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Study The Identification And Purifying Techniques For *Cymbopogon Flexuosus (Krishna), Centella Asiatica*, And *Cynodon Dactylon*

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Abstract

Traditional medicine, the pharmaceutical and herbal businesses, and the pharmaceutical industry all rely on accurately identifying and purifying medicinal plants. *Cymbopogon flexuosus*(krishna), often known as lemongrass, *Centella asiatica*, or gotu kola, and *Cynodon dactylon*, or Bermuda grass, are just a few of the many plant species that have medicinal qualities. In this paper study the identification and purifying techniques for *Cymbopogon Flexuosus (krishna)*, *Centella Asiatica*, and *Cynodon Dactylon***.**

Keywords: herbal, pharmaceutical, plant, industry, *Cymbopogon flexuosus (krishna).*

1. INTRODUCTION

Lemongrass, scientifically known as *Cymbopogon flexuosus (krishna)* is an adaptable plant with a strong citrus scent. In addition to its many uses in the kitchen, it makes a major contribution to the fragrance and pesticide markets. [1,2] The medical qualities of the plant make it significant beyond only its pleasing aroma. One of the many essential oils found in lemongrass is citronella, which has antimicrobial, anti-inflammatory, and antioxidant properties. [3,4] When it comes to traditional medicine, lemongrass is highly regarded for its ability to soothe gastrointestinal problems, lower stress levels, and improve skin health. To identify lemongrass, one must look at its leaves and other morphological traits, and then use sophisticated chemical analysis methods, such as chromatography, to determine its specific chemical make-up.[5]

Ayurveda and traditional Chinese medicine both put a heavy emphasis on *Centella asiatica*, most often known as gotu kola. With its reputation as the "herb of longevity," gotu kola is highly regarded for its ability to enhance cognitive function. [6,7] There is more to its significance than just herbal medicines; it is steeped in cultural customs and has historical weight. As a medicinal herb, gotu kola has anti-anxiety, wound-healing, and cognitive-enhancing properties. Because of its purported ability to decrease inflammation and increase collagen formation, it is used in cosmetic products. In order to identify gotu kola, it is necessary to use chemical analysis techniques to separate its bioactive chemicals and to carefully examine the plant's physical characteristics. [8,9]

Although *Cynodon dactylon*, or Bermuda grass, is technically a weed, its potential health benefits have not gone unrecognized. Because of its diuretic, anti-inflammatory, and anti-diabetic effects, this hardy grass is used in traditional medical systems. [10,11] A combination of chemical investigation to determine the specific chemical components of Bermuda grass and observation of morphological traits, such as the structure of its

leaves, allows for its identification. Traditional procedures like boiling and steeping, known as decoction and infusion, are used to extract therapeutic components from *Bermuda grass*.[12]
Ensuring the effectiveness and purity of the resulting products relies on accurately identifying these plants. The size, shape, and color of the leaves are morphological features that help identify these plants. In addition, modern methods like as spectroscopy and chromatography greatly aid in the chemical examination of bioactive substances, essential oils, and alkaloids that are unique to each plant.[13]
The separation and concentration of these plants' beneficial components rely heavily on purification methods. One typical technique for lemongrass is steam distillation, which involves passing steam over plant material in order to extract essential oils. Chemicals of medicinal value in *Centella asiatica* may be isolated by using extraction techniques including solvent and supercritical fluid extraction. These state-of-the-art methods guarantee the intact extraction of targeted chemicals with medicinal potential.[14]
Particularly for plants such as Bermuda grass, traditional techniques of purification such as decoction and infusion are still applicable. An all-encompassing method of extracting medicinal ingredients from plants is by boiling or steeping them in hot water, which is called a decoction or an infusion.[15]

2. METHODOLOGY

"We obtained the fresh aerial parts of three different plant species from the lush fields of Ambikapur, which is situated in the tranquil Surguja district of Chhattisgarh: *Cymbopogon flexuosus (krishna)*, also called *Krishna*; *Centella asiatica*, also called Brahmi; and *Cynodon dactylon*, also called Doob Grass. These carefully chosen plant materials would function as the basic components of the research we have planned.
After gathering these essential materials, we started getting them ready for the next study. To preserve their original essence and characteristics, the leaves of these plants were carefully allowed to air dry. Then, to make sure the dried leaves were prepared for use in our scientific endeavors, they were ground into a fine powder.
The first phase was carefully inspecting the plant samples taken from *Cymbopogon flexuosus (Krishna)*, *Centella asiatica*, and *Cynodon dactylon* for any indications of infection, spores, damage, discoloration, or deformation. After that, unharmed leaf samples underwent a comprehensive cleaning procedure that included rinsing with deionized water after being washed with tap water. The leaves were then meticulously air-dried at 37°C in a room with ambient conditions. To make the leaves ready for further examination, the midribs were cut off.

- **Purification & Identification Method**

(a) Isolation and Purification
Thin Layer Chromatography (TLC) was used for this crucial procedure, which was carried out under the direction of the technique developed by Bhakuni and associates in their seminal work that was published in 2005.
A 1 mg/ml sample dilution was made, and 1 ml of this solution was used to spot the samples onto the TLC templates. Run compound-loaded TLC plates after preparing a solvent mixture in 50 ml tubes.

