- Pictures of the results of the disc antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Candida albicans.



A

B

C

Figure 68.- Pictures of the results of the disc antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Pseudomonas aeruginosa.

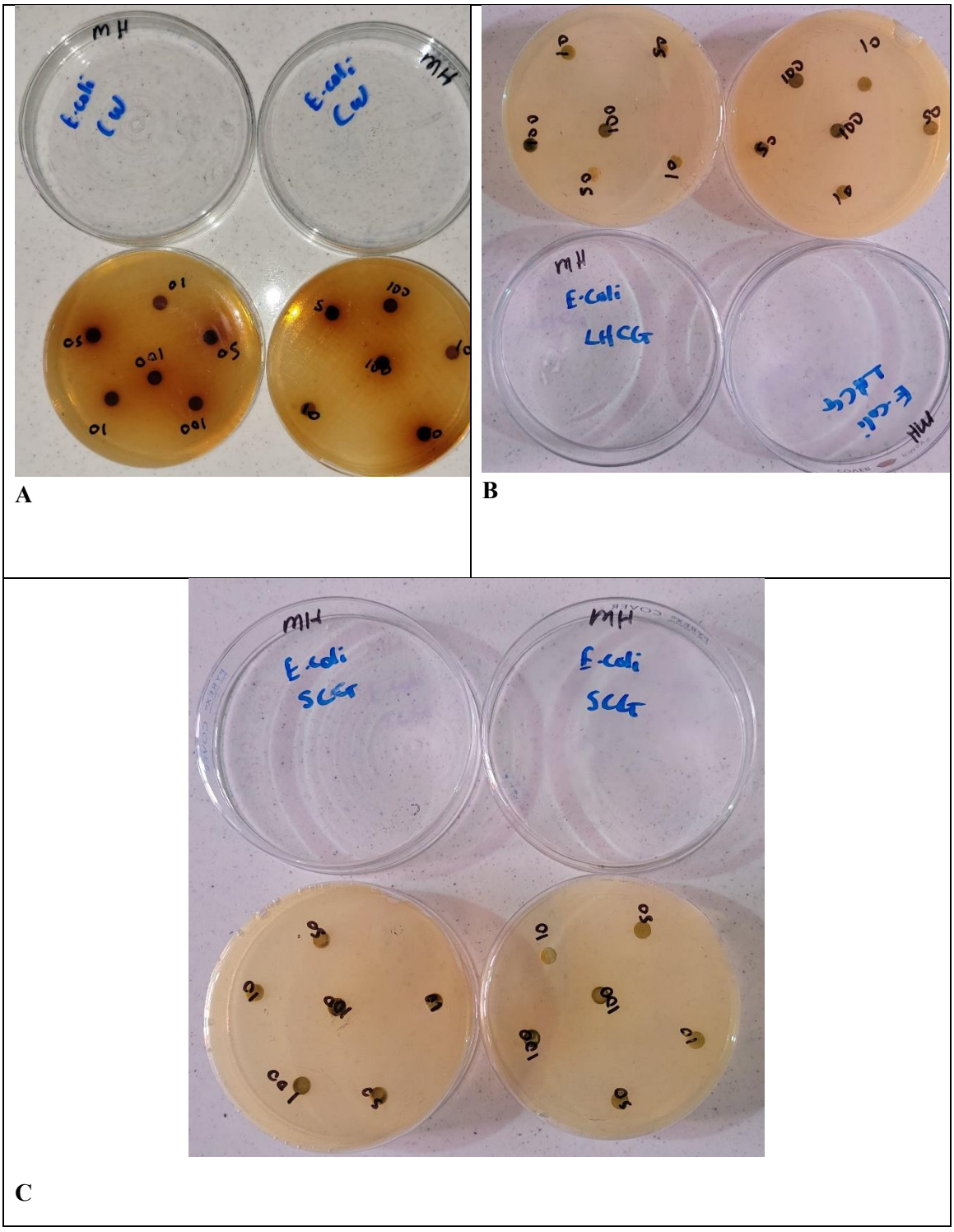
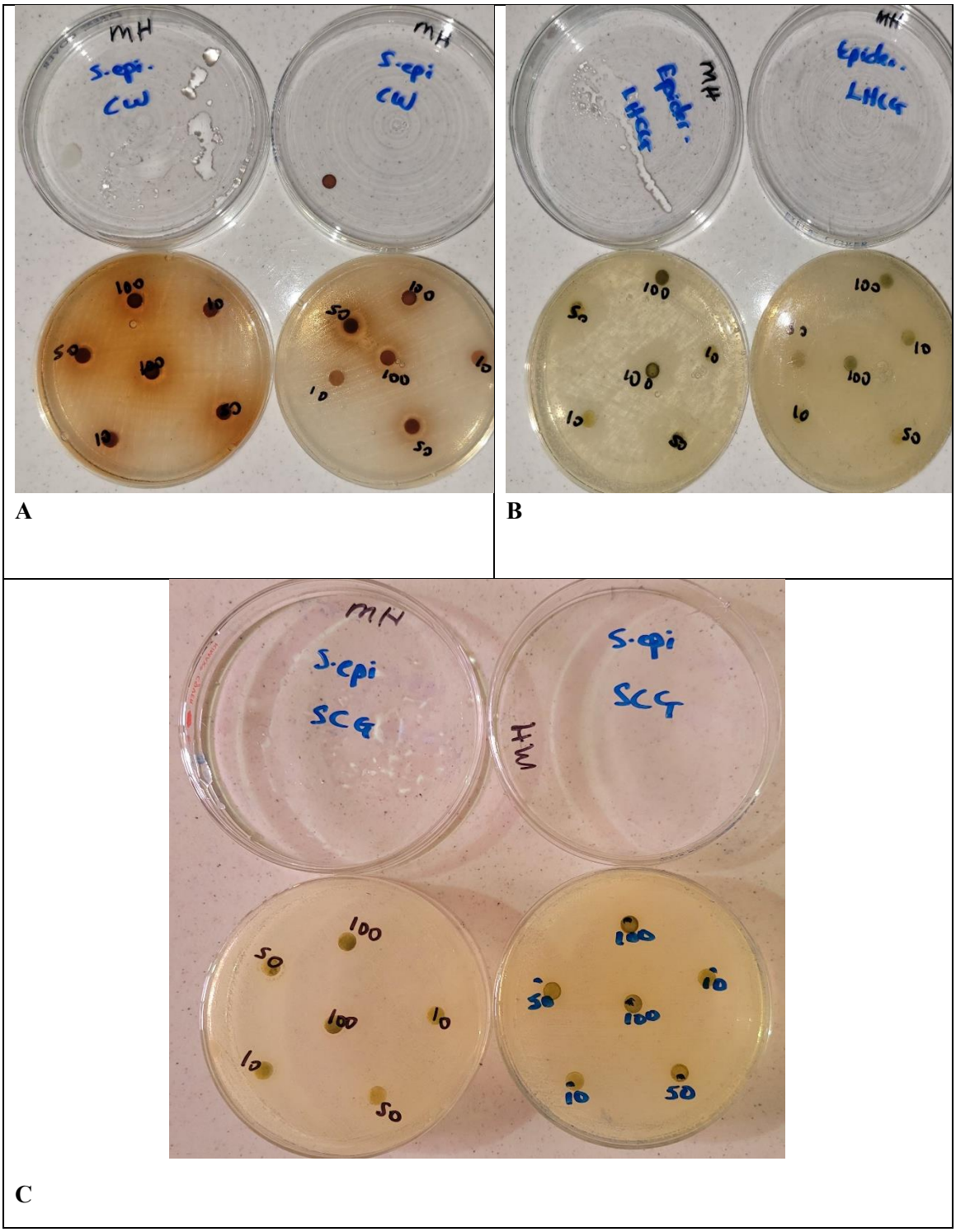


Figure 69.- Pictures of the results of the disc antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Escherichia coli.



A **B**

C

Figure 70. - Pictures of the results of the disc antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Staphylococcus epidermidis.

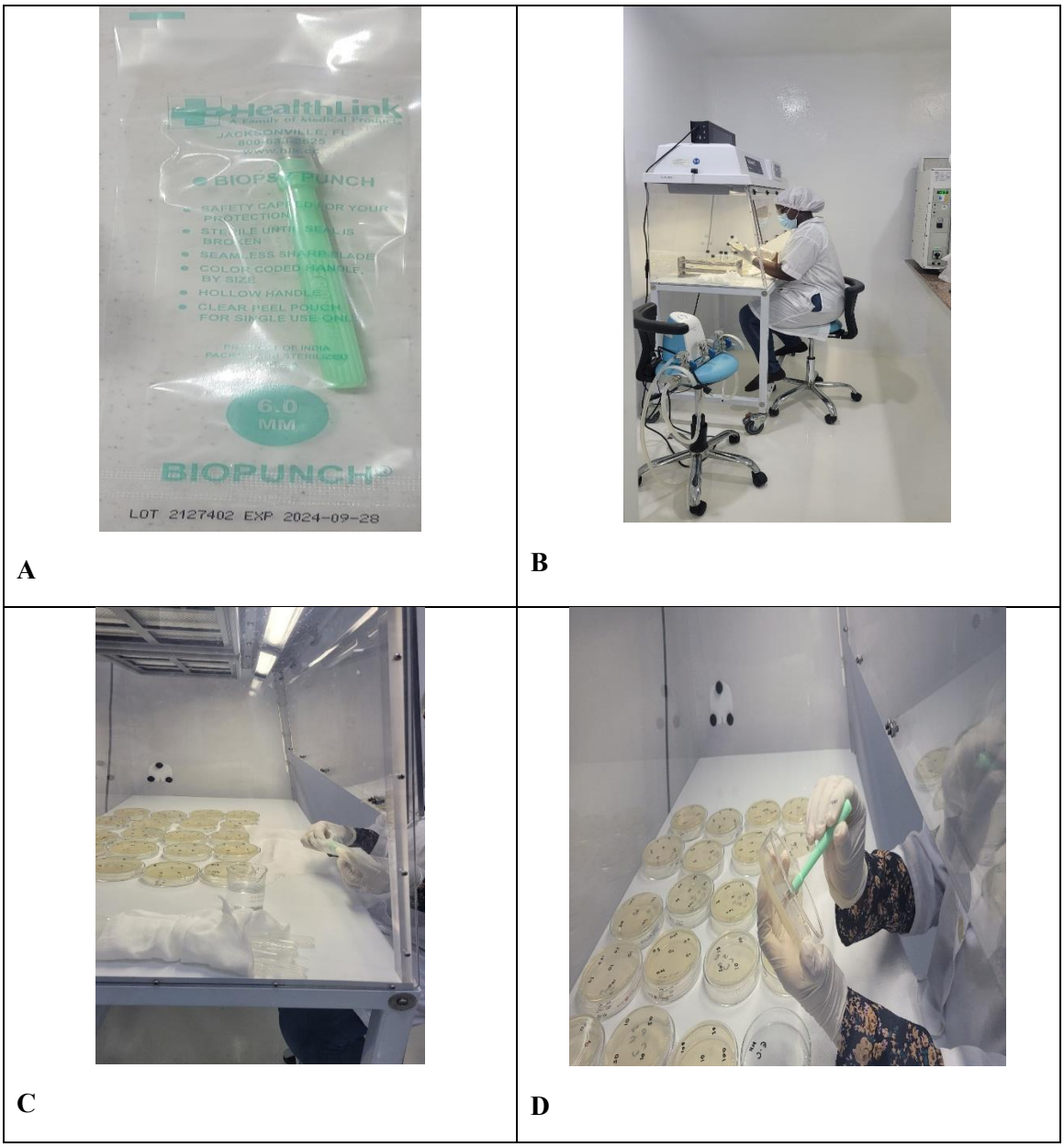
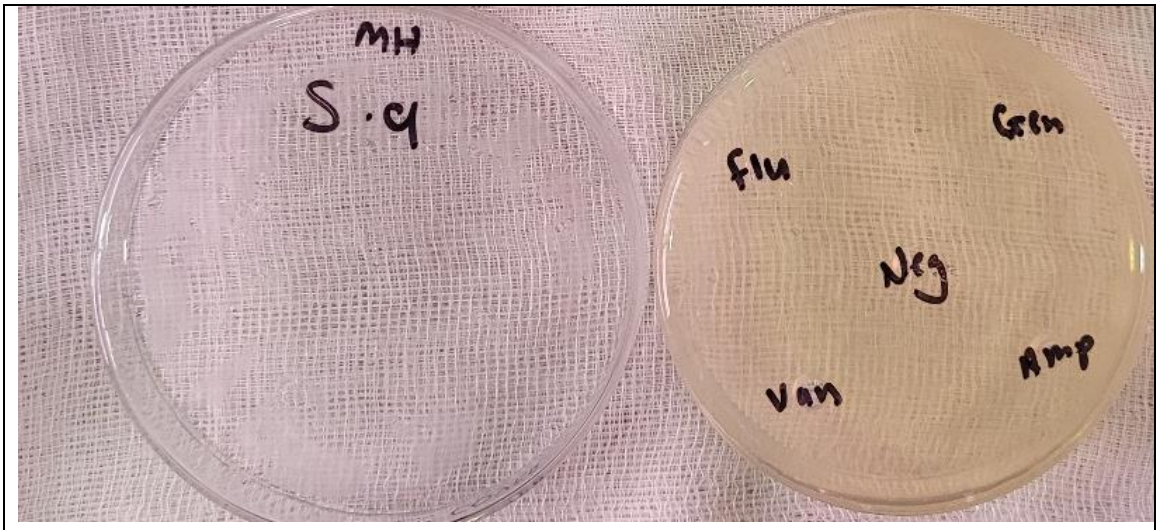
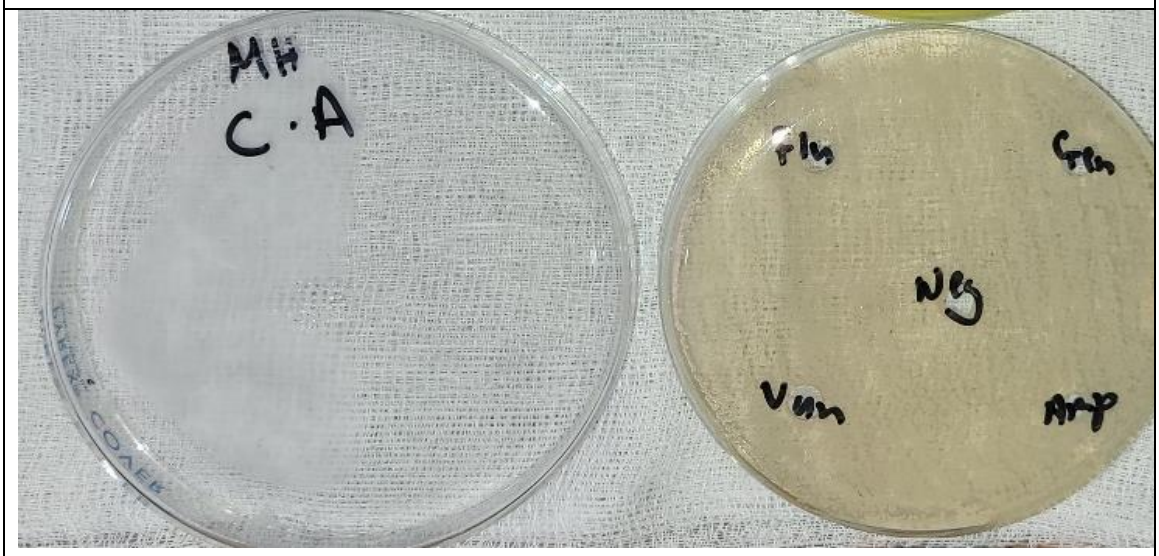


Figure 71.- Preparation of the plates in the well agar method – Cork borer (A), punching of the 6 mm holes in the plates (B,C&D).

