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RESEARCH PAPER

TITLE:

PHYTOCHEMICAL PROFILING AND POTENT ANTIBACTERIAL ACTIVITY OF *CYMBOPOGAN CITRATUS* EXTRACT, AS A NATURAL ANTIOXIDANT AGENT

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PHYTOCHEMICAL PROFILING AND POTENT ANTIBACTERIAL ACTIVITY OF *CYMBOPOGAN CITRATUS* EXTRACT, AS A NATURAL ANTIOXIDANT AGENT

ABSTRACT:

The practice of utilizing medicinal plants has been widespread since ancient times due to the ability of plants to produce chemical compounds. Five medicinal plants i.e., *Cymbopogan citratus, Piper longum, Althaea officinalis, Malva* and *Vasaka* were studied for observing their phytochemical potential and many other antibacterial activities. Phytochemical analysis of the methanol and ethyl acetate plant extracts showed the presence of different useful bioactive compounds such as steroids, carbohydrates, reducing sugars in these plants. Antibacterial activity against gram positive (*Bacillus* KC881030) and gram negative (*E. coli* GM2163) strains was determined. Among all extracts, lemon grass (*Cymbopogan citratus*) gave maximum inhibition zone of 17mm. Minimum inhibitory concentration of *Cymbopogan citratus* methanol extract was 31.25mg/ml. DPPH (2, 2-diphenyl 1-1 picrylhydrazyl) test was performed for checking the antioxidant potential that gave the best activity of 68.3% in case of lemon grass extract. Antimitotic studies revealed that lemon grass extract possesses antimitotic activity (53%). Due to all of these properties of lemon grass, thin layer chromatography (TLC) was performed to identify the bioactive compounds. Out of the 7 spots obtained from selected plant extract in methanol, only 1 spot exhibiting the best zone size (14mm) was analyzed for Gas chromatography and mass spectrophotometry (GCMS) analysis. GCMS revealed the presence of Citral as a bioactive component in selected plant extract. In conclusion, *Cymbopogan citratus* showed the best antibacterial, antioxidative and antimitotic potential. Further research should analyze phytochemical components for effective use of herbs in pharmaceutical industry.

KEYWORDS:

Phytochemical screening, Antibacterial activity, thin layer chromatography, Gas Chromatography and Mass Spectrophotometry.

1. INTRODUCTION:

For a long period of time, people have worked on natural remedies in order to cure their diseases. Since the beginning of their development, humans have fought for their survival because of starvation and unawareness that plants can also be a source of food. A lot of advancement in science and future studies claims the utilization of herbs for food and healthcare, and with the passage of time, man was able to fulfill his needs by adjusting to his surroundings (Jamshidi-Kia et al., 2017).

The study of ethno botany involves all forms of interactions between plants and human beings. It is the examination of how plants are used by members of a specific culture and geographic area. The study of plants with medicinal uses is included in this study (McClatchey et al., 2009). Since the beginning of time, medicinal plants have been utilized in healthcare. Any plant that has compounds in one or more of its structures that have therapeutic value or that serve as building blocks for the production of beneficial medications is considered medicinal (Sofowora et al., 2013).

Metabolism is the whole of the countless biochemical activities and reactions occurring simultaneously in every plant cell. Primary plant metabolites are more or less the same in all living cells because they are involved in fundamental life functions. In traditional medicine and folk applications, secondary plant metabolites were vital in treating a number of illnesses (Hussein & El-Anssary, 2019).