Table 1: TLC sample

Phytochemicals	Solvent system	Ratio	Confirmatory test
Alkaloids	Ethyl Acetate: Methanol : Water	10:1.35:1	Mayer's reagent spray
Flavonoids	Ethyl Acetate : Butanol : Formic Acid : Water	5: 3 : 1	3% Boric Acid + 10% oxalic acid spray
Tannins	Toluene : Ethyl Acetate : Formic Acid : methanol	3:3:0.8:0.2	FeCl3 Spray
Phenols	Ethyl acetate : Toluene : Formic acid	2.2:1.1:1.1	Folin Ciocalteau reagent
Essential Oil	Toluene and ethyl acetate	94 : 6	Anise Aldehyde : Sulphuric (VI) acid reagent

(b) Identification
A thorough analytical method was used to identify the chemicals that were isolated from our research subjects, *Cymbopogon flexuosus* (also known *as Krishna)*, *Centella asiatica*, and *Cynodon dactylon.* Our main techniques for identification were High-Performance Thin-Layer Chromatography (HPTLC) and Gas

Chromatography-Mass Spectrometry (GC-MS), both of which have shown to be useful in the field of chemical analysis.
The GC-MS method was well known for its accuracy in revealing the make-up and molecular structure of many substances. It achieved this by first isolating the constituent parts, after which mass spectrometry was used to identify and measure them. The precise chemicals present in our plant materials were clarified thanks in large part to this technique.

1. GS- MS (Gas Chromatography-Mass Spectrometry)
The experiment was conducted using a gas chromatograph from Agilent Technologies (Santa Clara, CA, USA) combined with a mass selective detector from Agilent Technologies (Santa Clara, CA, USA) (Model 5975). The analytical instrument used a 30-meter-long, 0.25-mm-diameter, and 0.25-micron-thick Agilent capillary column (HP-5 ms nonpolar 5% phenyl methyl polysiloxane). The carrier gas used was helium, with a flow rate of 1.1 mL/min. The gas chromatograph's oven was kept at a constant 300 °C. Starting at 60 °C and holding for 2 minutes, the temperature in the column was gradually raised to 250 °C, at a rate of 10 °C per minute. Consequently, the ideal temperature was maintained for a duration of 10 minutes. A split injection of 10:1 was used to inject one microliter of oil into the column. According to Conde-Hernández and Guerrero-Beltrán (2014), the temperature of the injector was 250 °C. The mass spectra of the components were analysed for fragmentation patterns and compared to data available in the NIST Mass Spectral database in order to identify them (NIST-MS, 2010).
In addition, we made use of High-Performance Thin-Layer Chromatography (HPTLC), a potent separation method that let us identify and measure the different substances in complicated combinations. Our methodology fully adhered to the protocol described by Visvanathan et al. in their 2008 article, guaranteeing the precision and dependability of our findings.

2. HPTLC (High-Performance Thin Layer Chromatography)
60 F254 (Merck) silica gel plates are used for HPTLC analysis during the normal phase. 20 × 10 cm were the dimensions of the TLC plates used for the development. Through the use of LINOMAT 5 (CAMAG®), 20 µL (10 mg/mL) were spread out. The spot was scraped off the HP-TLC plate, eluted in a tube, and analysed in the Bruker Daltonics micrOTOF-Q™ mass spectrometer in positive and negative mode using a CAMAG TLC-MS interface system. Development was performed using the mobile phase on the TLC plate, which covers three quarters of the plate.

- **Statistical Analysis**

Every experiment's data was run via a one-way analysis of variance (ANOVA), which was followed by post hoc analysis. The Bonferroni post hoc test is used after the two-way ANOVA and Tukey's multiple comparison test. Software called Graph Pad Prism 6.0 was used to do the analysis.

3. RESULT

- **Thin Layer Chromatography (TLC)**

After doing the analysis on the crude extract, when the TLC plates were used to run Sample-*CA(Centella asiatica)*,Ethyl acetate, methanol, and water at a ratio of 10:1.35:1 form phytochemicals, which are alkaloids. With Mayer's Reagent sprayed on, no bands were detected; the solvent system consisted of flavonoids in a 5:3:1 ratio of ethyl acetate, butanol, formic acid, and water. Phenols in a solvent solution consisting of ethyl acetate, toluene, and formic acid (in a ratio of 2.2:1.1:1.1) were sprayed with a mixture of 3% and 10% oxalic acid, and one band was seen. Use of Folin Ciocalteau Reagent and an essential oil solution with a ratio of 4.7:14 to ethyl acetate resulted in the observation of four bands. Seven bands were seen in sample *CA(Centella asiatica)* after spraying with anise aldehyde: sulphuric acid reagent. Sample *CA(Centella asiatica)* showed two bands for tannins and seven bands for phytochemicals essential oil when run on a TLC plate without spray.
Based on the results of the crude extract characterisation study, when Sample-*CD(Cynodon dactylon)* was conducted on the TLC plates Ethyl acetate, methanol, and water at a ratio of 10:1.35:1 form phytochemicals, which are alkaloids. Four distinct bands were detected when the flavonoids were sprayed with Mayer's Reagent in a solvent solution consisting of ethyl acetate, butanol, formic acid, and water in the ratio of 5:3:1. Two bands were identified when the sample was sprayed with a mixture of 3% oxalic acid and 10% phenols in a solvent system consisting of ethyl acetate, toluene, and formic acid in the ratio of 2.2: 1.1: 1.1. “The Folin Ciocalteau Reagent was used to get seven bands in the *CD(Cynodon dactylon)* sample. There were five bands in sample

CD(Cynodon dactylon) representing phytochemical tannins when the sample was processed on a TLC plate without spray.