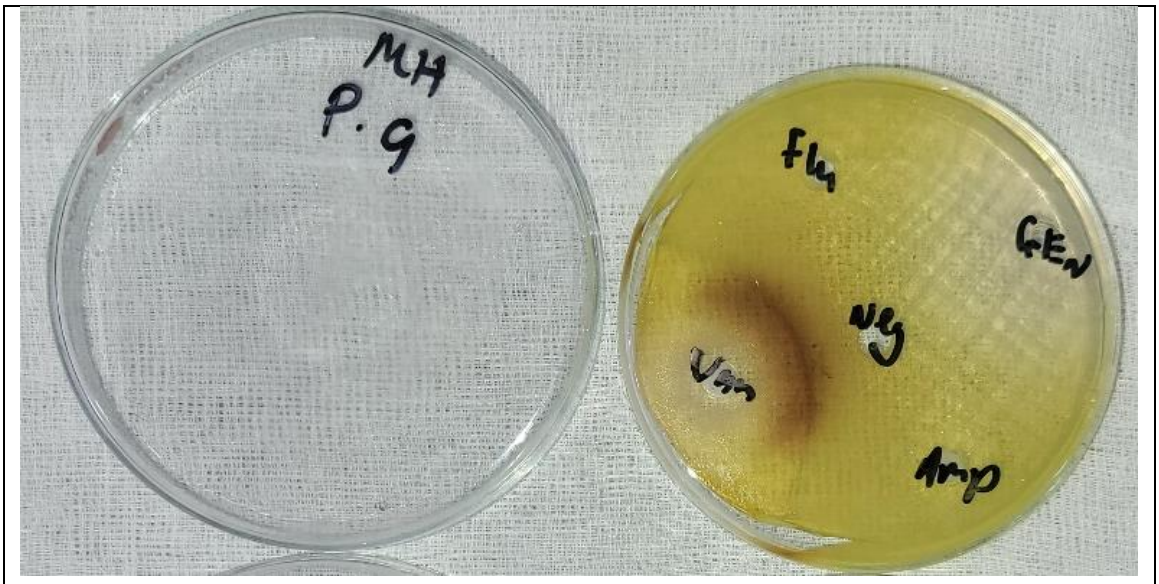


A

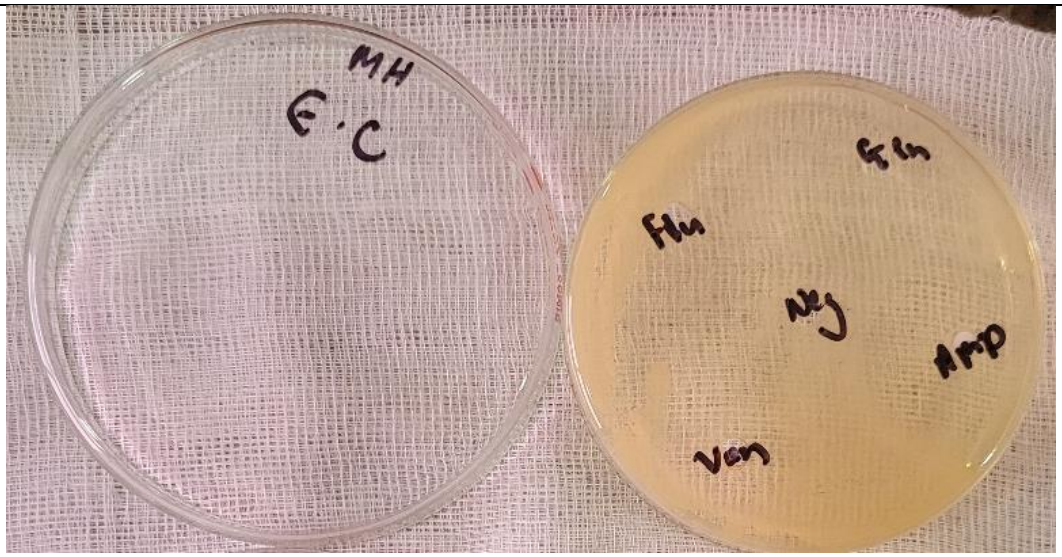


B

Figure 72.- Pictures of the positive and negative controls for the agar well method antimicrobial assays against Staphylococcus aureus (A) and Candida albicans (B).

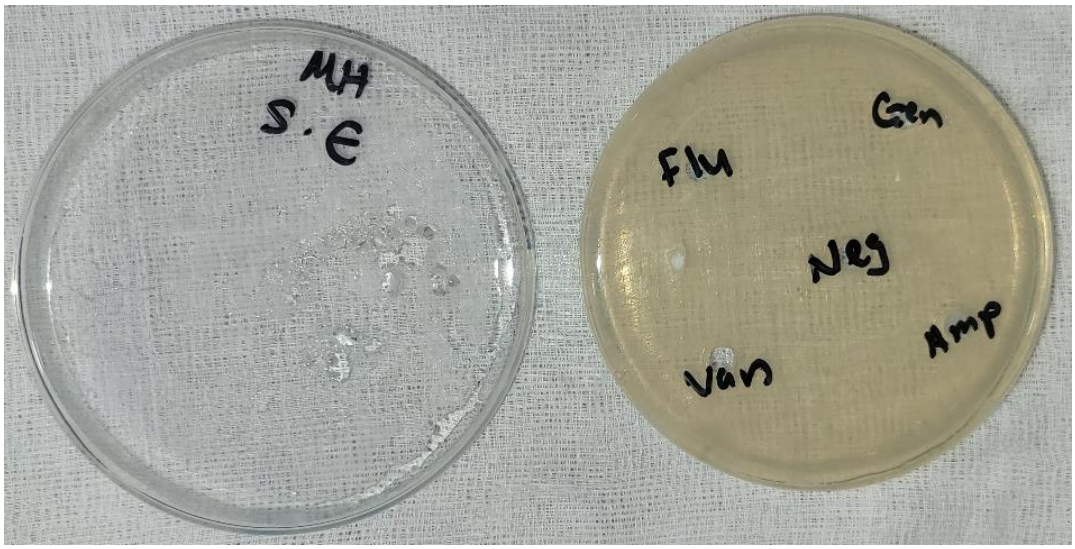


A



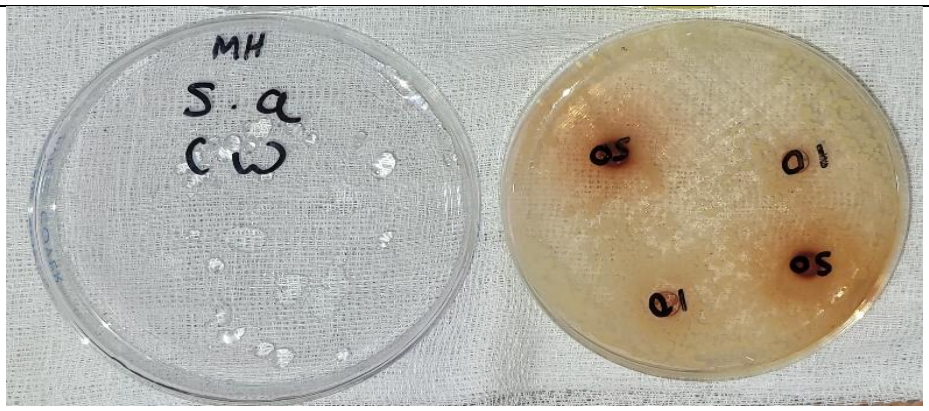
B

Figure 73.- Pictures of the positive and negative controls for the agar well method antimicrobial assays against Pseudomonas aeruginosa and Escherichia coli.

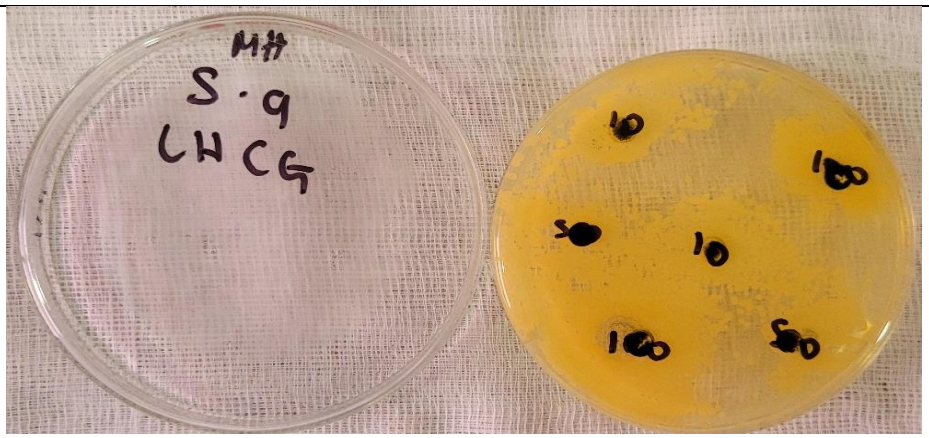


A

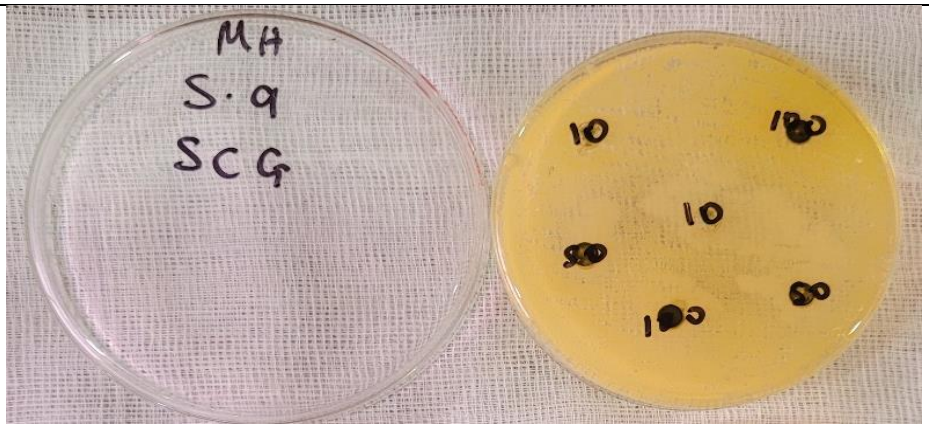
Figure 74.- Pictures of the positive and negative controls for the agar well method antimicrobial assays against Staphylococcus epidermidis.



A

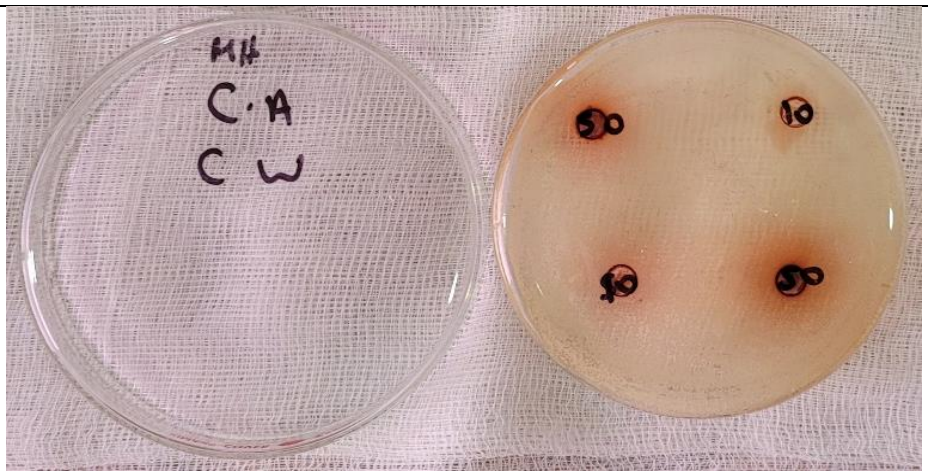


B

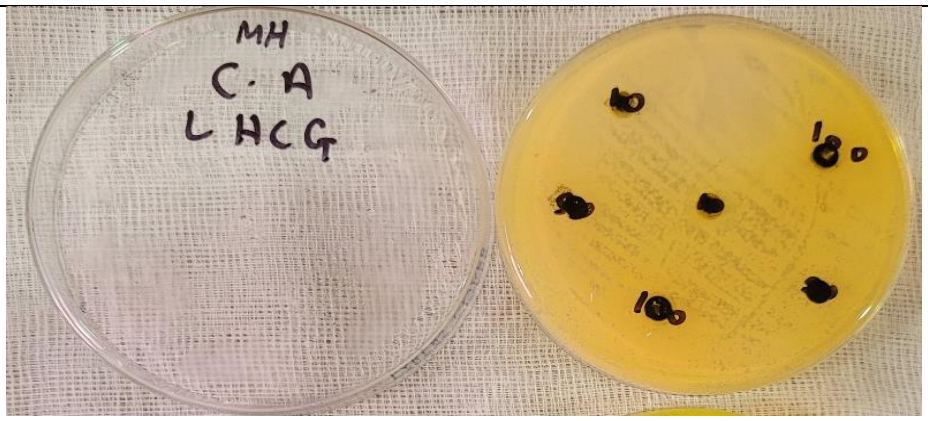


C

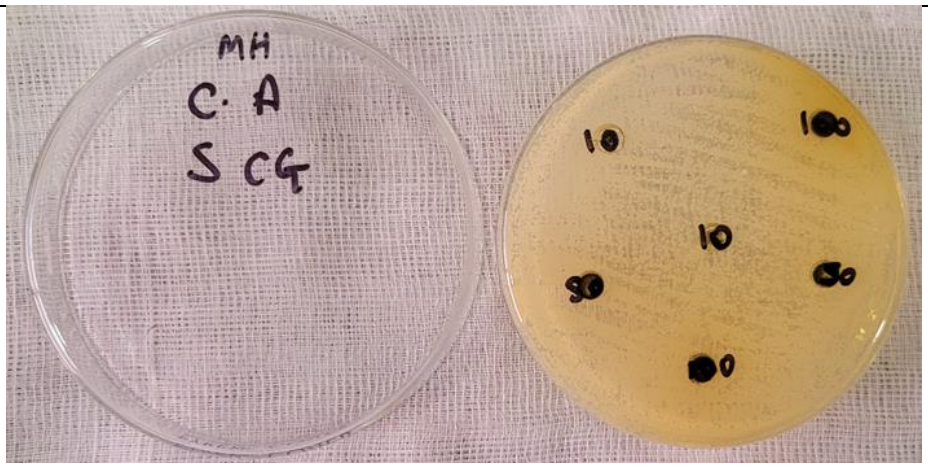
Figure 75.- Pictures of the results of the agar well antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Staphylococcus aureus.



A

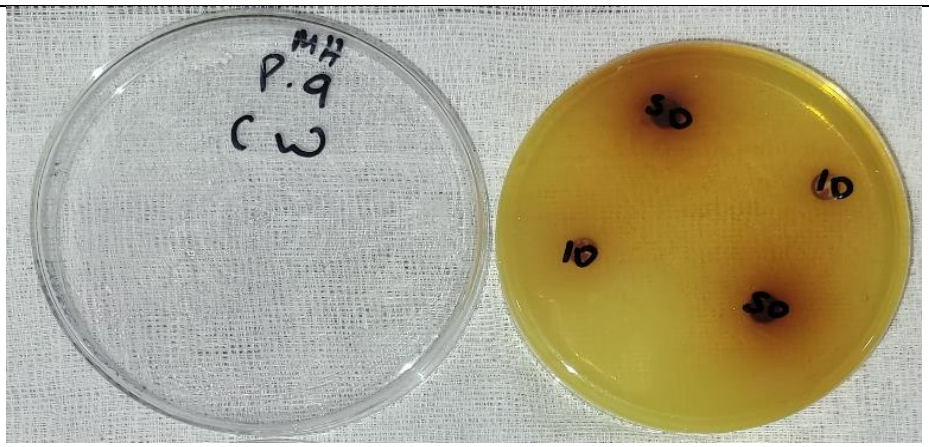


B



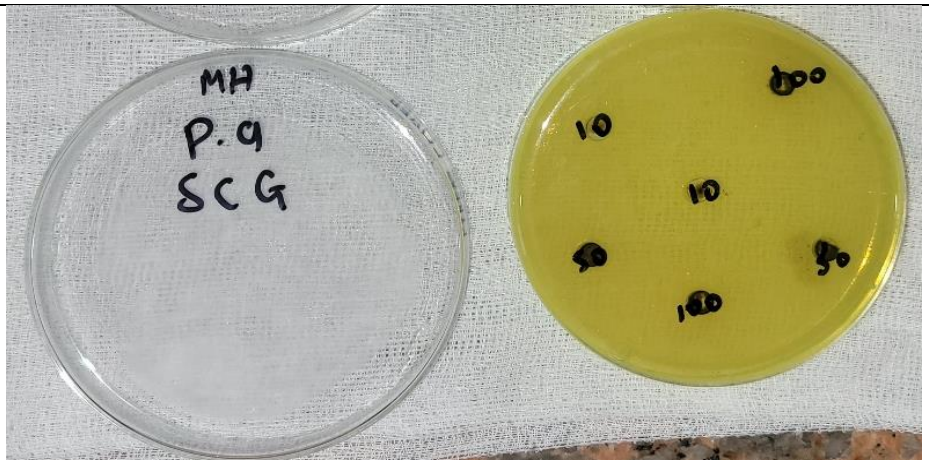
C

Figure 76.- Pictures of the results of the agar well antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Candida albicans.



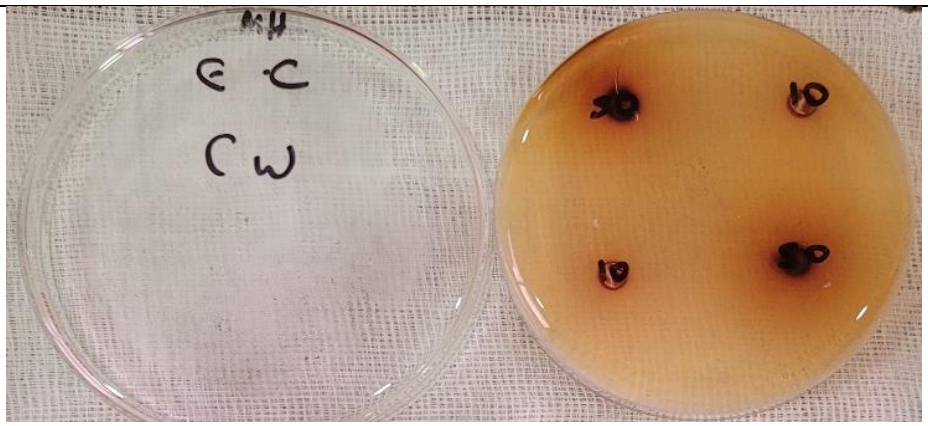
A

B

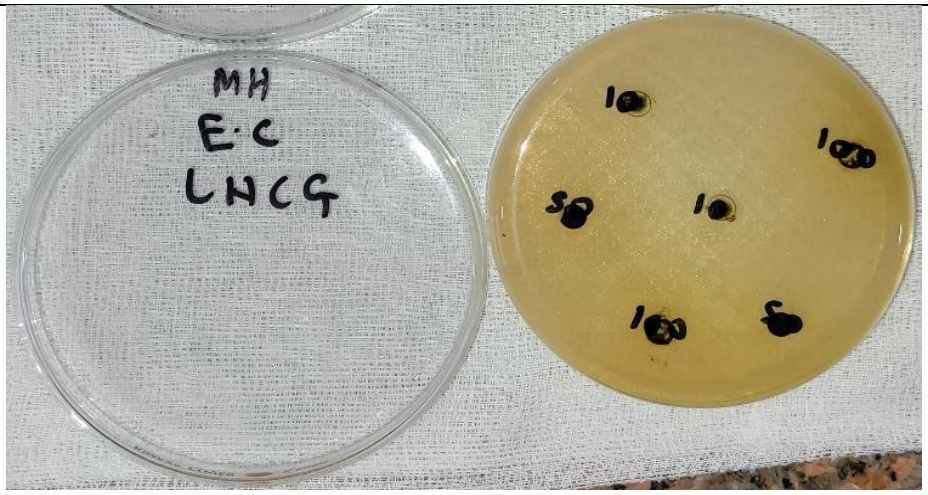


C

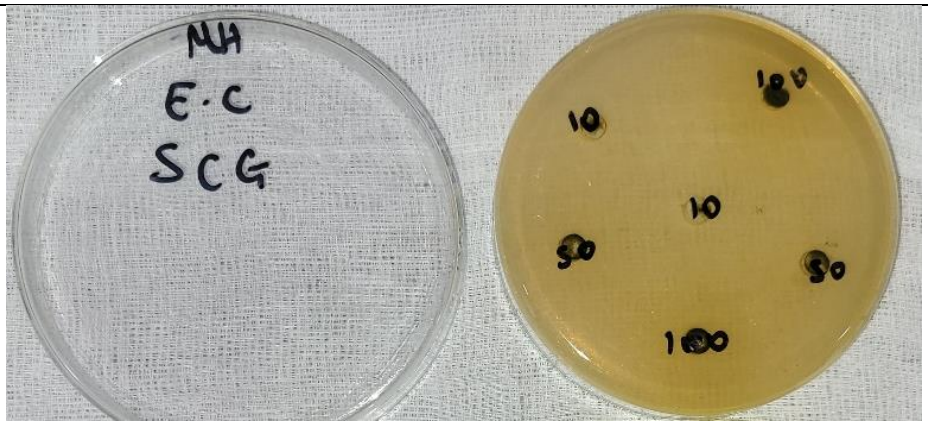
Figure 77.- Pictures of the results of the agar well antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Pseudomonas aeruginosa.