Lemon grass *(Cymbopogan citratus)* is a member of the Poaceae family (Singh et al., 2011). Because of its antibacterial, antifungal, and anti-inflammatory characteristics this kind of plant is frequently utilized by medical professionals (Kamaruddin et al., 2022). Lemon grass oil can be utilized to heal number of diseases, together with discomfort in the teeth and migraine. It is suitable to cure ringworm along with further fungal infections of the feet (Naik et al., 2010). Pepper (*Piper longum*) comes from the Sanskrit word for long pepper (pippali). Piperine, an alkaloid found in the fruits, adds to their pungency. Traditional uses include the entire plant as well as plant parts like the fruit. It is affordable, widely accessible, and beneficial for a variety of illnesses because it retains anti-amoebic activity, antiasthmatic activity, antifungal properties, antibacterial action, anti-diabetic properties, and also cancer-fighting properties (Kumar et al., 2011) (Zaveri, 2010). Marshmallow, denoted to as Althaea and Alcea, is an associate of the Malvaceae family (Mozaffarian, 2013). Marshmallow can be used to treat testicular edema with a plaster and compress, bladder stones with a sitz bath, delayed dental growth with a medicinal toothbrush, and constipation with a syrup, suppository, and enema (Kumar et al., 2013). Malva herb (Malvaceae) species has long been consumed due to its edible nature as well as its potential therapeutic benefits (Kaileh et al., 2007). The entire plant has shown therapeutic benefits; because its leaves have huge amount of vitamin-C, essential oils, tannins, mucus and flavonoids (Staff, 2017). It has been used as a therapeutic food as a tonic to cleanse liver, and to treat acidity. *Vasaka*, an Indian medicinal herb has been applied in traditional remedies for a variety of human complications for many years (Dash, 2017). The Punjabi name for this herb is Barg-e-Bansa associated to the family Acanthaceae. The plant's numerous components, especially its leaves and roots, are used for their many therapeutic uses, which include anti-inflammatory and respiratory health benefits (Sri S, 2023).

The major objective of this study was to find out the phytochemical components and antibacterial potential of selected local herbs such as lemon grass, long pepper, Khatmi Dana, Khabazi and Barg-e-bansa extracts. These medicinal herbs are locally used for the cure of respiratory problems. So, these potential phytochemicals can subsequently be used in the pharmaceutical industries to improve the efficacy, and to get novel synthetic drugs at the molecular level.

2. MATERIALS AND METHODS:

Sample Collection

Plants that are locally used to treat respiratory diseases mainly flu and cough were collected from the local market in Lahore, Pakistan. The plants collected were *Cymbopogan citratus, Piper longum, Althaea officinalis, Malva* herb and, *Vasaka.*

Table 1: Selected Medicinal Plants

Plant	Selected Plant	Common Name	Plant Part
A	*Cymbopogan citratus*	Lemon grass	Leaves
B	*Piper longum*	Long pepper/Maghan	Fruit
C	*Althaea officinalis*	Khatmi dana	Seeds
D	Malva herb/ Mallow	Khabazi	Seeds
E	*Vasaka*	Barg-e-Bansa	Leaves

Preparation of Selected Medicinal Plant Extracts

Medicinal plants were collected from local shop in Lahore and carefully cleaned with tap water. These plants were allowed to dry at room temperature away from direct sunlight, crushed to powder form and were sieved to obtain uniform sized particles. By putting, 10g of each plant material in 100 ml of solvents (methanol and ethyl acetate) the extracts were made. These extracts were then filtered with the help of Whatman filter paper No.1 and the extract was kept in the dark, in glass vials to avoid photo-inactivation.

Phytochemical Analysis of Selected Medicinal Plant Extracts

To confirm the occurrence of bioactive compounds in the plant extracts, various phytochemical tests were performed.

Alkaloids

Plant extract (0.5ml) was combined with 0.5ml of HCl in a test tube and placed in a boiling water bath. Wagner's reagent (0.5ml) was added that showed the production of red precipitates, indicating the presence of alkaloids (Yadav and Agarwala, 2011).

Carbohydrates

Plant extract (0.5ml) was combined with 0.5 ml of Molisch's reagent along with the addition of 0.5 ml of conc. H_2SO_4 and was allowed to stand for few minutes. A ring of bluish-purple color seen indicated the presence of carbohydrates (Morsy, 2014).

Cardiac Glycosides

A drop of ferric chloride, 0.25 ml of glacial acetic acid, and 0.5 ml of plant extract were added to a test tube. Addition of 0.25 ml of conc. H_2SO_4, created a brown ring at the interface indicating the occurrence of cardiac glycosides (Morsy, 2014).

Flavonoids

Extract (0.5ml) was treated with a 0.5ml solution of 20% sodium hydroxide (NaOH). The yellow color indicated the existence of a positive test, which vanished following the addition of diluted hydrochloric acid, leaving a colorless solution (Morsy, 2014).

Reducing Sugars

Plant extract (0.5ml) was dissolved in 0.5ml of distilled water. In a separate tube, 0.5 ml of Fehling's solutions A and B were combined and heated in a water bath. After being added to the extract, the solution underwent a color change, indicating the presence of reducing sugars (Wadood *et al.*, 2013).