Based on the results of the crude extract characterisation study, when the *CF(Cymbopogon flexousus- krishna)* sample was run on the Phytochemicals-Alkaloids TLC plates using a solvent system consisting of ethyl acetate, methanol, and water in a ratio of 10:1.35:1, Four distinct bands were detected when the flavonoids were sprayed with Mayer's Reagent in a solvent solution consisting of ethyl acetate, butanol, formic acid, and water in the ratio of 5:3:1. Spraying with a mixture of 3% oxalic acid and 10% phenols in a solvent system consisting of ethyl acetate, toluene, and formic acid resulted in the formation of four distinct bands. Sample *CF(Cymbopogon flexousus-krishna)*yielded six bands after being sprayed with Folin Ciocalteau Reagent. Sample *CF(Cymbopogon flexousus-krishna)* showed four bands of phytochemical tannins when run on a TLC plate without spray.

TLC Characterization of Crude Extract

- **Alkaloid**

Solvent system – Ethyl Acetate : Methanol : Water (10:1.35:1)

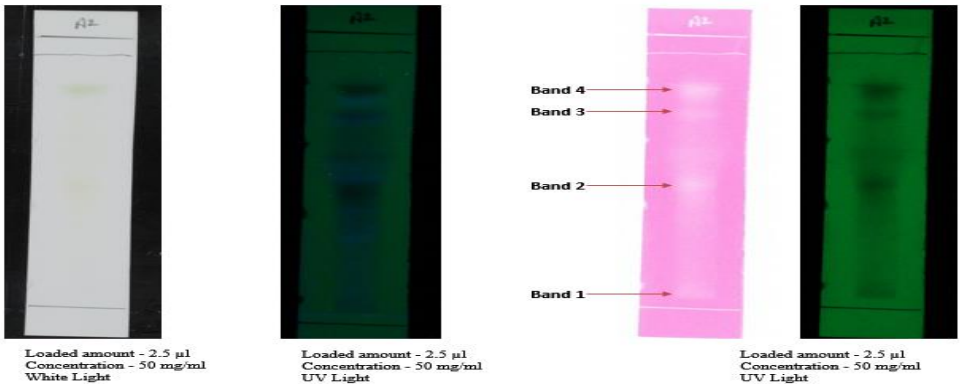


Figure 1: Sample Code- (*CD- Cynodon dactylon)*

Figure 2: Sample Code- *(CA- Centella asiatica)*

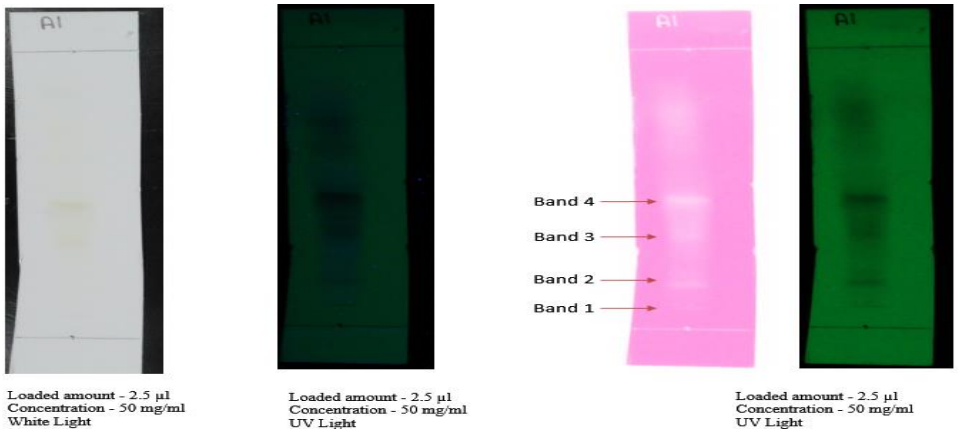


Figure 3: Sample Code- *(CF- Cymbopogon flexousus (krishna)*

- **Flavonoids**

solvent system – Ethyl Acetate : Butanol : Formic Acid : Water (5:3:1)