A

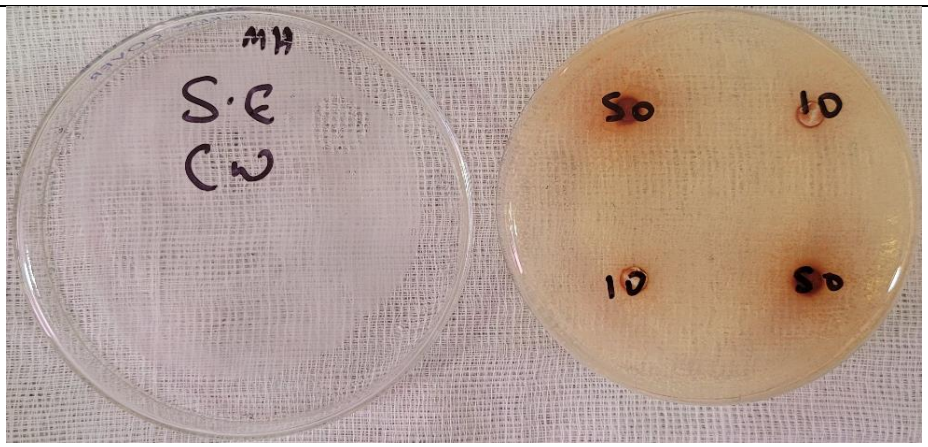


B

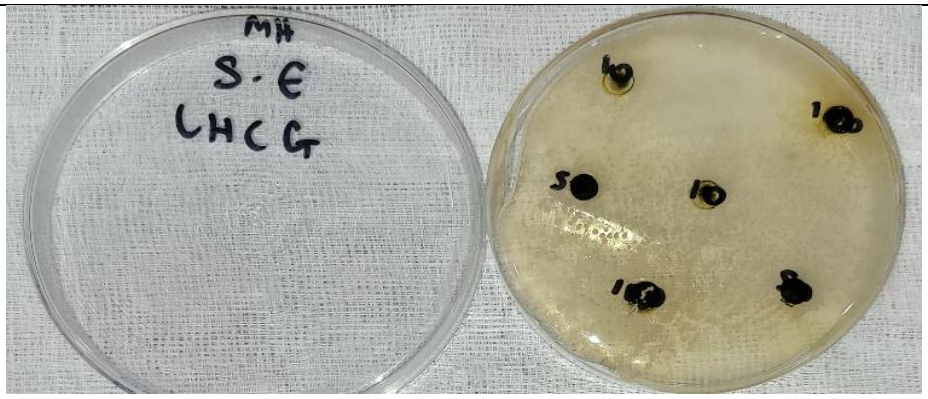


C

Figure 78.- Pictures of the results of the agar well antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Escherichia coli.



A



B

C

Figure 79.- Pictures of the results of the agar well antimicrobial assay for ethanol extract of crabwood (A), long hairy crabgrass (B) and smooth crabgrass (C) against Staphylococcus epidermidis.

Discussion on Antimicrobial testing:

Except for Fluconazole, all the other positive controls showed expected results. Fluconazole is a fungicide which was expected to inhibit the growth of Candida albicans. This was not observed in either the Disc or the Well agar antimicrobial testing methods. Initially, the tablet version (APO-Fluconazole-150) of Fluconazole was used in the Disc method, which revealed to be futile against Candida albicans. Subsequently, in Well agar method, the Fluconazole injection version was used instead of the tablets. This showed no inhibition of growth of the Candida albicans. It is interesting to state that it appears Candida albicans are or have become resistant to fluconazole. Literature later shows that this is true. According to (Centers for Disease Control and Prevention, 2020), "7% of all Candida blood samples tested at CDC are resistant to the antifungal drug fluconazole".

Organism growth was seen on all the negative controls. This indicated that the diluent used in this assay did not retard or affected the growth of the micro-organisms in any fashion.

All concentrations of Crabwood ethanol extract inhibited the growth of Staphylococcus aureus and Staphylococcus epidermidis in both Disc diffusion and Agar well antimicrobial assays. No noticeable inhibition zones were seen for the other organisms. This was very interesting since Crabwood was expected to retard the growth of Candida albicans because Crabwood oil helps with fungal infections on the skin. A study done on the bark of another species of crabwood, Carapa procera found in the Akim Oda forest in the Eastern Region of Ghana, showed that methanolic extract inhibited the growth of Candida albicans, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus pyrogen (Owusu, Afedzi, & Quansah, 2021).

All the concentrations of the ethanol extract of smooth crabgrass showed growth retardation with Staphylococcus epidermidis in the disc diffusion antimicrobial assay. No literature was found where smooth crabgrass antimicrobial activities were examined.

Ethanol extracts from long hairy crabgrass showed no inhibition of microbial growth in both assays. This was contrary to the publication by (Ibrahim, El-Hela, Dawoud, & Zhran, 2019), which indicated that n-hexane, ethyl acetate and n-butanol fractions of long hairy

crabgrass inhibited the growth of Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Candida albicans. The anomaly in outcomes maybe attributed to the differences in geographical locations since samples were from Guyana versus those from Egypt.

Additionally, as it relates to both crabgrasses, diffusion of the crude extract into the Muller Hilton agar in both assays were not noticeable. It was difficult to diffuse. Secondly, there may be a possibility of degradation since the extracts were approximately six months old. Lastly, since the crude samples were tested, it is conceivable that any likely antimicrobial agents may be present in low concentrations. Therefore, separation and concentration may prove a different outcome.

Gas Chromatography – Mass spectrometry and identification of compounds present in the fractions via the NIST library.

Gas Chromatography – Mass spectrometry analyses were carried out on 22 crabwood fractions, 26 Long hairy crabgrass fractions and 27 Smooth crabgrass fractions. The total analyses time was approximately 53 hours. A total of six hundred and eighty-two (682) compounds were found in all the fractions of the three ethanol crudes. Of this total, two hundred and ten (210) were from crabwood, two hundred and twenty-five (225) from long hairy crabgrass and two hundred and forty-seven (247) from smooth crabgrass. This extensive list of compounds can be found in the appendices of this thesis document. The list includes, the compound name, the separated fraction in which it was found, its molecular formula, its exact mass, a molecular structure, retention time, percentage area, classification of the compound and medicinal uses.

Extensive literature searches were done on each of the NIST hits and a list of compounds with medicinal properties was compiled. This is illustrated in tables 16, 17 and 18. Crabwood resulted in sixty (60) compounds with medicinal properties, Long hairy crabgrass with seventy (70) and Smooth crabgrass with sixty (60).

Crabwood NIST hits discussion:

The names and structures of some constituents in the ethanol extract are shown in Table 16. None of the compounds found in this study were the same as those published by any of the previous researchers but they were similar in classification. (Qi, Wu, Zhang, & Luo, 2004), isolated nine compounds from the twigs of Carapa guianensis Aubl plant using various instrumentation techniques. (Mecciaa, et al., 2013) isolated twenty-three compounds from the essential oil of the Carapa guianensis Aubl plant using gas chromatography mass spectrometry and flame ionization detection and found a majority of sesquiterpenes. The (Oliveira, et al., 2018) group on the other hand researched the oil but with another analytical technique known as electrospray ionization – mass spectrometry. Fatty acids and limonoids were among the two classes of compounds found. (Marcelle &

Mdotoo, 1975) and (Inoue, et al., 2013) also found tetranortriterpenoids and limonoid-rich classes of compounds in their studies.

A point to note is that similar classes of compounds were found in both the literatures and this study. These classes are fatty acids, terpenes, steroidal, phenolics and sugars. This is on par with the phytochemical study findings of the ethanol extracts in this research. Medicinal uses of the selected compounds range from Anti-inflammatory, insect repellent, skin moisturizer, antioxidant, antibacterial, anticancer, antidiabetic, anthelmintic, expectorant, antifungal, cholesterol lowering, treatment for acne, etc. Antibacterial activities determined in the disc diffusion and agar well methods could have been contributed by the following compounds: Phthalic acid, isobutyl octadecyl ester, Ethyl iso-allocholate, Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-, Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-, Benzyl Benzoate, Benzothiazole, Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate, γ-Sitostenone, Ethyl 2-hydroxybenzyl sulfone, 9-Octadecenamid, Estra-1,3,5(10)-trien-17β-ol, Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid, Phenyl-1-thio-α-d-glucopyranoside, 3H-Cycloocta[c]pyran-3-one, 5,6,7,8,9,10-hexahydro-4-isopropyl-1-phenyl. The structure of these compounds is found in Table 16.

Gas chromatogram and Mass spectrum of selected hits in Crabwood's bark.

Compound Target: Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-

Retention time: 28.860 – 28.995 minutes in Fraction 2 of Crabwood's bark ethanol crude.

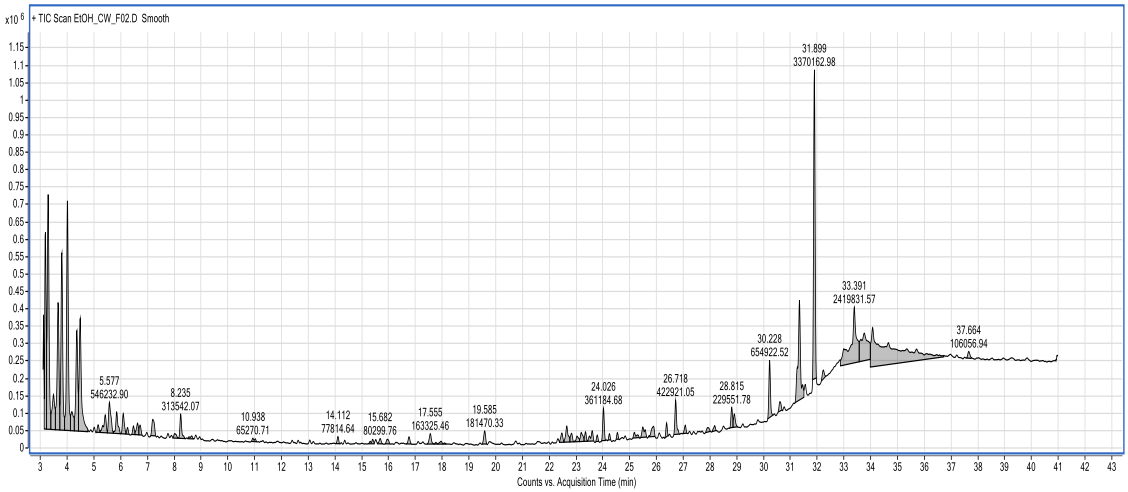
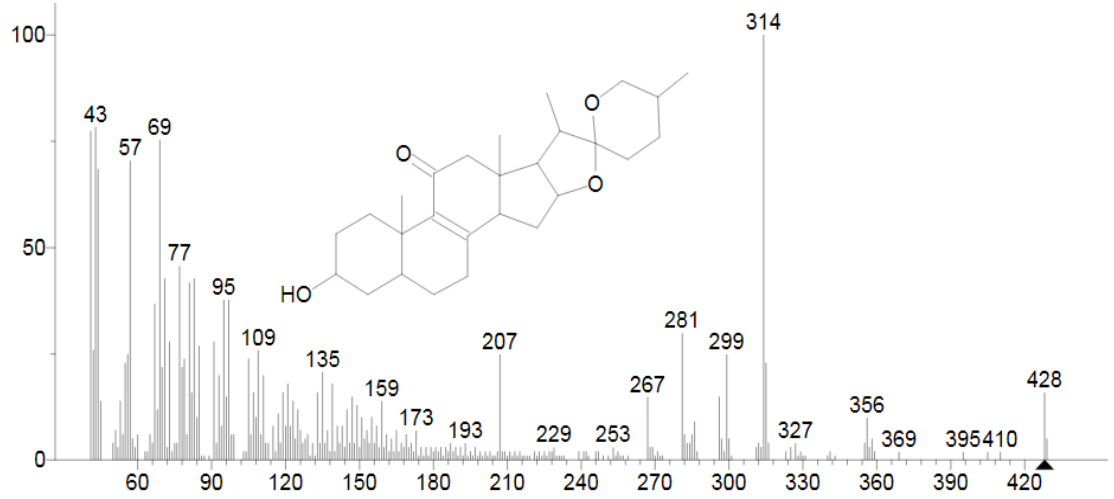


Figure 80 - GC chromatogram of Fraction 2 from the ethanol crude of Crabwood's bark.

Hit 1 : Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-
C27H40O4; MF: 625; RMF: 640; Prob 28.8%; CAS: 58072-54-1; Lib: mainlib; ID: 227869.



Name: Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-
Formula: $C_{27}H_{40}O_4$
MW: 428 Exact Mass: 428.29266 CAS#: 58072-54-1 NIST#: 48960 ID#: 227869 DB: mainlib
Other DBs: None
Contributor: CARL DJERASSI DEPT OF CHEM STANFORD UNIV STANFORD CALIF 94305
InChIKey: QDNYHEIMCUBLOI-UHFFFAOYSA-N Non-stereo
10 largest peaks:

314 999	43 782	41 772	69 752	57 703	44 683	77 455	71 425	83 425	81 415

241 m/z Values and Intensities:

41 772	42 257	43 782	44 683	45 138	50 39	51 69	52 29	53 138	54 59
55 227	56 247	57 703	58 49	59 29	60 59	63 19	64 19	65 59	66 39
67 366	68 118	69 752	70 217	71 425	72 29	73 277	74 19	75 39	76 39
77 455	78 217	79 237	80 59	81 415	82 158	83 425	84 99	85 267	86 9
87 9	89 9	91 277	92 39	93 198	94 79	95 376	96 148	97 376	98 59
99 59	103 19	104 19	105 237	106 59	107 158	108 99	109 257	110 59	111 198
112 39	113 39	115 79	116 19	117 108	118 39	119 158	120 79	121 178	122 79
123 138	124 49	125 118	126 69	127 39	128 49	129 59	130 9	131 39	132 9
133 158	134 39	135 207	136 39	137 69	138 19	139 178	140 19	141 79	142 39
143 79	144 29	145 118	146 39	147 148	148 39	149 128	150 29	151 99	152 49
153 69	154 39	155 99	156 39	157 79	158 29	159 138	160 29	161 59	162 19
163 49	164 19	165 69	166 19	167 39	168 29	169 59	170 29	171 39	172 19
173 69	174 9	175 29	176 9	177 29	178 9	179 29	180 19	181 29	182 19
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255 19	256 9	257 9	259 9	267 148	268 29	269 29	270 9	271 19	272 9
273 9	281 297	282 59	283 39	284 39	285 59	286 89	287 19	296 148	297 49
298 19	299 247	300 49	301 9	311 29	312 39	313 29	314 999	315 227	316 39
323 19	325 29	327 39	328 9	329 19	330 9	331 9	340 9	341 19	343 9
355 39	356 99	357 29	358 49	359 19	369 19	395 19	405 19	410 19	428 158
429 49									

Figure 81 - NIST hit and Mass spectrum of Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-

Compound Target: γ-Sitostenone.

Retention time: 33.324 – 33.559 minutes in Fraction 4 of Crabwood's bark ethanol crude.