Phlobatanins

Boiling a combination of 0.5ml of extract and 0.5ml of 1% hydrochloric acid was required to detect phlobatanins. The production of red precipitates revealed the existence of phlobatanins (Wadood *et al.*, 2013).

Phenols

When extract (0.5ml) was combined with 0.25ml of 5% ferric chloride solution, a deep blue or black color was observed indicating the presence of phenols in the sample (Ugochukwu *et al.,* 2013).

Steroids

About 0.5 ml of extract was mixed with equal amount of chloroform and concentrated H_2SO_4. The existence of steroids was observed by the appearance of red color in lower layer where chloroform was present (Kavit *et al.,* 2013).

Saponins

Foam creation showed the existence of saponins when plant extract (0.5ml) was mixed with 0.5ml of distilled water and shaken vigorously (Morsy, 2014).

Tannins

Few drops of ferric chloride were added to the mixture of 0.5 ml extract and 0.5ml of distilled water. The presence of tannins in the extracts was observed by the formation of green precipitates (Kavit *et al.,* 2013).

Terpenoids

Plant extract (0.5ml) was mixed in 0.5ml of chloroform and was allowed to evaporate. The mixture was provided heat after addition of 0.5ml of conc. H_2SO_4. Existence of terpenoids was confirmed by the presence of grey color (Kavit *et al.,* 2013).

Amines

0.5ml of plant extract was added in a test tube with a few drops of Ninhydrin reagent. This mixture was heated in a boiling water bath for few minutes. A purple or blue color showed the presence of amines.

Evaluation of antibacterial activity of medicinal plants

Agar well diffusion assay is a qualitative method for examining whether a substance has an antibacterial activity or not (Valgas *et al.,* 2007). The growth inhibition given by the compounds is observed as haloes because the compound diffuses equally from the well in all directions. The antibacterial potential of the plant's extracts of ethyl acetate and methanol was estimated by the agar well diffusion method (Ramakrishnan *et al.,* 2011). Muller Hinton (MH) agar plates were prepared and test bacterial cultures (gram positive (*(Bacillus* KC881030*)* and a gram-negative strain *(E. coli* GM2163*)* were spread on the agar surface. About 50µl of sample was placed in the wells and lastly, these plates were incubated for 24 hours at 37℃ and zones of inhibition (mm) were measured.

Antioxidative activity of selected medicinal plant extracts

The method of Ahmad et al was used to determine the free radical scavenging ability of the selected medicinal plants (Ahmad *et al.,* 2014). Whenever, DPPH (2,2-diphenyl-2-picryl hydrazyl) comes in contact with an antioxidant it changes its color from violet to pale yellow (Batool *et al.,* 2010). The test tube was incubated in dark for 30 minutes containing 100µl of sample and 3ml of working solution (made from stock solution). After incubation, the absorbance of the extracts and control was taken at 517nm by spectrophotometer. The percentage antioxidant (%RS) activity of these extracts was determined:

$$\%\ Antioxidant\ Activity = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{\text{Absorbance of Control}} \times 100$$

Antimitotic activity of selected medicinal plant extracts

The antimitotic activity of medicinal plants was estimated by applying the method of

Fiskesjo with little modifications. *Allium cepa* roots are typically used for this assay (Fiskesjö, 1988). The onion bulbs were cleaned properly under running tap water and were placed in the jars containing distilled water, allowing the roots to grow up to 3cm for 3 to 5 days. Later on, roots were dipped into plant extract and distilled water (control). When the roots reached the desired length, they were pulled out of the bulbs and were cleaned thoroughly with tap water. For about 24 hours, the roots were placed in the fixative. Later on, these roots were washed again with normal tap water and was placed in a preservative, containing (70% ethanol) for future usage. For the activation of roots, they were given the incubation of 15 minutes in a water bath at 55-60℃, by placing the roots in 1N HCl in an eppendorf. The activated roots were stained with Giemsa stain for 30 minutes with the application of heat. The roots that were stained, washed and the tip of the root was cut with a sharp blade and it was set on a glass slide. By using the needle of syringe, the tip of root was broken into pieces so that the cells are detached and are not overlapping. The slide was observed under the light microscope by placing a cover slip on top of smashed cells. By counting the number of dividing and non-dividing cells, the mitotic index of cells was calculated (Shachi, 2012)

$$Mitotic\ index = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells} \times 100$$

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of an antimicrobial agent is its lowest concentration which is required to entirely stop the bacterial growth. It is measured in mg/l (Kowalska-Krochmal and Dudek-Wicher, 2021). This assay was done by using the method of Sarkar et al and Klancik et al with some modifications (Sarkar *et al.,* 2007; Klančnik *et al.,* 2010).