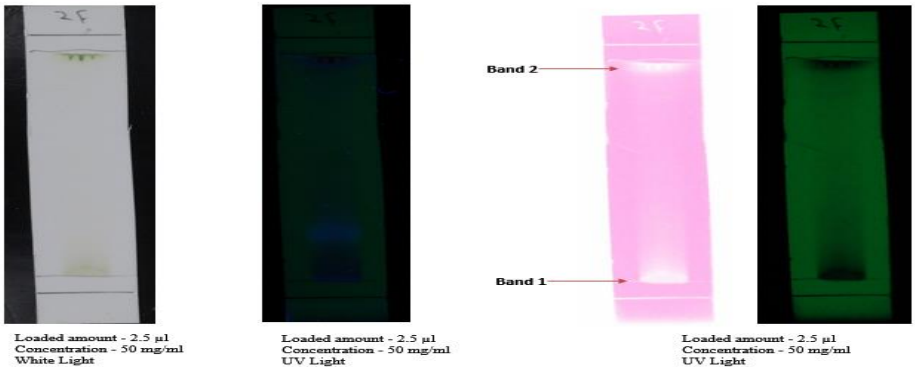


Figure 4: Sample Code- (*CD- Cynodon dactylon)*

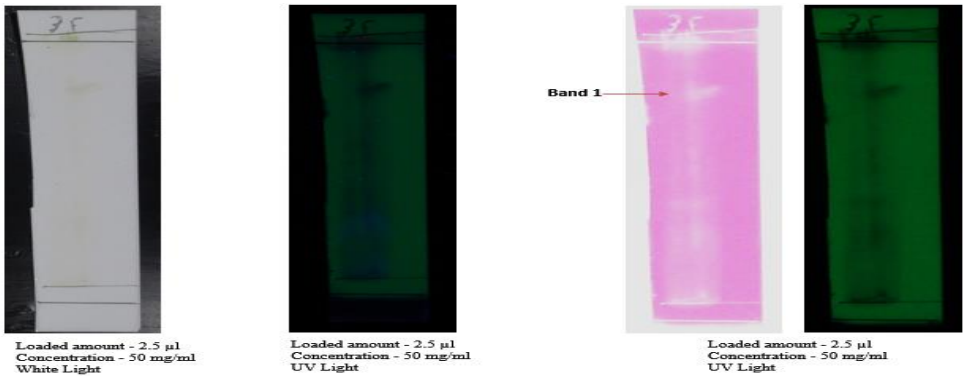


Figure 5: Sample Code- *(CA- Centella asiatica)*

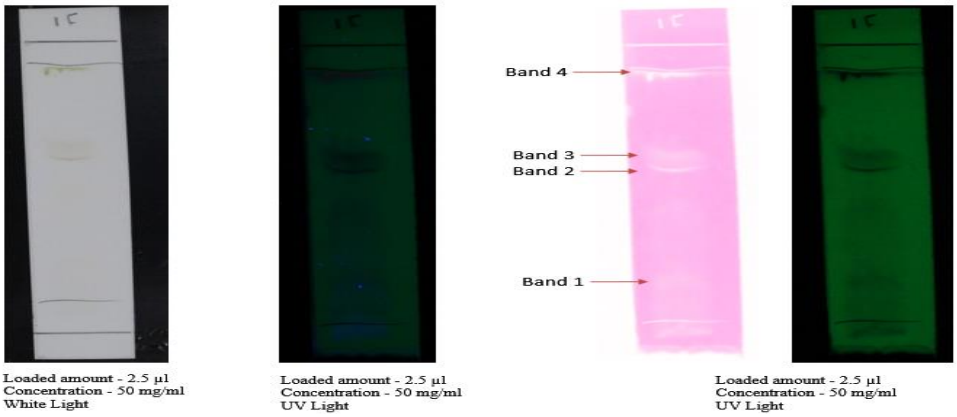


Figure 6: Sample Code- *(CF – Cymbopogon flexousus (krishna)*

- **Phenols**

Solvent system – Ethyl Acetate : Toluene : Formic Acid (2.2:1.1:1.1)