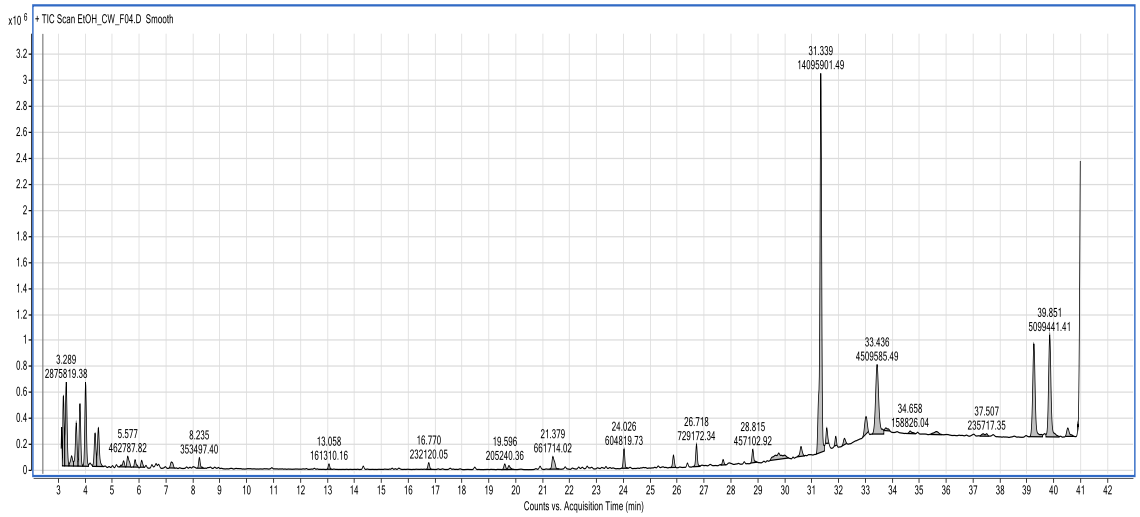
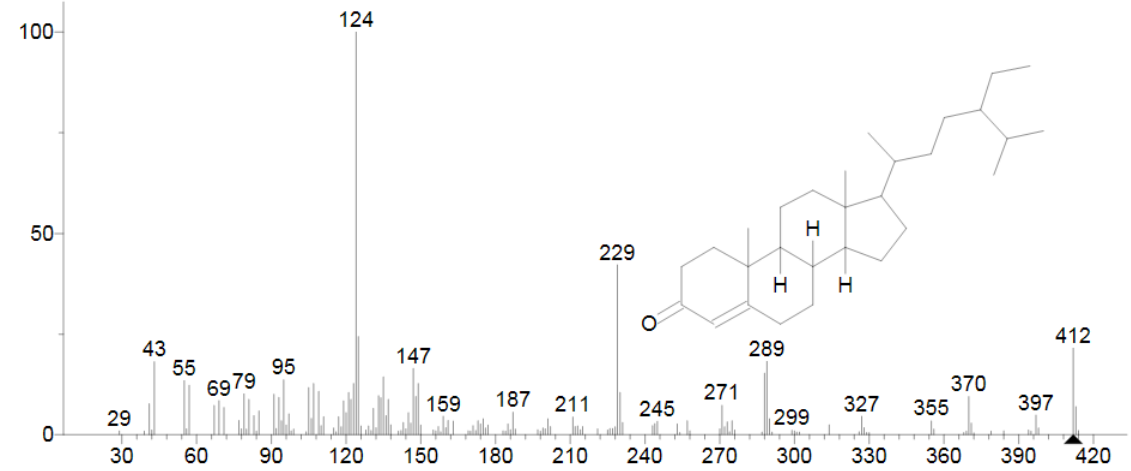


Figure 82 - GC chromatogram of Fraction 4 from the ethanol crude of Crabwood's bark.

Hit 1 : γ-Sitostenone
C29H48O; MF: 625; RMF: 850; Prob 29.8%; CAS: 84924-96-9; Lib: mainlib; ID: 108791.



Name: γ-Sitostenone
Formula: $C_{29}H_{48}O$
MW: 412 Exact Mass: 412.370516 CAS#: 84924-96-9 NIST#: 414394 ID#: 108791 DB: mainlib
Other DBs: None
Contributor: NIST Mass Spectrometry Data Center
InChIKey: RUVUHIUYGJBLGI-UHFFFAOYSA-N Non-stereo
10 largest peaks:

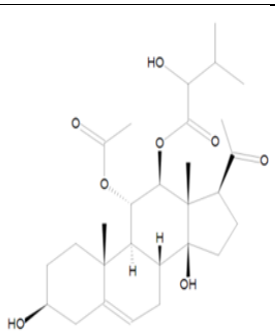
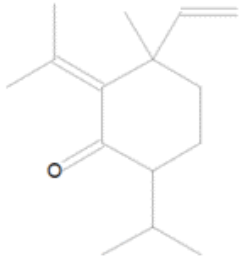
124 999	229 422	125 243	412 215	289 182	43 181	147 165	288 152	135 143	95 136

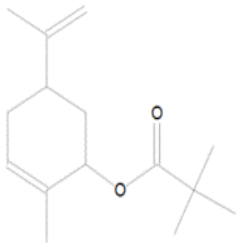
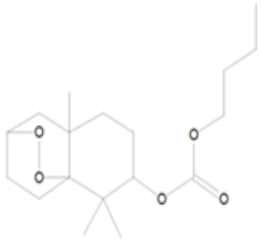
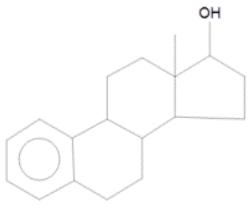
155 m/z Values and Intensities:

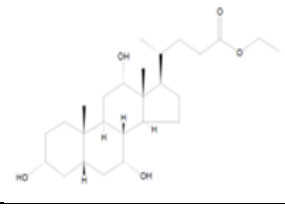
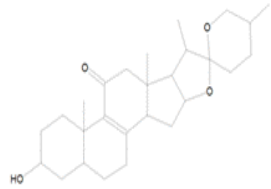
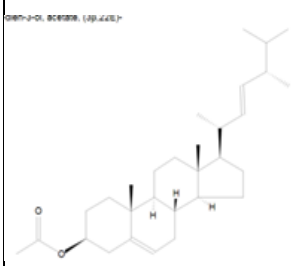
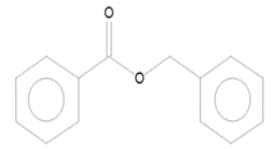
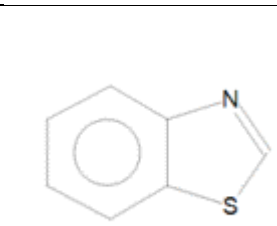
29	10	39	8	41	76	42	11	43	181	55	134	56	14	57	122	67	72	69	85
71	67	77	35	78	14	79	101	80	13	81	87	83	47	84	8	85	58	91	100
92	15	93	92	94	34	95	136	96	23	97	51	98	9	99	13	104	7	105	116
106	40	107	126	109	107	110	22	111	44	115	16	116	7	117	44	118	19	119	83
120	54	121	104	122	87	123	126	124	999	125	243	126	21	128	11	129	21	130	10
131	65	132	17	133	96	134	91	135	143	136	47	137	87	138	24	141	8	142	10
143	30	144	14	145	54	146	28	147	165	148	94	149	126	150	23	155	11	156	9
157	20	158	7	159	46	160	17	161	35	163	33	169	9	170	8	171	22	172	10
173	34	174	26	175	39	176	16	177	23	183	9	184	8	185	26	186	12	187	56
188	13	197	12	198	8	199	16	200	14	201	39	202	20	211	44	212	20	213	21
214	12	215	20	221	14	225	11	226	15	227	15	228	19	229	422	230	104	231	30
243	22	244	27	245	34	253	26	254	5	257	34	258	9	270	14	271	73	272	19
273	31	274	7	275	34	276	11	287	6	288	152	289	182	290	39	291	6	299	11
300	9	301	6	302	6	314	24	326	7	327	46	328	17	329	5	330	5	355	35
356	13	368	5	369	8	370	95	371	28	372	4	379	8	384	9	394	11	395	8
397	49	398	16	412	215	413	69	414	10										

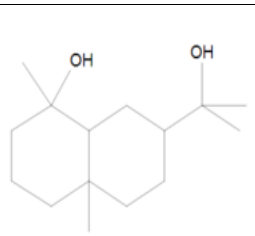
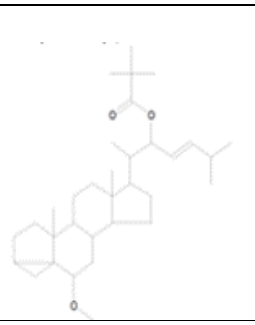
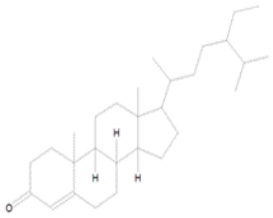
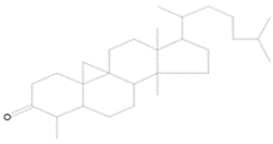
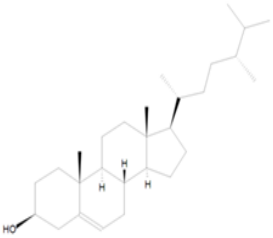
Figure 83 - NIST hit and Mass spectrum of γ-Sitostenone.

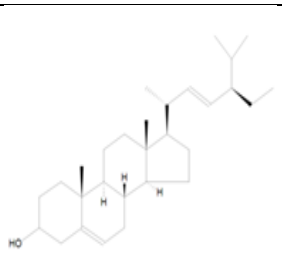
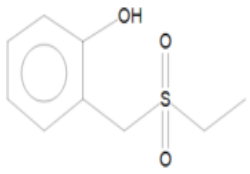
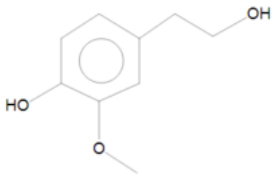
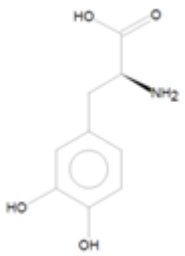
GC-MS NIST Hits for Crabwood fractions:

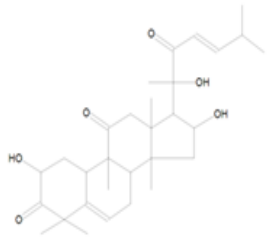
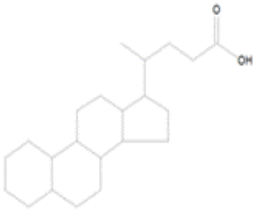
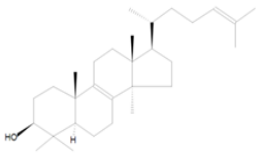
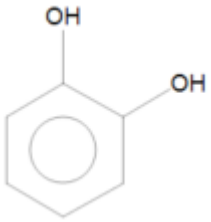
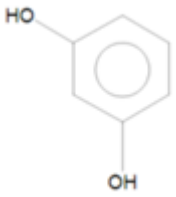
Fraction	Compound	Molecular Structure	Compound nature	Activity
1	Pregn-5-en-20-one, 11-(acetyloxy)-3,14-dihydroxy-12-(2-hydroxy-3-methyl-1-oxobutoxy)-, (3β,11α,12β,14β)- Drebyssogenin		Steroidal	Anti inflammatory
2	Isoshyobunone		Sesquiterpenoids – 3 isoprene units	Insecticide and repellent properties
2	Falcarinol		Fatty Acid	Natural pesticide – protects against fungal diseases
2	Isopropyl myristate		Fatty acid ester	Skin moisturizer

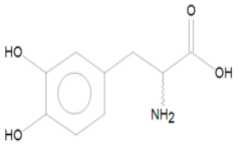
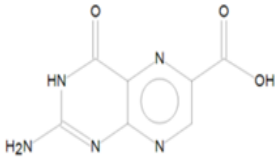
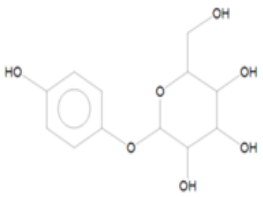
2	Limonen-6-ol, pivalate		Monoterpene hydrocarbon	Antioxidant and anti-inflammatory
2	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin		Epoxide	Diverse functional groups
2	Phthalic acid, isobutyl octadecyl ester		Dicarboxylic acid	Antimicrobial, Antifouling
2	Estra-1,3,5(10)-trien-17β-ol		Steroid	Anti-inflammatory
2	Ethyl iso-allocholate		Steroid	Antibacterial, anti-inflammatory

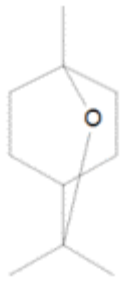
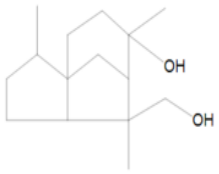
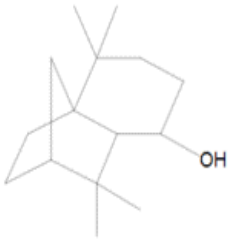
				
2	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-		Steroid	Antibacterial, anti-inflammatory
2	Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-		Steroid	Antibacterial, anti-inflammatory
3	Benzyl Benzoate		Benzoic acid esters	Antibacterial, anti-inflammatory, insect repellent
4	Benzothiazole		Sulfenamids	antimicrobial, anticancer, anthelmintic, and anti-diabetic activities
4	Cryptomeridiol		Sesquiterpenoids	Expectorant, septic prevention

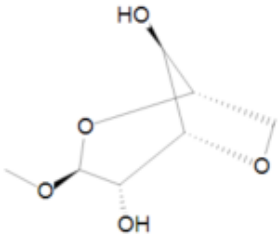
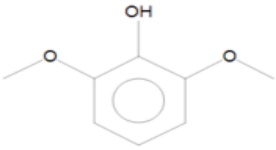
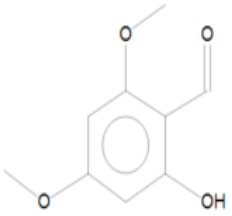
				
4	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate		Steroid	Antimicrobial, anti-inflammatory, antiarthritic, antiuretic, antiasthmatic
4	γ-Sitostenone		Steroid	Antibacterial, antifungal
4	9,19-Cyclocholestan-3-one, 4,14-dimethyl-		Triterpenoids	Antioxidant and anti-inflammatory
4	Ergost-5-en-3-ol, (3β)-		Campesterol	Liver disease, jaundice, arthrosclerosis Lowers LDL cholesterol levels
4	Stigmasterol		Steroid	Synthetic progesterone

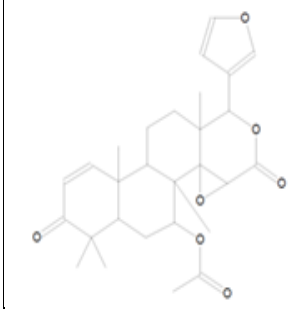
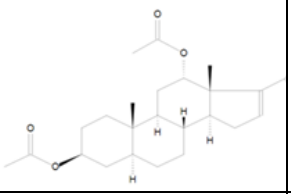
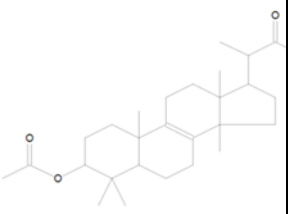
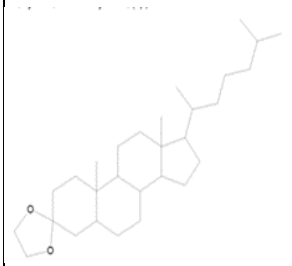
				
5	Ethyl 2-hydroxybenzyl sulfone		Phenolic - Sulfone	Antibacterial
5	Homovanillyl alcohol		Phenolic	protects red blood cells (RBCs) from oxidative injury and has protective effect on cardiovascular disease.
5	Levodopa		Amino acid	treat the symptoms of Parkinson's disease and Parkinson's-like symptoms

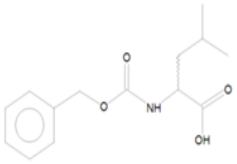
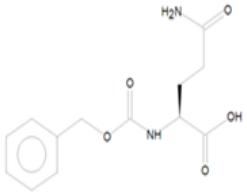
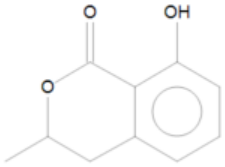
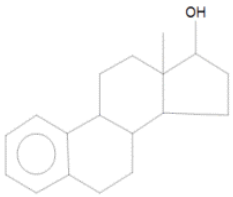
5	Cucurbitacin b, 25-desacetoxy-		Triterpenoids	Antioxidant and anti-inflammatory
5	Bisnorallocholanic acid		Steroid	Antifungal
5	Lanosterol		tetracyclic triterpenoid	Precursor for steroids
6	Catechol		Phenolic	Various skin issues
6	Resorcinol		Phenolic	treat acne, seborrheic dermatitis, eczema, psoriasis, and other skin disorders

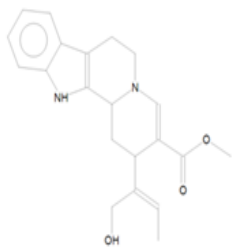
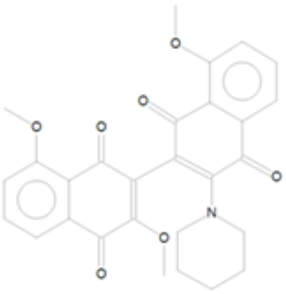
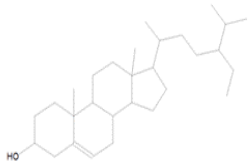
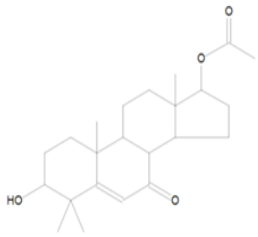
6	Hydroquinone		Phenolic	lighten the dark patches of skin (also called hyperpigmentation, melasma
6	Dihydroxyphenylalanine		Amino acid	Blood pressure
6	Pterin-6-carboxylic acid		Alkaloids	Anaesthetics, cardioprotective, and anti-inflammatory agents
6	2-Myristynoyl-glycinamide		Amino acid	Antifungal
7	Arbutin		Phenolic glycosides	Skin