Thin layer chromatography (TLC)

TLC was applied to separate the various components in our plant extract. This technique was performed by means of the method of Zaki et al., (1975). The components in ethyl acetate and methanol extracts were differentiated by TLC using silica gel-coated plates. The mobile phase, called the solvent system contained Hexane and Ethyl acetate in a ratio of 6:8. Selected plant extract in methanol and ethyl acetate had the same solvent system as already reported by Zaki *et al.,* (1975). The extracts were spotted on the TLC plate with the help of a Pasteur pipette. It must be concentrated enough for the components to get separated easily. The extract of *Cymbopogan citratus* was marked on a line, 1.5cm above the base line of the TLC plate. After the entire TLC plate had run, the plate was then removed and was allowed to dry. The spots on the plate were marked with the lead pencil and were observed under high and low UV rays. The plate was then placed in a jar containing iodine crystals, to observe the iodine-activated compounds. Observed spots were labelled and R_f values were calculated.

Extraction of components from TLC plate

Marked spots were scrapped off from the TLC plate. From the plant extract containing methanol, 07 components were marked, while from the plant extract containing ethyl acetate, 8 components were marked. Scratched substance was placed in the

respective vials containing methanol and ethyl acetate separately. After 24 hours, this extract was filtered and the filtrate was left at room temperature to evaporate.

Antibacterial Activity

The antibacterial activity of the selected spots was determined against the gram-positive (*(Bacillus* KC881030*)* and a gram-negative strain *(E. coli* GM2163*)*. A disc diffusion assay was implemented to check the antibacterial activity. Discs were immersed in extract for five minutes so that partially purified spot could stick to disc. These discs were dried at room temperature. For this assay, test strains were swabbed on MH-agar plates, and the discs were placed on the plates. The plates were incubated of 24 hours at 37℃ and zones of inhibition (mm) were recorded. The negative controls were the discs that were dipped in respective solvents. The component that showed the best antibacterial activity was sent for GCMS analysis.

Gas Chromatography Mass Spectrophotometry (GCMS)

Gas chromatography and mass spectrophotometry analysis of the spot that had maximum antibacterial activity was done following the standard temperature and pressure conditions (Uraku, 2015; Dilshad *et al.*, 2018).

Statistical Analysis

All the experiments were performed in triplicates. SPSS personal (version 16, SPSS Inc. Chicago) was used for statistical analysis of data.

3. RESULTS:

Phytochemical analysis of selected medicinal plants

Herbal plants that were used for curing respiratory problems mainly flu, were collected from local store of Lahore, Pakistan. Phytochemical analysis revealed the presence of different bioactive compounds in these plants. The results obtained are mentioned in table 2.

Table 2: Phytochemical Analysis of Selected Medicinal Plants

Tests name	Extract	A	B	C	D	E
Alkaloids	**Ethyl Acetate**	+	+	+	+	+
	Methanol	+	+	+	+	+
Carbohydrates	**Ethyl Acetate**	+	+	+	+	+
	Methanol	+	+	+	+	+
Cardiac Glycosides	**Ethyl Acetate**	+	-	+	+	-
	Methanol	+	-	-	+	-
Flavonoids	**Ethyl Acetate**	+	-	+	+	+
	Methanol	+	-	+	+	+
Phenols	**Ethyl Acetate**	-	+	+	-	+
	Methanol	-	+	+	-	+
Phlobatanins	**Ethyl Acetate**	-	+	-	-	-
	Methanol	-	-	+	+	-
Reducing sugars	**Ethyl Acetate**	+	+	+	+	+
	Methanol	+	-	-	+	-
Saponins	**Ethyl Acetate**	-	-	-	-	-
	Methanol	-	-	-	-	-
Steroids	**Ethyl Acetate**	+	+	+	+	+
	Methanol	-	-	+	+	-
Tannins	**Ethyl Acetate**	-	-	-	-	+
	Methanol	-	-	-	-	-
Terpenoids	**Ethyl Acetate**	+	-	+	-	+
	Methanol	+	-	-	-	-
Amines	**Ethyl Acetate**	+	+	-	-	+
	Methanol	+	-	-	-	+