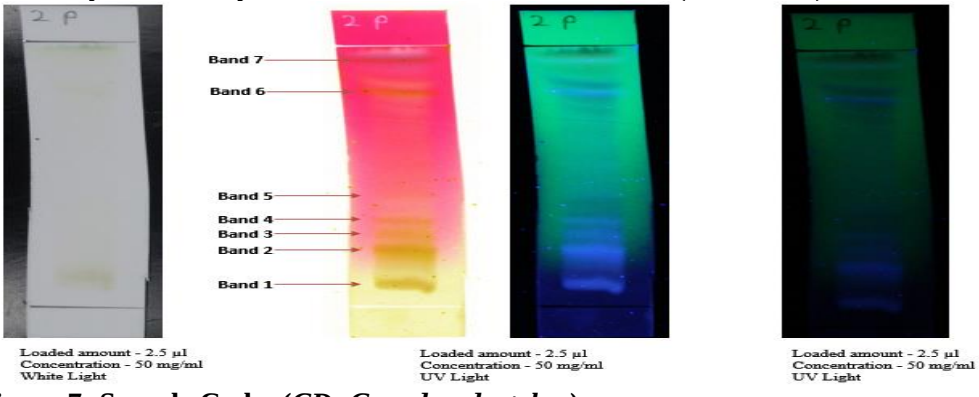


Figure 7: Sample Code- (*CD- Cynodon dactylon)*

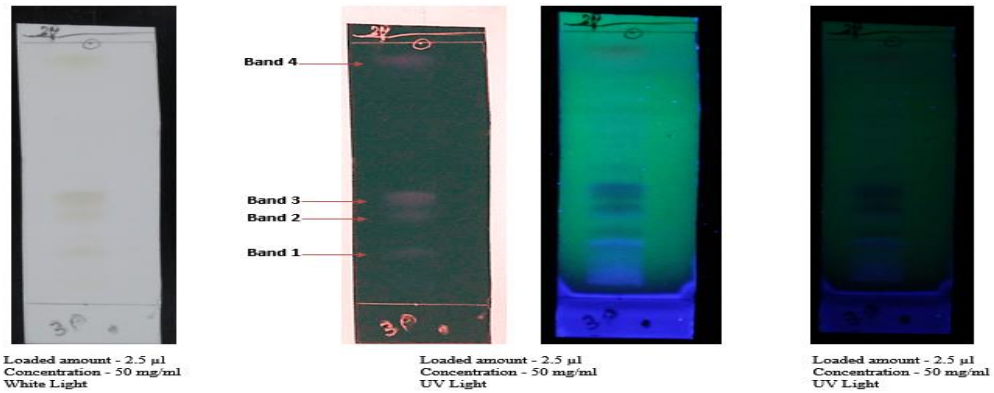


Figure 8: Sample Code- ***(CA- Centella asiatica)***

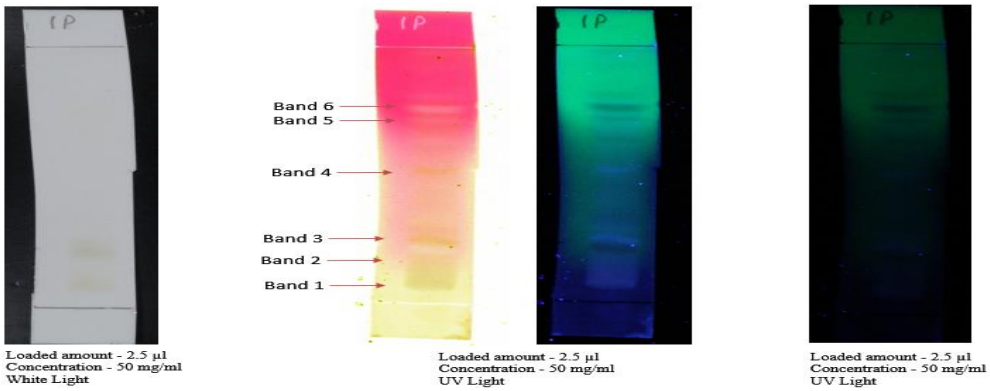


Figure 9: Sample Code- ***(CF – Cymbopogon flexousus (krishna)***

- **Without Spray – Tannins**

Solvent system – Toluene: Ethyl Acetate : Formic acid : Methanol (4:6)