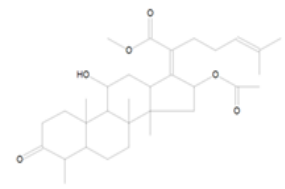
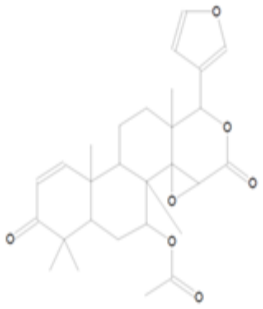
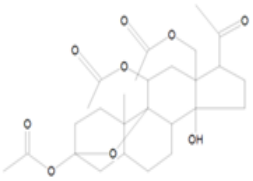
7	Eucalyptol		Monoterpenoid	Cough suppressant
7	Cedran-diol		Sesquiterpenoids	anti cancer, immunosuppressive and anti inflammatory agents.
7	Isolongifolan-8-ol		Terpenoid	Fragrance woody
7	9-Octadecenamid		Hydrocarbon	Anti-inflammatory and antibacterial

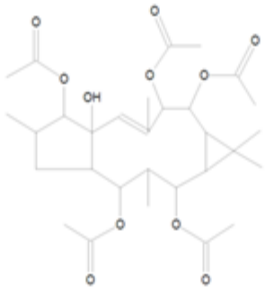
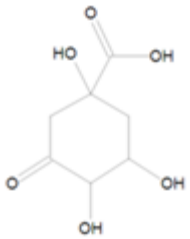
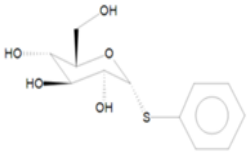
8	α-D-Mannopyranoside, methyl 3,6-anhydro-		Carbohydrate	Cell growth and energy
8	Phenol, 2,6-dimethoxy-		Phenol	Antioxidant, anticancer, antiinflammatory and sex hormoneactivity
8	4,6-Dimethoxysalicylaldehyde		Aldehyde	Antifungal

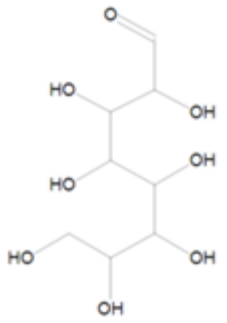
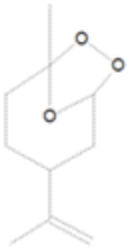
9	D-Homo-24-nor-17-oxachola-1,20,22-triene-3,16-dione, 7-(acetyloxy)-14,15:21,23-diepoxy-4,4,8-trimethyl-, (5α,7α,13α,14β,15β,17aα)-**Gedunin**		Tetranortriterpenoid	naturally occurring Hsp90 inhibitor antiproliferative activity antimalarial activity
9	5α-Pregn-16-en-20-one, 3β,12α-dihydroxy-, diacetate		Steroid	corticosteroid hormone
10	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-		Steroid	Protein tyrosine phosphatase 1B (PTP 1B) inhibitor
10	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5β)-		Steroid	Antioxidant and anti-inflammatory

11	dl-Leucine, N-[(phenylmethoxy)carbonyl]-		Amino Acid	Synthesis of Proteins
11	L-Glutamine, N2-[(phenylmethoxy)carbonyl]-		Amino Acid	Synthesis of Proteins
11	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-3-methyl-		Isocoumarin	Anti-cancerous
11	Estra-1,3,5(10)-trien-17β-ol		Steroid	Antibacterial, anti-inflammatory

11	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15β,16E)-		Alkaloids	Male fertility
11	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone		Steroid	Antioxidant and anti-inflammatory
12	β-Sitosterol		Steroid	Lowering cholesterol levels and improving symptoms of an enlarged prostate (BPH), hair growth
12	Acetic acid, 3-hydroxy-4,4,10,13-tetramethyl-7-oxo-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-ol		Steroid	Anti-inflammatory

12	2-(16-Acetoxy-11-hydroxy-4,8,10,14-tetramethyl-3-oxohexadecahydrocyclopenta[a]phenanthren-17-ylidene)-6-methyl-hept-5-enoic acid, methyl ester		Steroid	Anti-inflammatory
13	D-Homo-24-nor-17-oxachola-1,20,22-triene-3,16-dione, 7-(acetyloxy)-14,15:21,23-diepoxy-4,4,8-trimethyl-, (5α,7α,13α,14β,15β,17aα)-		Steroid	Anti-inflammatory
13	3,9-Epoxypregnan-14-ol-20-one, 3,11,18-triacetoxy-		Steroid	Anticancer

13	7aH-Cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecahydro-1,1,3,6,9-pentamethyl-, 2,4,7,10,11-pentaacetate			
17	Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid		Carboxylic acid	Anti-microbial
19	Phenyl-1-thio-α-d-glucopyranoside		Thio sugar	Antibacterial

21	l-Gala-l-ido-octose		Saccharides	Skin enhancement
22	R-Limonene		Monoterpenoids	Reduce cholesterol, gastric neutralizing effect, heart burn relief, chemo preventive activity against cancers.

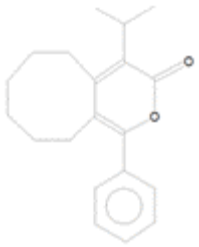
22	3H-Cycloocta[c]pyran-3-one, 5,6,7,8,9,10-hexahydro-4-isopropyl-1-phenyl-		Pyrone	Antimicrobial activity, retroviral

Table 16 - Table of the GC-MS NIST Hits for Crabwood fractions, their retention time, percentage area, compound name, chemical formula, molecular structure, exact mass, classification, and medicinal use.

Long Hairy crabgrass NIST hits discussion:

Only one literature reference was found on the chemical composition of long hairy crabgrass; investigated in Cairo Egypt. (Ibrahim, El-Hela, Dawoud, & Zhran, 2019) attained pure fractions and subjected them to ultraviolet, infrared, ^{1}H and ^{13}C nuclear magnetic resonance and mass spectrometry analyses. They found seven compounds. Two of the steroidal compounds found in Ibrahim's study were also found in this research; stigmasterol and β sitosterol. This showed some correlation with the composition of long hairy crabgrass even though samples were from two different geographical location. The classes of compounds found in this research were fatty acids, terpenes, steroidal, phenolics and sugars, which corresponded to the phytochemical assays. A more detailed list of the medicinal compounds can be found in Table 17. Medicinal uses of the selected compounds range from anti-inflammatory, insect repellent, skin moisturizer, antioxidant, antibacterial, anticancer, antidiabetic, anthelmintic, expectorant, antifungal, cholesterol lowering, treatment for acne, etc. Anti – bacterial properties found in the antimicrobial assays must have been related to one or some combination of these compounds: 17-Norkaur-15-ene, 13-methyl-, (8β,13β)- (Hibaene), Benzothiazole, geranyl-α-terpinene, Cucurbitacin b, 25-desacetoxy, Phthalic acid, butyl tetradecyl ester, Ethyl iso-allocholate, Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-, 2-Pentadecanone, 6,10,14-trimethyl-, Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, Cholestan-3-one, cyclic 1,2-ethanediyl aetal, Neophytadiene, Glycidyl palmitate, 1-Dodecanol, 1H-[1,2,4]Triazole-3-carboxylic acid [2-(4-tert-butyl-phenoxy)-ethyl]-amide, Isoquinoline, decahydro-, 1-Heptatriacotanol, Estra-1,3,5(10)-trien-17β-ol, 6-Methoxy-2-benzoxazolinone, 2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.1.0(1,6)]decan-5-ol, Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-, trans-Z-α-Bisabolene epoxide, Imidazole, 2-amino-5-[(2-carboxy)vinyl]-, 6-epi-shyobunol, Thunbergol, 2-Furanmethanol. The characteristics of these compounds can be found in Table 17.

Gas chromatogram and Mass spectrum of selected hits in Long Hairy Crabgrass:

Compound Target: Benzothiazole.

Retention time: 13.013 – 13.103 minutes in Fraction 1 of Long Hairy Crabgrass ethanol crude.

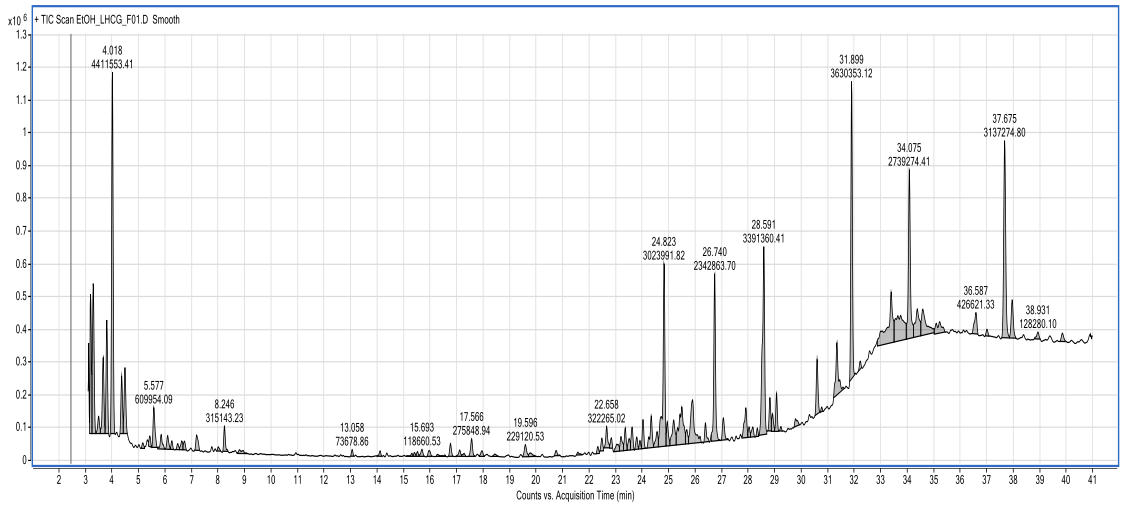
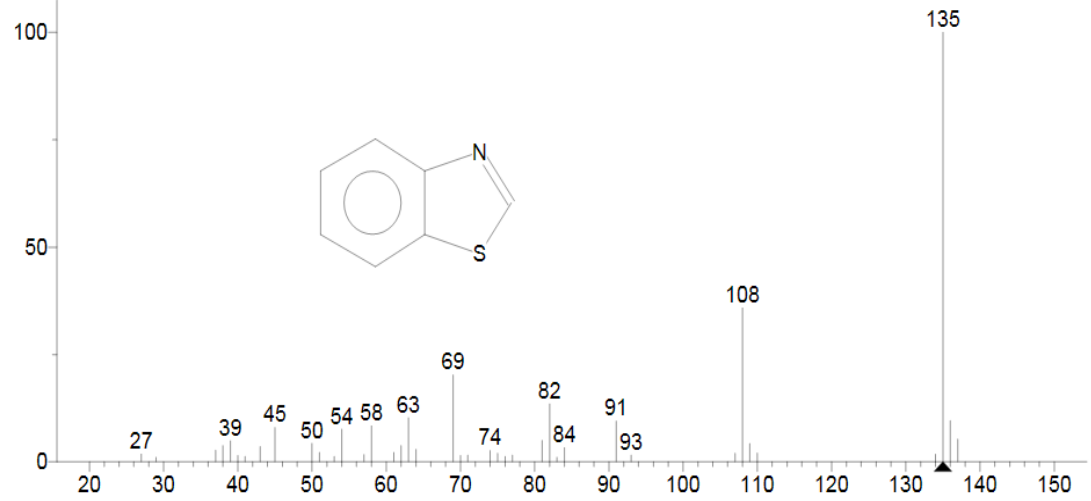


Figure 84 - GC chromatogram of Fraction 4 from the ethanol crude of Long hairy crabgrass.

Hit 1 : Benzothiazole
C7H5NS; MF: 797; RMF: 882; Prob 66.8%; CAS: 95-16-9; Lib: replib; ID: 21472.



Name: Benzothiazole
Formula: C_7H_5NS
MW: 135 Exact Mass: 135.01427 CAS#: 95-16-9 NIST#: 73061 ID#: 21472 DB: replib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS, IRDB
Contributor: V.A. KOPTYUG, VOL.4, ATLAS OF MASS SPECTRA OF ORGANIC COMPOUNDS
InChIKey: IOJUPLGTWVMSFF-UHFFFAOYSA-N Non-stereo
10 largest peaks:

135 999	108 358	69 202	82 135	63 103	91 95	136 95	58 83	45 80	54 76

40 m/z Values and Intensities:

27	18	29	10	37	26	38	37	39	49	40	14	41	12	43	35	45	80	50	43
51	21	53	12	54	76	57	16	58	83	61	21	62	37	63	103	64	28	69	202
70	14	71	15	74	26	75	19	76	12	77	15	81	49	82	135	83	10	84	33
91	95	93	16	107	19	108	358	109	42	110	20	134	17	135	999	136	95	137	52

Figure 85 - NIST hit and Mass spectrum of Benzothiazole.

Compound Target: Thunbergol.

Retention time: 25.047 – 25.260 minutes in Fraction 19 of Long hairy crabgrass ethanol crude.

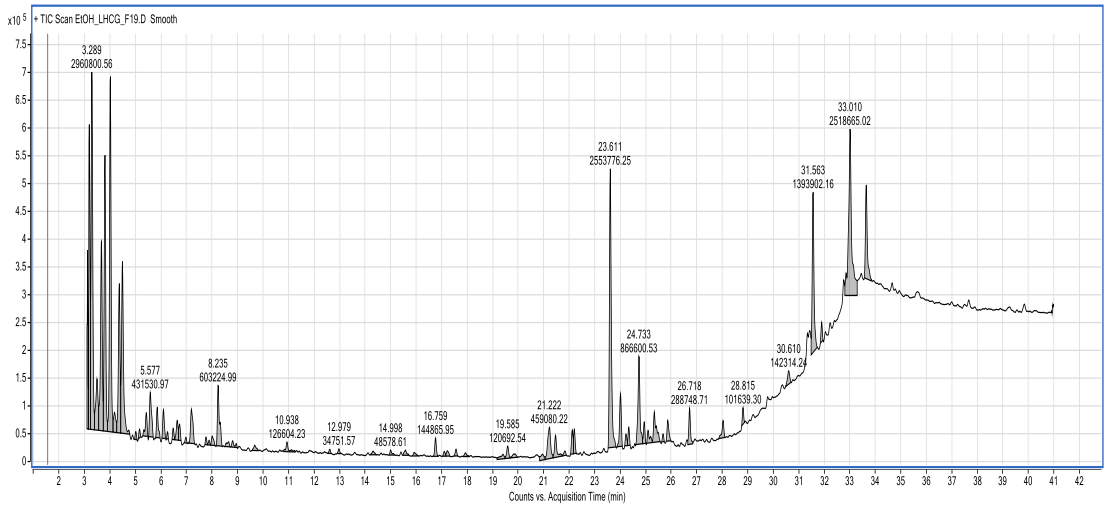
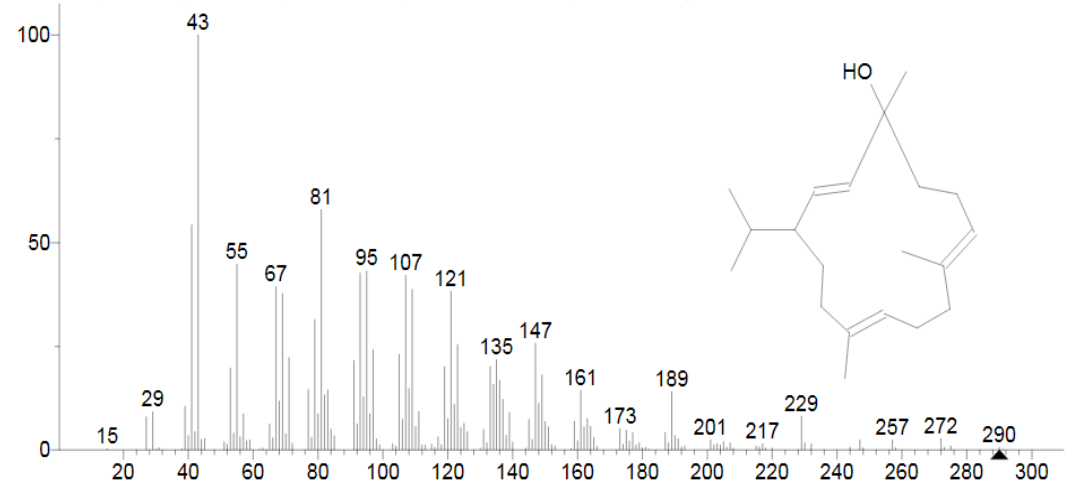


Figure 86 - - GC chromatogram of Fraction 19 from the ethanol crude of Long hairy crabgrass.

Hit 1 : Thunbergol
C20H34O; MF: 708; RMF: 747; Prob 18.4%; CAS: 25269-17-4; Lib: mainlib; ID: 9470.