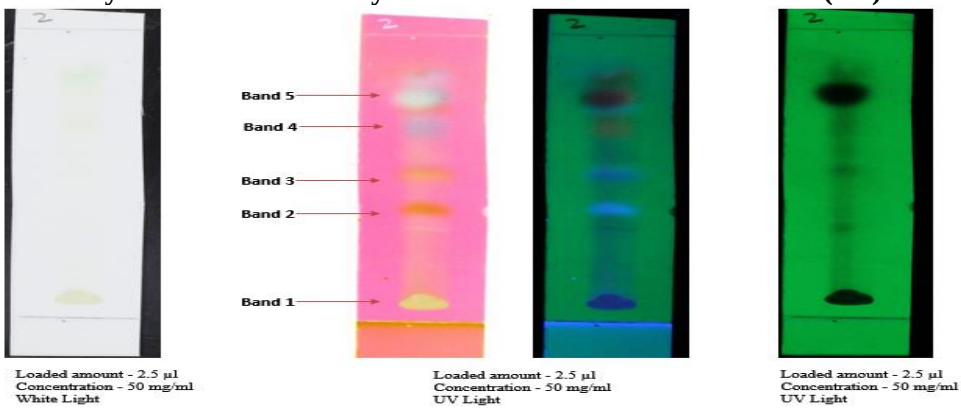


Figure 10: Sample Code- (*CD- Cynodon dactylon)*

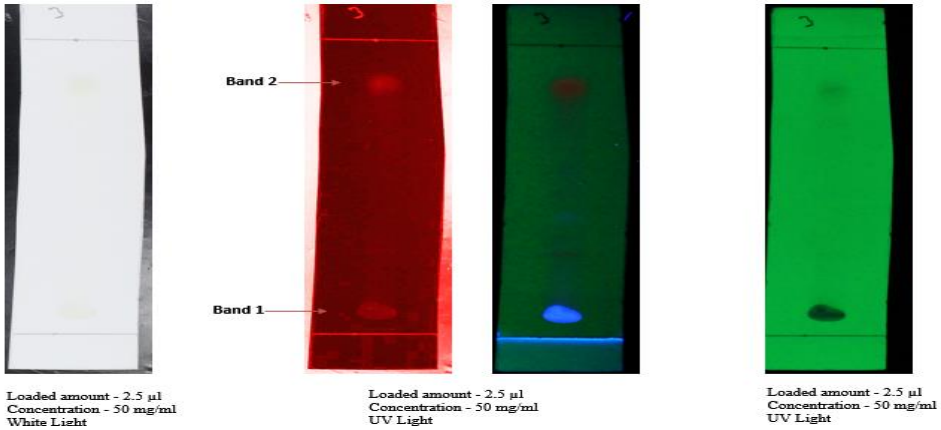


Figure 11: Sample Code- ***(CA- Centella asiatica)***

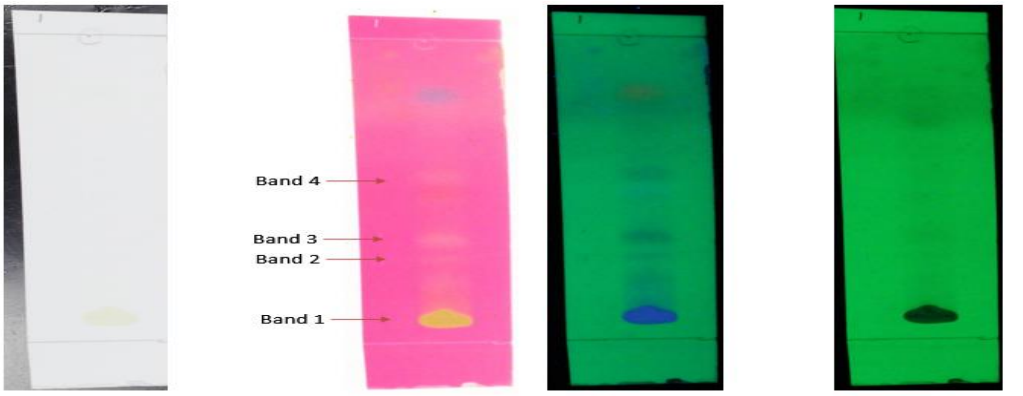


Figure 12: Sample Code- ***(CF – Cymbopogon flexousus (krishna)*****With Spray-Oil**

Solvent system – Ethyl Acetate : Toluene (4.7:14)

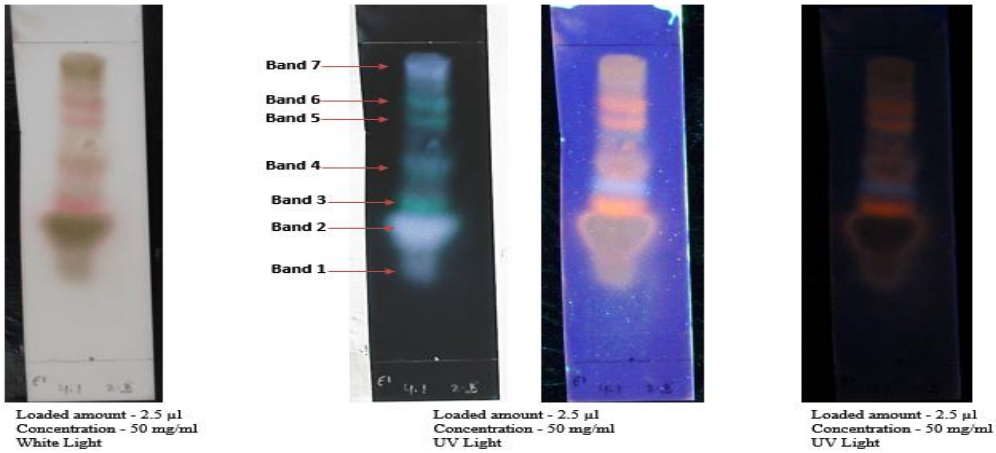


Figure 13: Sample Code- ***(CA- Centella asiatica)***

- **Without Spray-Oil**

Solvent system – Ethyl Acetate : Toluene (4.7:6)

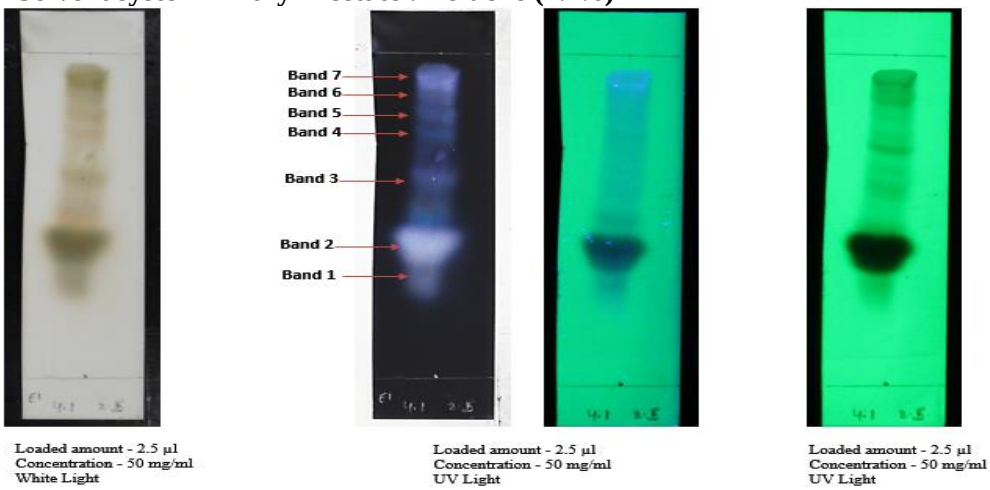


Figure 14: Sample Code- ***(CA- Centella asiatica)***

- **GS- MS (Gas Chromatography-Mass Spectrometry)**

Tthe GC-MS method was able to identify the primary constituents of the essential oils of the plants that were harvested.