Name: Thunbergol
Formula: $C_{20}H_{34}O$
MW: 290 Exact Mass: 290.260965 CAS#: 25269-17-4 NIST#: 384002 ID#: 9470 DB: mainlib
Other DBs: None
Contributor: NIST Mass Spectrometry Data Center, 2010
InChIKey: YAPXSYXFLHDPCK-MAUDFNNOSA-N Non-stereo
10 largest peaks:

43 999	81 578	41 541	55 448	95 431	93 425	107 420	67 393	109 386	121 382

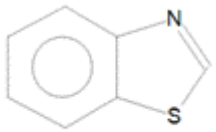
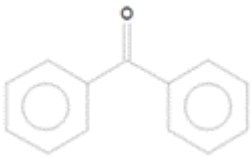
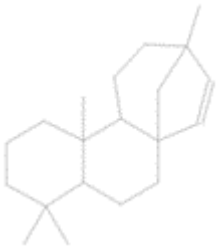
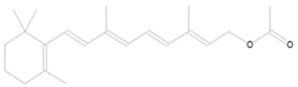
146 m/z Values and Intensities:

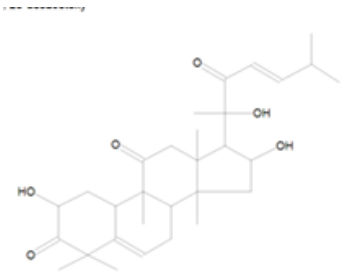
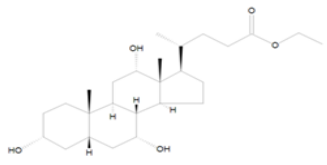
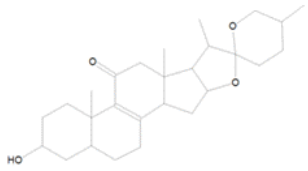
15	3	27	79	29	92	30	2	31	5	38	3	39	105	40	34	41	541	42	43
43	999	44	25	45	27	51	19	52	13	53	196	54	40	55	448	56	31	57	86
58	22	59	24	62	1	63	4	65	62	66	28	67	393	68	117	69	376	70	38
71	222	72	16	77	145	78	30	79	313	80	86	81	578	82	132	83	144	84	50
85	33	91	215	92	62	93	425	94	127	95	431	96	86	97	241	98	26	99	12
103	15	104	9	105	229	106	73	107	420	108	147	109	386	110	56	111	92	112	12
113	11	115	13	116	6	117	31	118	12	119	200	120	75	121	382	122	109	123	253

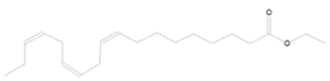
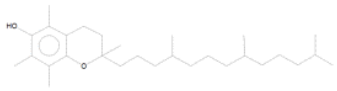
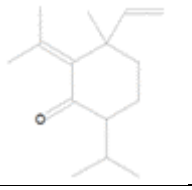
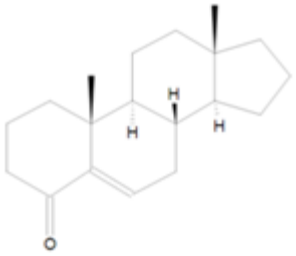
124	53	125	63	126	43	130	4	131	49	132	17	133	200	134	157	135	217	136	167
137	121	138	35	139	90	140	18	144	4	145	73	146	25	147	257	148	112	149	180
150	68	151	55	152	12	153	10	158	3	159	68	160	22	161	144	162	55	163	75
164	56	165	30	166	8	173	52	174	13	175	47	176	21	177	41	178	11	179	17
180	5	181	6	182	1	187	42	188	17	189	141	190	34	191	26	192	7	193	9
201	25	202	12	203	15	204	11	205	19	206	6	207	16	208	4	215	9	216	7
217	15	218	4	220	4	229	81	230	17	232	14	244	6	247	23	248	5	257	24
258	5	272	28	273	6	275	9	290	7	291	1								

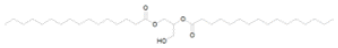
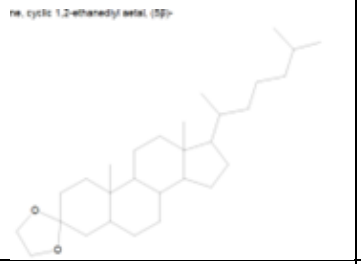
Figure 87 - NIST hit and Mass spectrum of Thunbergol.

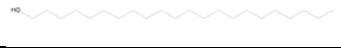
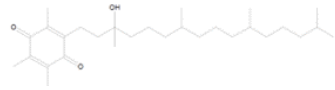
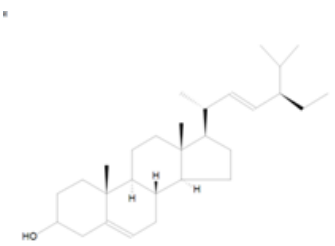
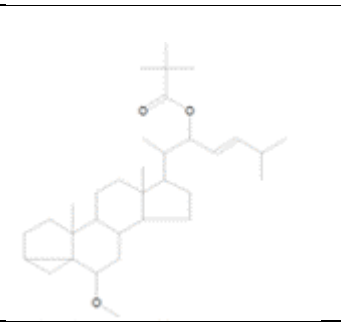
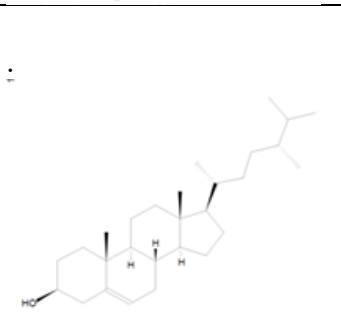
GC-MS NIST Hits for Long Hairy Crabgrass fractions:

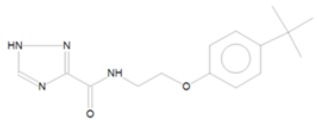
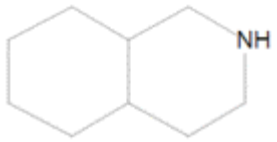
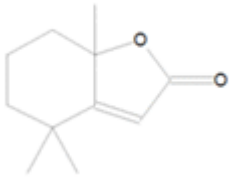
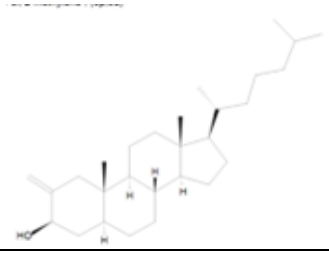
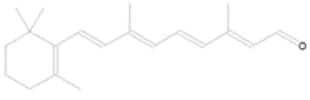
Fraction	Compound	Molecular Structure	Compound nature	Activity
1	Benzothiazole		Sulfenamids	antimicrobial, anticancer, anthelmintic, and anti-diabetic activities
1	Naphthalene, 1,7-dimethyl-		Benzenoid polycyclic aromatic hydrocarbon	Anti-inflammatory
1	Benzophenone		Benzophenones	inhibitory activity against Staphylococcus aureu
1	17-Norkaur-15-ene, 13-methyl-, (8β,13β)-Hibaene		Diterpenoids	Antibacterial activity, anti-cancerous
1	Retinol, acetate		retinoids	metabolic functioning of the retina, the growth of and differentiation of epithelial tissue, the growth of bone,

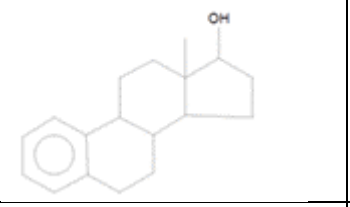
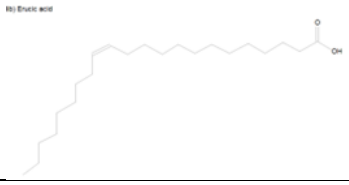
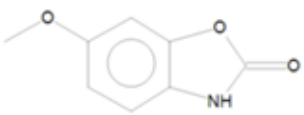
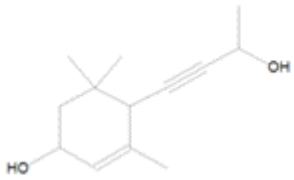
				reproduction, and the immune response.
1	geranyl-α-terpinene		Terpinene	Antibacterial
1	Cucurbitacin b, 25-desacetoxy		Terpene	Cytotoxic, anti-tumor properties, hepatoprotective, anti-inflammation, antimicrobial, anthelmintic, cardiovascular and anti-diabetic effects
1	Phthalic acid, butyl tetradecyl ester		lipophilic chemicals	allelopathic, antimicrobial, insecticidal,
1	Ethyl iso-allocholate		Steroid	Antibacterial, anti-inflammatory
1	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-		Steroid	Antibacterial, anti-inflammatory

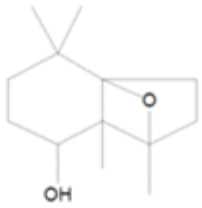
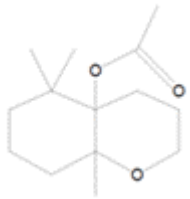
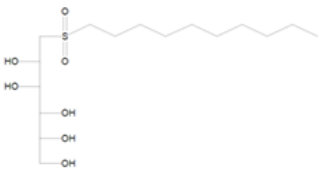
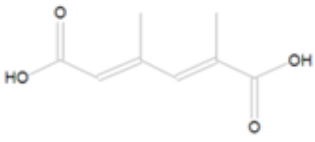
1	Ethyl 9,12,15-octadecatrienoate Linolenic acid		Esters	Prevents hypertension, anti-inflammatory, antitumor
1	Vitamin E		Tocopherols	Vision, reproduction, antioxidant
2	Isoshyobunone		Sesquiterpenoids	Repellent, stomach issues, skin issues
2	2-Pentadecanone, 6,10,14-trimethyl-		Ketone	Antioxidant, Antibacterial and Antischistosomal
2	Androst-5-en-4-one		Steroid	Boost testosterone levels
2	Phytol		isoprenoid alcohol	Antinociceptive and antioxidant activities, anti-inflammatory and antiallergic effects

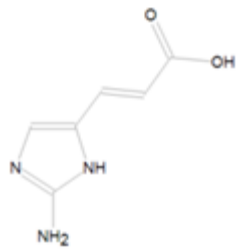
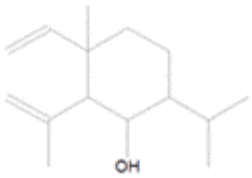
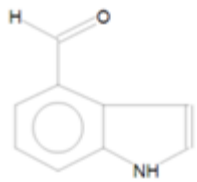
2	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-		Ester	Anti-inflammatory
2	Oleic acid, 3-(octadecyloxy)propyl ester		Fatty acid	Skin aid
2	Squalene		Alkene	Reduce cholesterol, skin aid
3	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester		Ester	antioxidant, antimicrobial, and anti-inflammatory activities
3	Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5β)-		Steroid	antioxidant, antibacterial activities
4	1-Nonadecene		Hydrocarbon alkene	antituberculosis activity as well as antifungal activity
4	Neophytadiene		Hydrocarbon alkene	Antibacterial
4	2-Pentadecanone, 6,10,14-trimethyl-		Ketone	Anti-inflammatory
4	Glycidyl palmitate		Ether	antioxidant, antimicrobial and antitumor agent

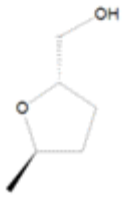
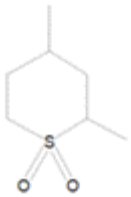
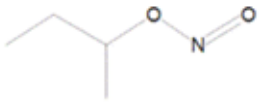
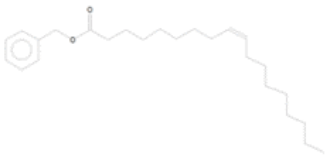
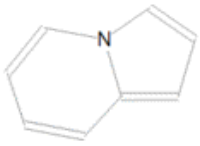
5	1-Dodecanol		Long chain alcohol	antibacterial activity
5	Cetene		Hydrocarbon Alkene	Antifungal
5	Behenic alcohol		Long chain alcohol	antiviral agent
5	α-Tocopherolquinone		Tocopherols	Reduced form of vitamin E – anticlotting agent
5	Stigmasterol		Steroid	Inhibit the development of various cancerous
6	Cholest-22-ene-21-ol		Steroid	Lower cholesterol, prevent heart diseases
6	Campesterol		Steroid	Controlling cholesterol and lowering the risk of heart disease

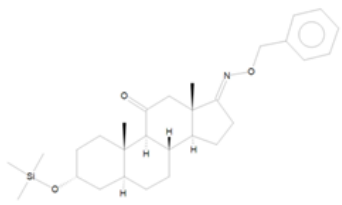
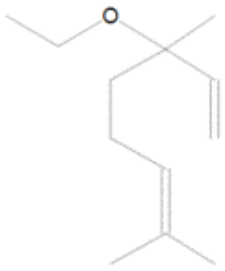
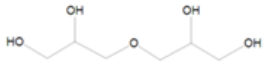
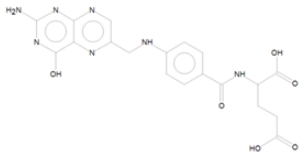
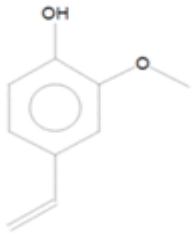
7	1H-[1,2,4]Triazole-3-carboxylic acid [2-(4-tert-butyl-phenoxy)-ethyl]-amide		Amide	Antimicrobial (new)
7	Isoquinoline, decahydro-		Alkaloids	Anti-inflammatory, antimicrobial, and analgesic effects
7	2(4H)-Benzofuranone		Benzofurans	Anti-cancerous
7	1-Heptatriacotanol		Long chain alcohol	Antimicrobial
8	Cholestan-3-ol, 2-methylene-, (3β,5α)-		Steroid	Cholesterol lowering, testosterone increasing
9	Retinal		Vitamin A Retinaldehyde	Maintain cornea

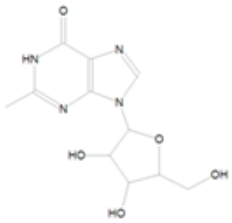
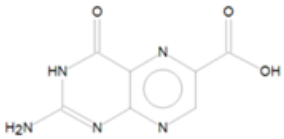
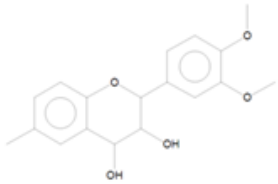
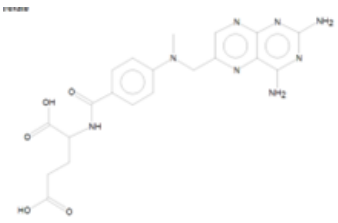
11	Oleic Acid		Fatty acid	Anti cancer, autoimmune and inflammatory diseases
11	Estra-1,3,5(10)-trien-17β-ol		Steroid	Antibacterial, anti-inflammatory
11	Erucic acid		Carboxylic acid	Reduce the risk of heart disease
12	6-Methoxy-2-benzoxazolinone		Benzoxazolones	Antimicrobial and anti-tumor
16	3-Hydroxy-7,8-dihydro-β-ionol		Alcohol	Antioxidant

17	2,2,6,7-Tetramethyl-10-oxatricyclo[4.3.1.0(1,6)]decan-5-ol		Tetrahydropyran	Antimicrobial
18	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-		Tetrahydropyran	Antimicrobial
18	2-Myristynoyl-glycinamide		Amino acid – fatty acid	Antifungal
18	d-Mannitol, 1-decylsulfonyl-		Sugar	Sweetener
19	trans-Z-α-Bisabolene epoxide		Sesquiterpenes	antimicrobial, anti-inflammatory, and antioxidant properties.
19	Dimethylmuconic acid		Fatty Acid	fatty acid synthesis inhibitor

19	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-		Imidazole	antifungal, antimicrobial and antibiotic activities
19	6-epi-shyobunol		Sesquiterpenoid	Anti-bacterial
19	1H-Indole-4-carboxaldehyde		Indole	Anti-inflammatory
19	Thunbergol		Heterocyclic compound	antibacterial effects, used orally in the treatment of various enteric infections, especially bacterial dysentery

21	2-Furanmethanol, tetrahydro-5-methyl-, trans-		Oxolane	Antibacterial and antimicrobial
22	trans-2,4-Dimethylthiane, S,S-dioxide		Thio	Anti inflammatory
22	sec-Butyl nitrite		Nitrite	treatment of angina (chest pain), and has been used for the treatment of cyanide poisoning
23	9-Octadecenoic acid (Z)-, phenylmethyl ester		Ester	anti-inflammatory, antiandrogenic, and anemiagenic
24	Indolizine		Indolizine	antitumor, antimycobacterial, antagonist, and antiproliferative activities

24	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-		Steroid	Reduce cholesterol, testosterone booster
25	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-		acyclic monoterpenoids	to treat diabetes, lumbago, wounds, arthritis, anemia,
26	Diglycerol		Esters of glycerol	Reduce cholesterol
26	Folic Acid		Methoxyphenols	Preventing and treating anemia
26	2-Methoxy-4-vinylphenol		Phenol	Anti-inflammatory

26	2-Methyl-9-β-d-ribofuranosylhypoxanthine		Purine	Cholesterol-lowering
26	Pterin-6-carboxylic acid		Purine	Anesthetics, cardioprotective, and anti-inflammatory agents
26	2H-1-Benzopyran-3,4-diol, 2-(3,4-dimethoxyphenyl)-3,4-dihydro-6-methyl-, (2α,3α,4α)-			
26	Methotrexate		Glutamic Acid	rheumatoid arthritis, thickened skin in psoriasis or damage to your bowel in Crohn's disease
26	Benzenepropanoic acid, 4-hydroxy-		Carboxylic acid	Antioxidant

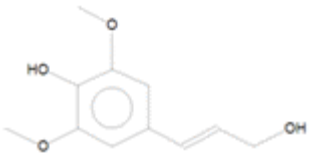
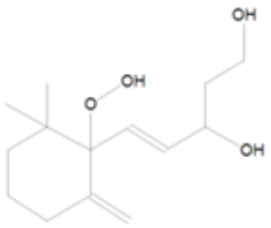
26	trans-Sinapyl alcohol		Phenolic alcohol	heart condition, asthma, arteriosclerosis, viral and bacterial infections
26	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol		Hydrocarbon	Anti inflammatory

Table 17 - GC-MS NIST Hits for Long Hairy Crabgrass fractions, their retention time, percentage area, compound name, chemical formula, molecular structure, exact mass, classification, and medicinal use.

Smooth Crabgrass NIST hit discussion:

No literature reference was found on the composition of smooth crabgrass. (Kanupriya, Sharma, & Dhiman, 2021) indicated some medicinal properties of this species of grass in their publication. These include anti-inflammatory, anti-diabetic, and anti-ulcer. Classes of compounds found in this study were terpenoids, steroids, phenolic, alkaloids and sugars. A more detailed list of the medicinal compounds can be found in Table 18. Some of the compounds found were; Linoleic acid ethyl ester, Neophytadiene, Vitamin E, Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-, Campesterol, Stigmasterol, l-(+)-Ascorbic acid 2,6-dihexadecanoate, Geranyl isovalerate, Melibiose, and d-Mannose.

Gas chromatogram and Mass spectrum of selected hits in Smooth Crabgrass.

Compound Target: Neophytadine.

Retention time: 20.605 – 20.639 minutes in Fraction 1 of Smooth Crabgrass ethanol crude.

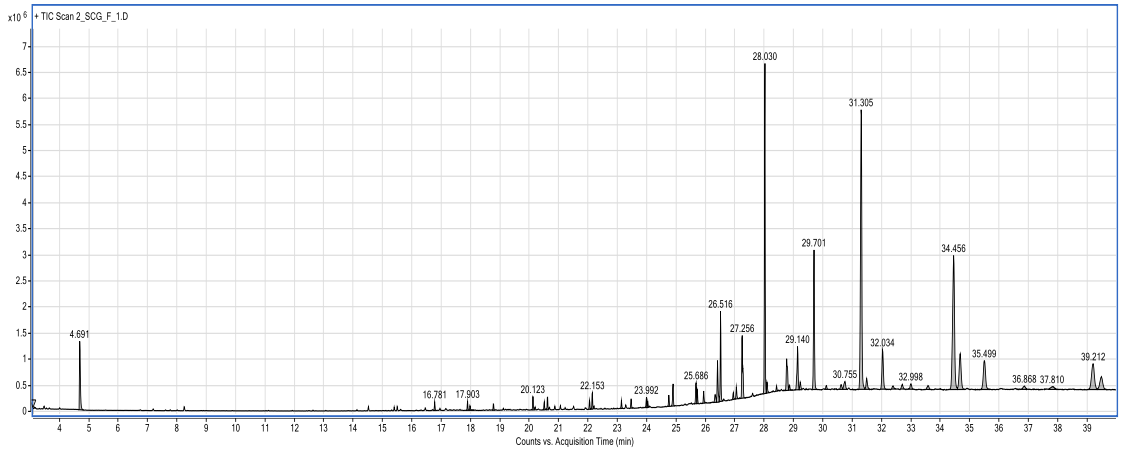
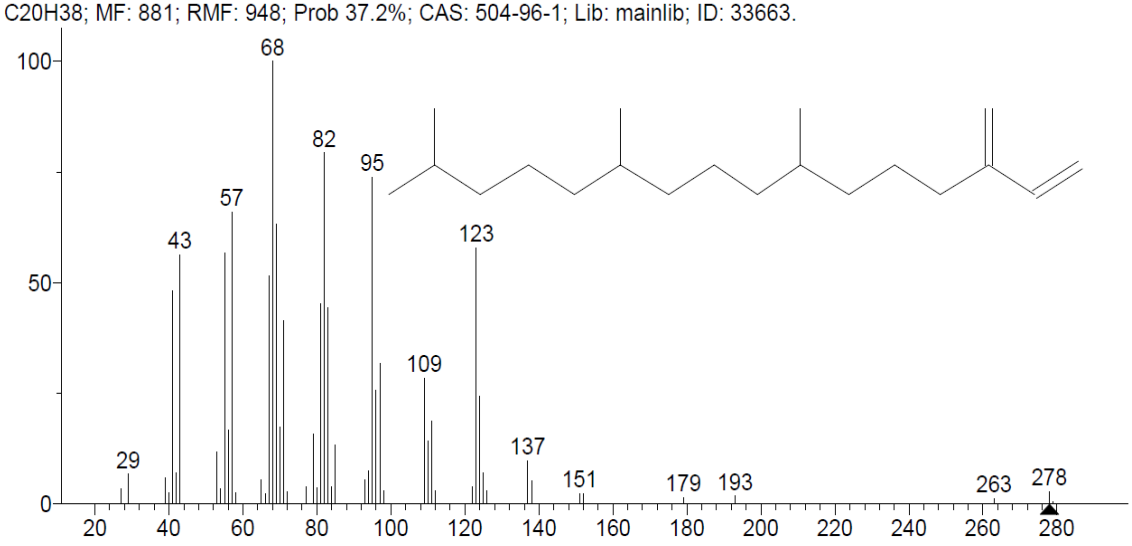


Figure 88 - GC chromatogram of Fraction 1 from the ethanol crude of Smooth crabgrass.

Hit 1 : Neophytadiene
C20H38; MF: 881; RMF: 948; Prob 37.2%; CAS: 504-96-1; Lib: mainlib; ID: 33663.



Name: Neophytadiene
Formula: $C_{20}H_{38}$
MW: 278 Exact Mass: 278.297352 CAS#: 504-96-1 NIST#: 412348 ID#: 33663 DB: mainlib
Other DBs: None
Contributor: NIST Mass Spectrometry Data Center
InChIKey: NIDGCIPAMWNKOA-UHFFFAOYSA-N Non-stereo
10 largest peaks:

68 999	82 793	95 738	57 660	69 630	123 580	55 566	43 563	67 514	41 481

53 m/z Values and Intensities:

27 33	29 70	39 60	40 25	41 481	42 71	43 563	53 118	54 33	55 566
56 166	57 660	58 26	65 55	66 23	67 514	68 999	69 630	70 173	71 413
72 28	77 38	79 157	80 36	81 450	82 793	83 441	84 38	85 133	93 55
94 74	95 738	96 257	97 317	98 30	109 286	110 142	111 186	112 30	122 40
123 580	124 243	125 69	126 30	137 99	138 52	151 26	152 24	179 17	193 20
263 15	278 30	279 5							

Figure 89 - NIST hit and Mass spectrum of Neophytadine.

Compound Target: Campesterol.

Retention time: 34.254 – 34.411 minutes in Fraction 8 of Smooth Crabgrass ethanol crude.

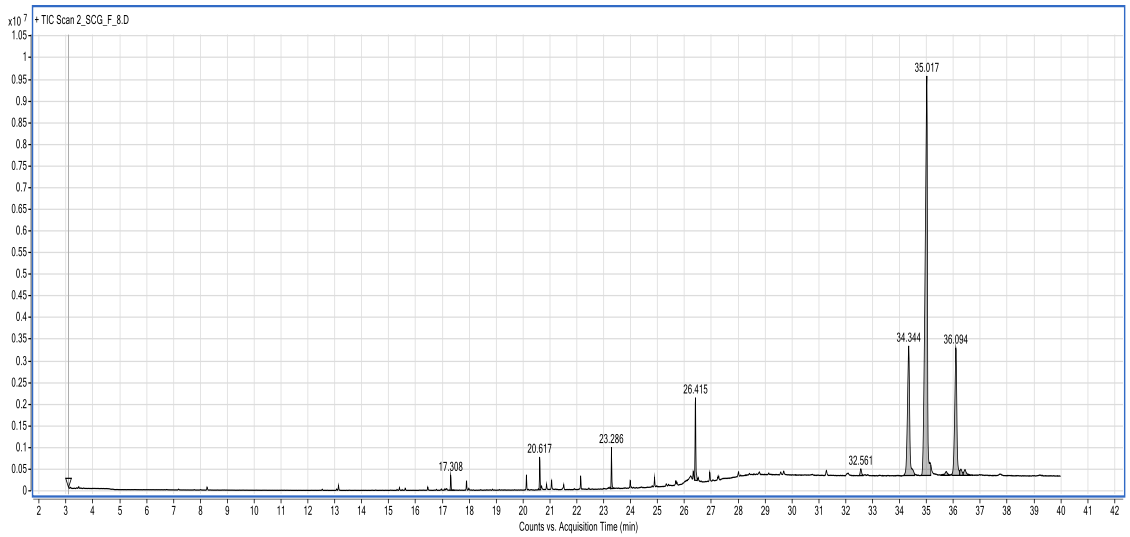
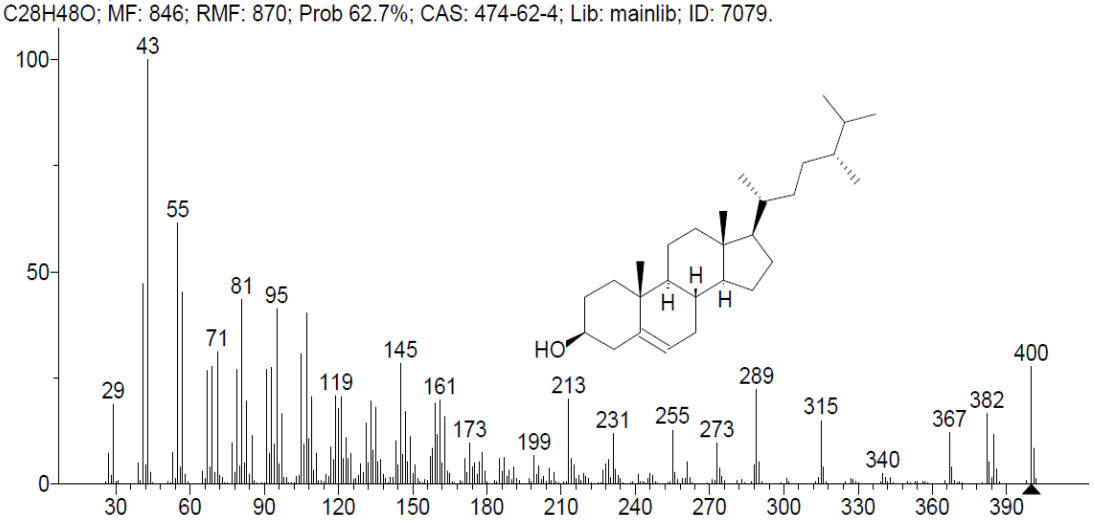


Figure 90 - GC chromatogram of Fraction 8 from the ethanol crude of Smooth crabgrass.

Hit 1 : Campesterol
C28H48O; MF: 846; RMF: 870; Prob 62.7%; CAS: 474-62-4; Lib: mainlib; ID: 7079.



Name: Campesterol
Formula: $C_{28}H_{48}O$
MW: 400 Exact Mass: 400.370516 CAS#: 474-62-4 NIST#: 151556 ID#: 7079 DB: mainlib
Other DBs: Fine, HODOC, NIH, EINECS
Contributor: Chemical Concepts
Related CAS#: 137764-28-4
InChIKey: SGNBVLSWZMBQTH-PODYLUTMSA-N Non-stereo
10 largest peaks:

43 999	55 617	41 470	57 452	81 436	95 414	107 403	71 312	105 306	145 285

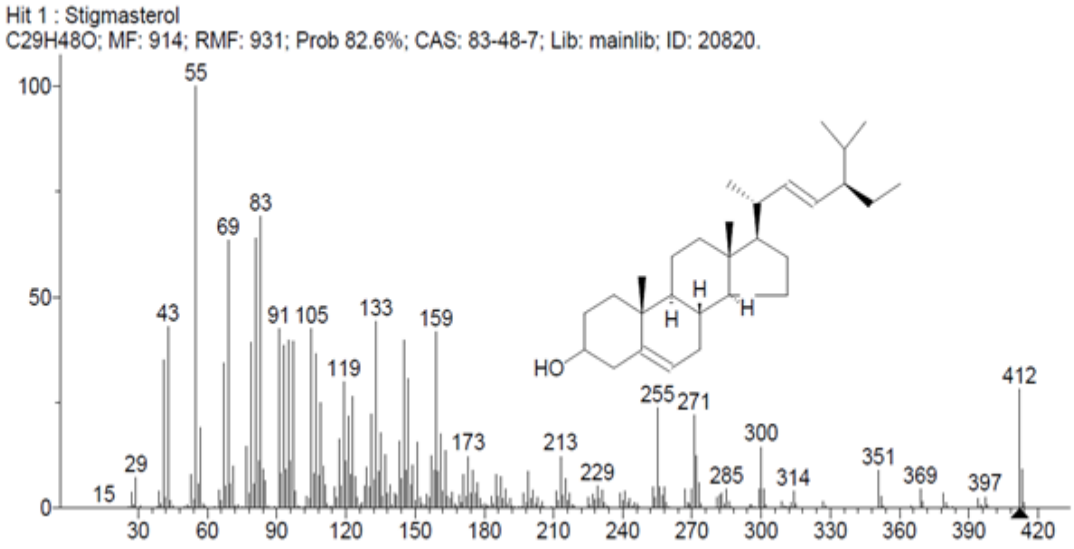
284 m/z Values and Intensities:

26	7	27	71	28	20	29	190	30	5	31	8	38	2	39	51	40	9	41	470
42	44	43	999	44	28	45	4	51	7	52	4	53	74	54	13	55	617	56	39
57	452	58	22	59	6	60	2	61	1	63	2	65	31	66	14	67	266	68	41
69	277	70	29	71	312	72	20	73	16	74	3	75	4	76	2	77	96	78	27
79	269	80	43	81	436	82	51	83	194	84	23	85	114	86	9	87	3	88	2
89	3	90	3	91	268	92	73	93	274	94	94	95	414	96	48	97	166	98	16
99	15	100	4	101	3	102	3	103	17	104	20	105	306	106	94	107	403	108	106
109	206	110	32	111	73	112	8	113	8	114	5	115	23	116	17	117	86	118	57
119	209	120	177	121	206	122	60	123	110	124	60	125	71	126	10	127	13	128	21
129	48	130	29	131	143	132	49	133	195	134	79	135	179	136	52	137	58	138	24
139	14	140	4	141	15	142	15	143	101	144	46	145	285	146	70	147	170	148	53
149	112	150	25	151	45	152	13	153	6	154	3	155	10	156	9	157	65	158	83
159	189	160	117	161	200	162	49	163	159	164	30	165	26	166	7	167	4	168	2
169	9	170	6	171	60	172	27	173	100	174	40	175	49	176	24	177	53	178	74
179	30	180	5	181	2	183	8	184	5	185	60	186	31	187	61	188	17	189	33
190	10	191	41	192	13	193	9	194	2	195	2	197	14	198	7	199	70	200	24
201	42	202	11	203	19	204	6	205	37	206	8	207	29	208	6	209	4	210	2
211	7	212	6	213	203	214	60	215	45	216	12	217	21	218	9	219	26	220	18
221	13	222	4	225	3	226	3	227	33	228	47	229	57	230	16	231	121	232	34
233	21	234	14	235	3	238	3	239	7	240	2	241	23	242	6	243	5	244	4
245	14	246	25	247	20	248	7	249	4	253	4	254	5	255	130	256	29	257	11
258	2	259	14	260	14	261	52	262	16	263	3	267	2	269	4	270	1	271	10
272	8	273	99	274	37	275	19	276	9	281	8	282	2	283	11	284	3	285	2
287	6	288	46	289	225	290	52	291	6	297	2	299	4	300	2	301	12	302	7
313	5	314	16	315	152	316	41	317	6	325	6	326	2	327	12	328	11	329	5
330	3	339	6	340	27	341	15	342	7	343	15	344	4	350	6	351	3	353	5
354	6	355	2	356	6	357	7	358	3	365	8	367	124	368	40	369	8	370	4
371	5	372	3	379	2	380	3	382	167	383	53	384	15	385	116	386	35	387	5
398	8	400	278	401	83	402	12												

Figure 91- NIST hit and Mass spectrum of Campesterol.

Compound Target: Stigmasterol.

Retention time: 34.894 – 35.051 minutes in Fraction 8 of Smooth Crabgrass ethanol crude.