Table 2: *Cymbopogon flexousus (krishna)* essential oil chemical components isolated by steam distillation from several species.

Chemical	Area (%)
Cymbopogon flexousus(krishna)	
Cis-linalool oxide	0.7
Linalool	0.64
2-hydroxy-1, 1, 10-trimethyl-6,9-decalin epidioxi	8.09
Oxirane methanol, 3-methil-3-(4-methyl-3-pentenyl)	28.4
Dihydronopol	2.52
Neral	19.35
Geranial	15.97
Geranic acid	5.76
Neric acid	9.15
2-tridecanone	1.21
7-Methyl-z-tetradecen-1-ol- acetate	2.37
1-allyl-2-hydroxy-6-methyl-cyclohexanecarboxylic acid	1.1
9-methyl-z10-tetradecen-1-ol-acetate	4
Cholestan-3-ol, 2-methylene (3*β*, 5*α*)	0.3
Ethyl-linoleate	0.43
Centella asiatica.	
Cis-linalool oxide	1.2
Linalool	0.75
2-hydroxy-1, 1, 10-trimethyl-6,9-decalin epidioxi	7.5
Oxirane methanol, 3-methyl-3-(4-methyl-3-pentenyl)	32.0
Dihydronopol	2.0
Neral	18.0
Geranial	16.5
Geranic acid	6.0
Neric acid	8.5
2-tridecanone	1.5
7-Methyl-z-tetradecen-1-ol- acetate	2.0
1-allyl-2-hydroxy-6-methyl-cyclohexanecarboxylic acid	0.9
9-methyl-z10-tetradecen-1-ol-acetate	4.5
Cholestan-3-ol, 2-methylene (3β, 5α)	0.5
Ethyl-linoleate	
Cynodon dactylon	
Cis-linalool oxide	0.9
Linalool	0.55
2-hydroxy-1, 1, 10-trimethyl-6,9-decalin epidioxi	6.8
Oxirane methanol, 3-methyl-3-(4-methyl-3-pentenyl)	30.2
Dihydronopol	2.3
Neral	17.2
Geranial	15.1
Geranic acid	5.5
Neric acid	8.0
2-tridecanone	1.3
7-Methyl-z-tetradecen-1-ol- acetate	1.8
1-allyl-2-hydroxy-6-methyl-cyclohexanecarboxylic acid	1.0
9-methyl-z10-tetradecen-1-ol-acetate	4.2
Cholestan-3-ol, 2-methylene (3β, 5α)	0.4
Ethyl-linoleate	0.5

Table 2: Compounds found in the essential oils of plants that have been distilled with the help of microwaves

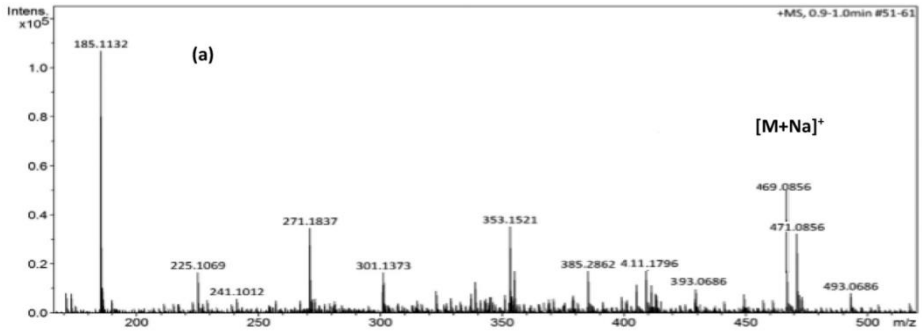
Chemical	Area (%)
Cymbopogon flexousus (krishna)	
3-Methyl-2-butenal	0.16
Nerol	1
Limonene	0.46
Citronellal	0.21
2-cyclohexen-1-one	0.33
Cis-linalool oxide	0.4
Linalool	0.86
Neral	29
Geranial	22.63
Methyl acetate	2.56
Oxirane carboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)	25.29
Cis-pulegone oxide	3.25
Neric acid	9.19
Carotol	0.88
7-methyl-z-tetradecen-1-ol-acetate	0.25
Centella asiatica	
3-Methyl-2-butenal	0.2
Nerol	1.2
Limonene	0.5
Citronellal	0.3
2-cyclohexen-1-one	0.4
Cis-linalool oxide	0.5
Linalool	0.9
Neral	30.5
Geranial	23.0
Methyl acetate	2.8
Oxirane carboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)	26.0
Cis-pulegone oxide	3.5
Neric acid	9.5
Carotol	0.9
7-methyl-z-tetradecen-1-ol-acetate	0.3
Cynodon dactylon	
Nerol	1.1
Limonene	0.48
Citronellal	0.25
2-cyclohexen-1-one	0.38
Cis-linalool oxide	0.42
Linalool	0.82
Neral	28.5
Geranial	21.2
Methyl acetate	2.7
Oxirane carboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)	24.5
Cis-pulegone oxide	3.15
Neric acid	9.0
Carotol	0.85
7-methyl-z-tetradecen-1-ol-acetate	0.28