Name: Stigmasterol
Formula: $C_{29}H_{48}O$
MW: 412 Exact Mass: 412.370516 CAS#: 83-48-7 NIST#: 352610 ID#: 20820 DB: mainlib
Other DBs: Fine, HODOC, NIH, EINECS
Contributor: NIST Mass Spectrometry Data Center
InChIKey: HCXVJBMSMIARIN-DJCTUNKVSA-N Non-stereo
10 largest peaks:

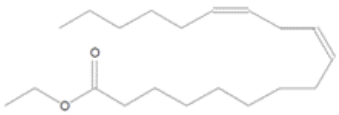
55 999	83 692	81 638	69 636	133 444	43 431	91 427	105 427	159 418	95 398

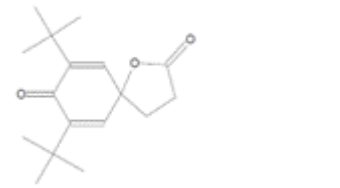
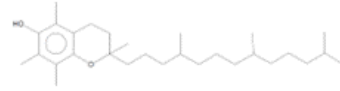
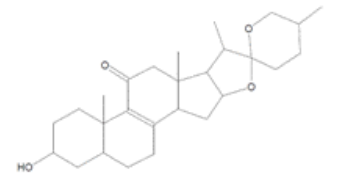
293 m/z Values and Intensities:

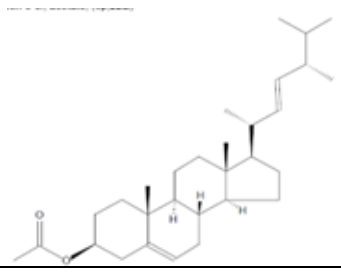
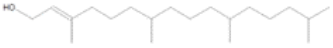
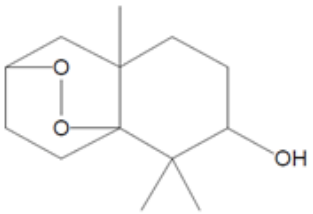
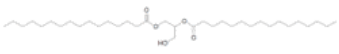
15	4	25	1	26	4	27	38	28	9	29	74	30	2	31	6	37	2	38	3
39	39	40	10	41	350	42	25	43	431	44	19	45	7	50	3	51	8	52	4
53	79	54	20	55	999	56	57	57	191	58	11	59	7	61	1	62	1	63	4
64	1	65	42	66	17	67	342	68	52	69	636	70	58	71	98	72	7	73	9
75	3	77	145	78	34	79	392	80	58	81	638	82	113	83	692	84	92	85	65
86	5	87	2	89	3	91	427	92	82	93	384	94	91	95	398	96	111	97	394
98	39	99	7	100	4	102	3	103	29	104	24	105	427	106	81	107	366	108	76
109	249	110	99	111	55	112	10	113	3	114	5	115	51	116	25	117	162	118	53
119	300	120	113	121	217	122	79	123	264	124	74	125	26	126	8	127	13	128	52
129	97	130	51	131	223	132	67	133	444	134	86	135	178	136	28	137	127	138	36
139	55	140	9	141	34	142	31	143	158	144	70	145	397	146	89	147	306	148	54
149	102	150	18	151	156	152	25	153	10	154	8	155	32	156	25	157	125	158	90
159	418	160	87	161	175	162	40	163	136	164	27	165	24	166	37	167	11	168	7
169	30	170	14	171	79	172	27	173	125	174	35	175	88	176	35	177	60	178	22
179	8	180	10	181	7	182	5	183	27	184	11	185	78	186	27	187	74	188	23
189	44	190	11	191	24	192	5	193	4	194	1	195	3	196	3	197	36	198	13
199	87	200	24	201	42	202	11	203	25	204	6	205	15	206	3	209	3	210	2
211	41	212	19	213	125	214	30	215	70	216	17	217	35	218	9	219	7	220	2
223	2	224	1	225	25	226	9	227	33	228	21	229	54	230	23	231	42	232	12
233	7	234	3	235	1	237	2	238	2	239	34	240	17	241	39	242	12	243	24
244	7	245	14	246	10	247	4	248	1	251	2	253	49	254	25	255	240	256	50
257	30	258	49	259	13	260	4	266	1	267	45	268	12	269	11	270	45	271	222
272	125	273	59	274	10	275	1	281	26	282	31	283	34	284	10	285	48	286	15
287	3	288	1	289	1	293	1	294	1	295	8	296	8	297	3	298	2	299	46
300	147	301	44	302	9	303	1	309	15	310	5	311	3	312	3	313	12	314	43
315	11	316	2	323	1	326	1	327	15	328	6	329	2	335	1	336	1	339	2
340	2	342	1	349	1	350	1	351	91	352	29	353	6	354	2	365	5	366	2
367	1	368	2	369	47	370	16	371	2	379	36	380	12	381	3	383	4	384	1
392	1	393	1	394	24	395	9	396	3	397	29	398	8	399	2	410	2	411	1
412	283	413	92	414	14														

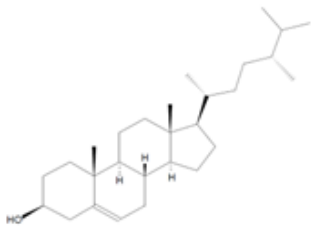
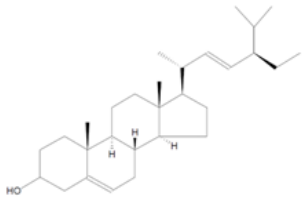
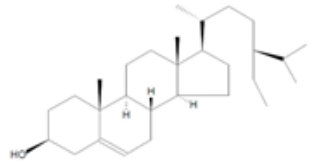
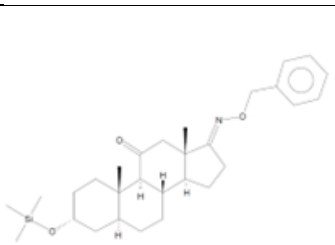
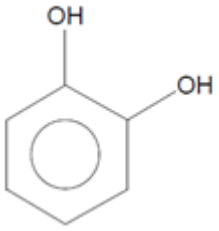
Figure 92- NIST hit and Mass spectrum of Stigmasterol.

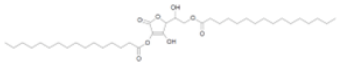
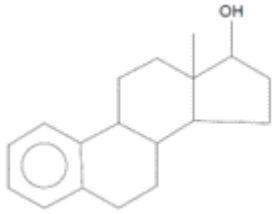
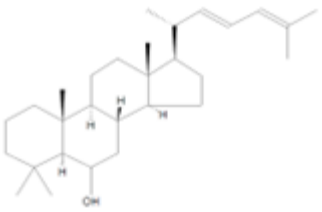
GC-MS NIST Hits for Smooth Crabgrass fractions:

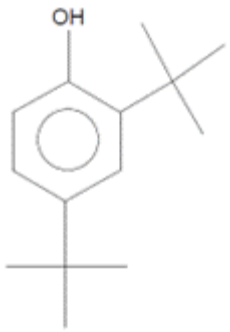
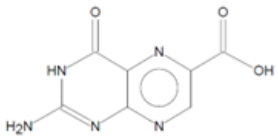
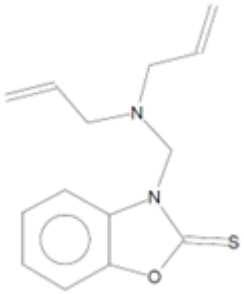
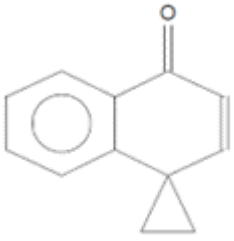
Fraction	Compound	Molecular Structure	Compound nature	Activity
1	Cetene		Hydrocarbon – alkene	Antifungal
1	1-Hexadecanol		Hydrocarbon – alcohol	Skin aid
1	Neophytadiene		Diterpenoid	Antimicrobial, antifungal, anti-inflammatory, antioxidant
1	Octacosanal		Fatty Aldehyde	Cholesterol lowing effects
2	Hexadecanoic acid, ethyl ester		Ester	antioxidant, antimicrobial, and anti-inflammatory activities
2	Linoleic acid ethyl ester		Fatty acid	Anti-inflammatory
2	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-		Fatty acid	Antiinflammatory, Insectifuge Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-

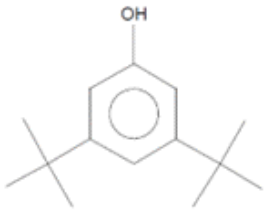
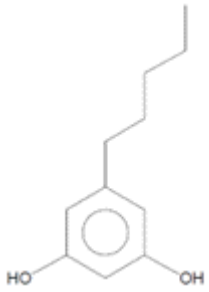
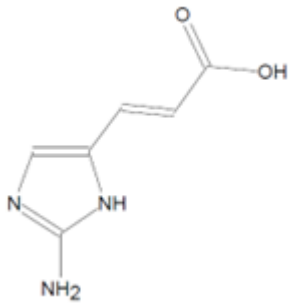
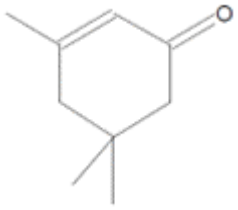
				Alpha reductase inhibitor, Antiandrogenic, Antiarthritic and Anticoronary
3	3-Eicosene, (E)-		Alkene	antimicrobial activities
3	Hexahydrofarnesyl acetone 2-Pentadecanone, 6,10,14-trimethyl-		Sesquiterpenoid	Antibacterial and antifunga
3	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione		Oxaspiro	treat skin diseases, gonorrhea, migraine, intestinal parasites, and warts
3	9,12-Octadecadienoic acid, ethyl ester		Fatty Acid	anti-inflammatory and antimicrobial activities
3	Vitamin E		Tocopherols	Vision, reproduction, antioxidant
4	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-		Steroid	Anti-cancerous

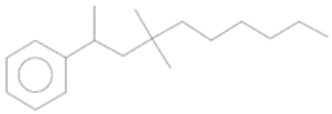
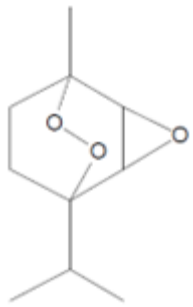
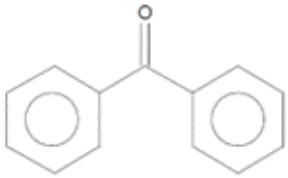
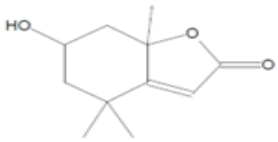
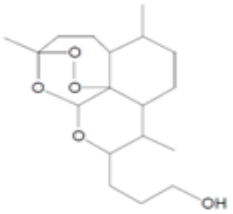
5	Ergosta-5,22-dien-3-ol, acetate, (3β,22E)-		Steroid	Antifungal and antibacterial
6	10-Heneicosene (c,t)		Hydrocarbon alkene	Gonorrhoea, Syphilis, Tuberclosis, edema and injuries
6	Phytol		isoprenoid alcohol	Antinociceptive and antioxidant activities, anti-inflammatory and antiallergic effects
7	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin		Phytosterols	Lower cholesterol
7	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester 19.7 C35H68O5		Fatty acid	antioxidant, hypocholesterolemic, antiandrogenic, hemolytic and alpha reductase inhibitor activity
8	Campesterol		Steroid	Inhibit the development of various cancerous

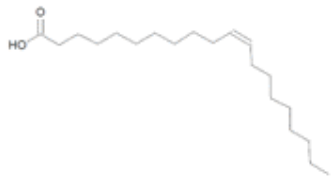
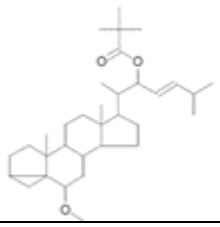
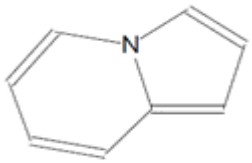
				
8	Stigmasterol		Steroid	Inhibit the development of various cancerous
8	γ-Sitosterol		Steroid	Controlling cholesterol and lowering the risk of heart disease
9	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-		Steroid	antagonist activity against rat prostatic androgen receptor and favors the reduction of estrogens and androgens.
11	Catechol		Phenol	reatment of various central and peripheral nervous system disorders, including Parkinson's disease, depression, schizophrenia, and other dopamine

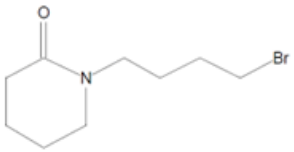
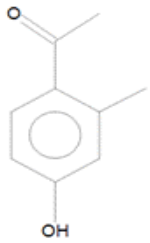
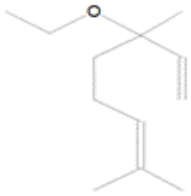
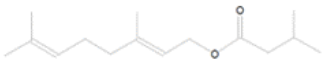
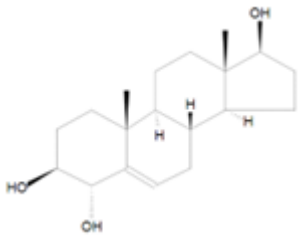
				deficiency-related disease
11	l-(+)-Ascorbic acid 2,6-dihexadecanoate		Acid	Anti oxidant, anti scorbutic, anti inflammatory, anti nociceptive, anti mutagenic, wound healing property
11	Estra-1,3,5(10)-trien-17β-ol		Steroid	Reduce cholesterol, testosterone booster
11	Oleic Acid		Fatty acid	Anti cancer, autoimmune and inflammatory diseases
11	Cholesta-22,24-dien-5-ol, 4,4-dimethyl-		Steroid	Lower cholesterol, prevent heart diseases

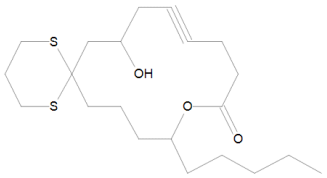
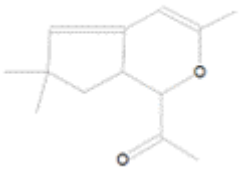
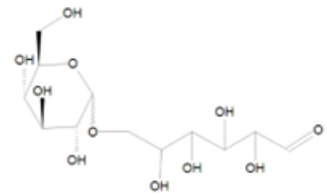
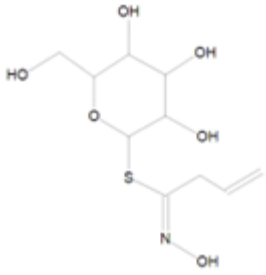
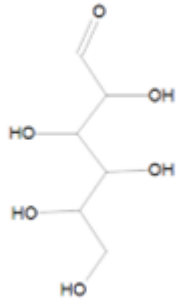
12	2,4-Di-tert-butylphenol		Pheonl	antifungal activities and antioxidant
12	Pterin-6-carboxylic acid		Purine	Anesthetics, cardioprotective, and anti-inflammatory agents
13	Benzoxazol, 2,3-dihydro-2-thioxo-3-diallylaminomethyl-		Oxazole	Antibacterial, Antifungal
13	Spiro[cyclopropane-1,1'(4'H)-naphthalen]-4'-one		Naphthalene	Anticancer

13	Phenol, 3,5-bis(1,1-dimethylethyl)-		Phenol	antioxidant and anti-cancer properties
13	1,3-Benzenediol, 5-pentyl-		Resorcinol	Carcinogenic
14	Imidazole, 2-amino-5-[(2-carboxy)vinyl]		Imidazole	antifungal, antimicrobial and antibiotic activities
15	Isophorone		Ketone	**
15	Behenic alcohol		Hydrocarbon alcohol	Topical antiviral

16	Benzene, (1,3,3-trimethylnonyl)-		Benzene	Anti-microbial
17	Ascaridole epoxide		Epoxide	An antiprotozoal drug used to treat or prevent infections caused by protozoan parasites that belong to the genus Leishmania
17	Benzophenone		Benzophenones	anticancer, anti-inflammatory, antimicrobial, and antiviral
17	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one		Benzofurans	Anti-inflammatory
17	12-β-(3'-Hydroxypropyl)deoxy artemisinin		Sesquiterpene lactones – peroxide bridge.	Anti malaria

17	cis-11-Eicosenoic acid		Carboxylic acid	Skin care
17	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate		Steroid	Cholesterol lowering abilities, antimicrobial, anti-inflammatory, antiarthritic.
19	Oleic acid, 3-(octadecyloxy) propyl ester		Fatty acid	Anti cancer, autoimmune and inflammatory diseases
20	Indolizine		Indolizine	Antitumor, antimycobacterial, antagonist, and antiproliferative activities
20	4-Cyclopropylcarbonyloxytridecane		Ester – epoxide	Anti-angiogenic, antibacterial and antiulcer

20	2-Piperidinone, N-[4-bromo-n-butyl]-		Lactam	Antibacterial
20	3-Chloropropionic acid, 3-pentadecyl ester		Ester	Antimicrobial
21	4-Hydroxy-2-methylacetophenone		Alkyl-phenyl ketones	Antibacterial
22	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-		Ether - alkene	dysentery, diarrhea, craw-craw, scabies, rheumatism
22	Geranyl isovalerate		Ester	Anti-cancer
22	5-Androsten-3,4,17-triol, (3β 4α 17β)-		Steroid	Lower cholesterol, prevent heart diseases

25	18-Hydroxy-10-pentyl-11-oxa-1,5-dithia-spiro[5.13]nonadec-15-yn-12-one		Spiro	Malarial and Filarial Vector
26	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl)ethenone		Ketone - ether	Antioxidant, antimicrobial
27	Melibiose		o-glycosyl compounds	Skin care
27	Desulphosinigrin		Thio-sugar	Anti-bacterial
27	d-Mannose		Carbohydrates	Slows down protein degeneration, improve liver function, decrease blood sugar.

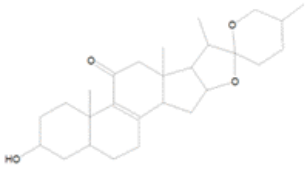
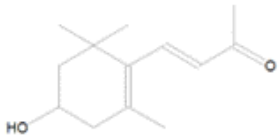
28	Benzaldehyde, 4-hydroxy-		Aldehyde	Flavor, anti-inflammatory
29	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-		Steroid	Lower cholesterol, prevent heart diseases
30	3-Hydroxy-α-ionene		Sesquiterpenoids	Anticancer, chemopreventive, cancer-promoting, melanogenesis, anti-inflammatory

Table 18 - GC-MS NIST Hits for Smooth Crabgrass fractions, their retention time, percentage area, compound name, chemical formula, molecular structure, exact mass, classification, and medicinal use.

6.0 - CONCLUSION

Digitaria *sanguinalis (Long Hairy crabgrass), Digitaria ischaemum (Smooth crab grass)* and the bark from *Carapa guianensis (crabwood)* were collected, identified, and tagged by a botanist affiliated with the University of Guyana. The 25 minutes reflux extraction process with ethanol proved to be the best method of extraction. Phytochemical analyses of the ethanol crude extracts revealed that they consist of Terpenes, Flavonoids, Steroids, Tannins, Phenolic, Proteins, Cardiac Glycoside, Reducing Sugars, and Carbohydrates. Conversely, Saponins were absent in Smooth Crabgrass and Crabwood's bark but present in Long Hairy Crabgrass. After column chromatography, the pooling of fractions resulted in 27 fractions for smooth crabgrass, 26 for long hair crabgrass and 22 for crabwood's bark. Antimicrobial assays showed promising results for Crabwood's bark against *Staphylococcus aureus* and *Staphylococcus epidermis* in both antimicrobial testing methods, while Smooth crabgrass indicated inhibition for only *Staphylococcus epidermis* in the Disc diffusion assay. Some of the compounds found via GC-MS NIST determination were Eucalyptol, Isolongifolan-8-ol, Limonen-6-ol, pivalate, Estra-1,3,5(10)-trien-17β-ol, Ethyl iso-allocholate, Cryptomeridiol, γ-Sitostenone, Stigmasterol, Levodopa, Glycidyl palmitate, 2-Myristynoyl-glycinamide, Androst-5-en-4-one, Cholestan-3-one, cyclic, Retinol, acetate, Linoleic acid ethyl ester, Neophytadiene, Vitamin E, Geranyl isovalerate, Melibiose, and d-Mannose. These plants are promising source for active ingredients that would be potential therapeutic modalities in different clinical settings.

7.0 - REFERENCES

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