The results of Mahanta et al. (2007) and Negrelle and Gomes (2007) are in agreement with the primary component of *Krishna* (*Cymbopogon flexousus Sp.)* essential oil, which was produced using two distinct distillation techniques (2007).” Andrade et al. (2012) and Adukwu, Allen, and Phillips (2012) both noted that citral was the most abundant chemical in C. flexousus. In 2012, researchers Chanthai, Prachakoll,

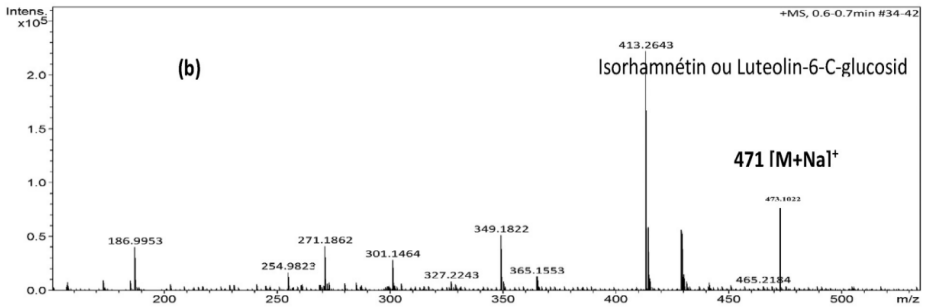
Ruangviriyachai, and Luthria found that citral concentration varied between 65 and 80%. Essential oils isolated from plants' leaves and dried in various environments (sun, shade, and oven) included varying quantities of geranial (31.53%, 39.86%, and 37.24%), neral (30.08%, 34.52%, and 31.28%), and myrcene (16.61%, 14.49%, and 15.42%), according to research by Mohamed Hanaa, Sallam, El-Leithy, and Aly (2012). Because plants were grown in different climates, there may be discrepancies in the components reported by different studies.

- **HPTLC (High-Performance Thin Layer Chromatography)**

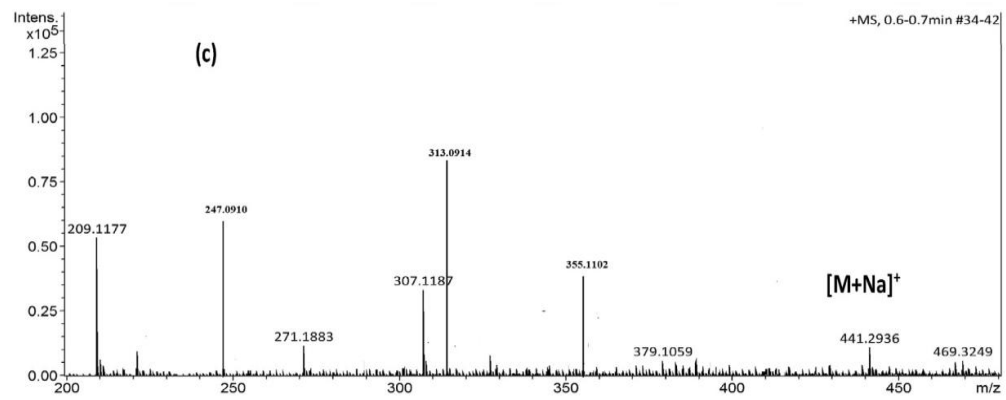
Raw material extract Out of the five advertisements that were produced using the TLC profile, three of them were majority spots. Spots 3 and 4 display a yellow fluorescence, whereas spot 1 is yellow-orange, when seen under UV 365 nm after being sprayed with Mayer's reagent. Research has linked these spots to flavonol derivatives that have been glycosylated (Wagner and Bladt, 1996). This study compares the TLC profile of crude extracts to those of standards. Following the TLC analyses of the methanol extracts, the mass spectrometer was used in conjunction with the CAMAG interface to examine the majority of the stains (1, 3, and 4). the bulk of spots' mass spectra. These three spots are relevant: spot 1 (Rf = 0.37), spot 3 (Rf = 0.67), and spot 4 (Rf = 0.7). We can see distinctive molecular ion peaks at m/z 441 ½M þ Naþ, m/z 471 ½M þ Naþ, and m/z 473 ½M þ Naþ on these mass spectra from the electrospray positive mode ionisation (ESIþ) technique. Each of these molecular peaks corresponds to a known flavonoid's molecular weight distinctive mass.



(a) Spot 4 (Rf ¼ 0.7)



(b) Spot 3 Rf ¼ 0.67



(c) Spot 1 Rf ¼ 0.37

Figure 15: HPTLC-MS mass spectra of Spot 4 Rf ¼ 0.7 (a) Spot 3 Rf ¼ 0.67 (b) Spot 1 Rf ¼ 0.37 (c).

4. CONCLUSION

The complex procedures involved in identifying and purifying *Cymbopogon flexuosus (krishna), Centella asiatica, and Cynodon dactylon* integrate ancient wisdom with cutting-edge scientific understanding. A diverse array of therapeutic plants is enhanced by these botanical entities, each of which has its own distinct set of qualities. It is crucial to have reliable methods of identification and purification in order to meet the ever-increasing demand for herbal goods and natural medicines. This approach is more than just a scientific procedure; it's a perfect union of old knowledge and new technology that will guarantee the ongoing study and use of nature's medicine.

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