(+) presence of phytochemicals, (-) absence of phytochemicals, A=is lemon grass, B= long pepper, C= Khatmi dana, D= Khabazi, E= Barg-e-Bansa

Antibacterial activity

Antibacterial activity is the capacity of any chemical or compound to suppress growth shown by bacteria while remaining non-toxic to adjacent tissues. Agar well diffusion method was used to determine the activity of selected medicinal plants. The growth of inhibition of test strains was shown by all plant extracts but Lemon grass (A) had shown maximum zone of inhibition (Table 3).

Antimitotic Activity

Only lemon grass was selected for the antimitotic activity in both ethyl acetate and methanol extracts because of its significant antibacterial potential. Results showed that the activity of lemon grass in methanol, ethyl acetate extract was 44% and 36%, respectively. In both of the extracts, minimum mitotic index was given by lemon grass extract in ethyl acetate (Figure 2).

Antioxidative Activity

Plant extract in methanol had antioxidative potential activity in the range of 68.3±1.49 while that of ethyl acetate had their activity in the range of 9.5±0.17, only for lemon grass. Extract in methanol were observed to have greater potential than that in ethyl acetate.

Table 3: Antibacterial Activity of Selected Medicinal Plant Extracts

Extract Type	Bacterial Strain Used	Zone of Inhibition (mm)					
		Control	A	B	C	D	E
Ethyl Acetate	Gram Positive	0.0±0.0	12±0.88	0.0±0.0	0.0±0.0	04±0.66	10±0.57
	Gram Negative	0.0±0.0	11±0.33	0.0±0.0	0.0±0.0	03±0.35	11±0.88
Methanol	Gram Positive	09±0.48	16±0.34	15±0.68	12±0.12	23±0.17	16±0.57
	Gram Negative	02±0.08	17±0.56	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Mean of triplicates, ± standard error of mean, Where, A= lemon grass, B= long pepper, C= Khatmi dana, D= Khabazi, E= Barg-e-Bansa

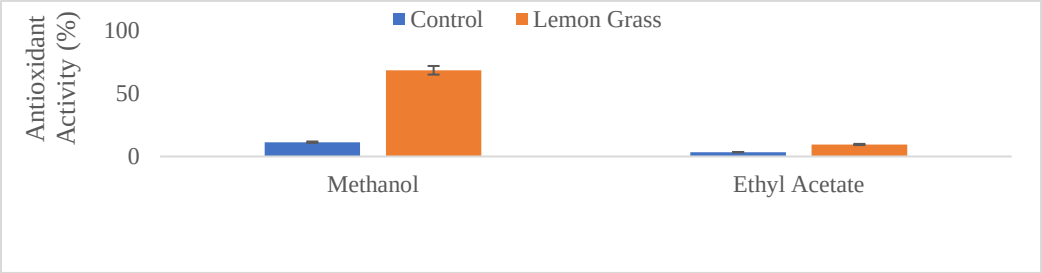

Fig. 1: Antioxidant activity of selected plant extract (Lemon grass)

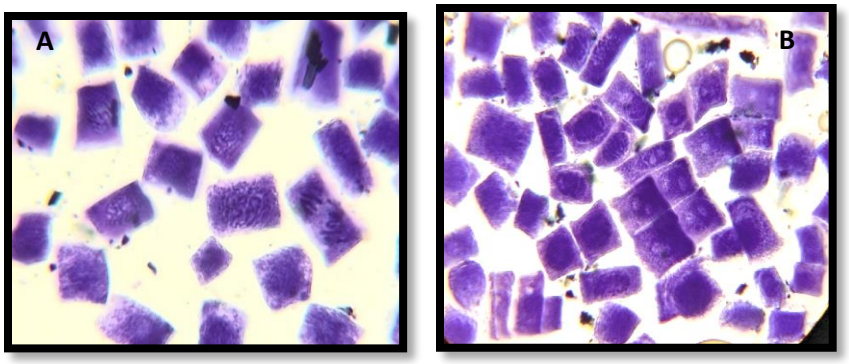

Fig. 2: Antimitotic activity of selected plant extract (A): Untreated root cells. (B): Lemon grass extract treated root cells showed antimitotic activity

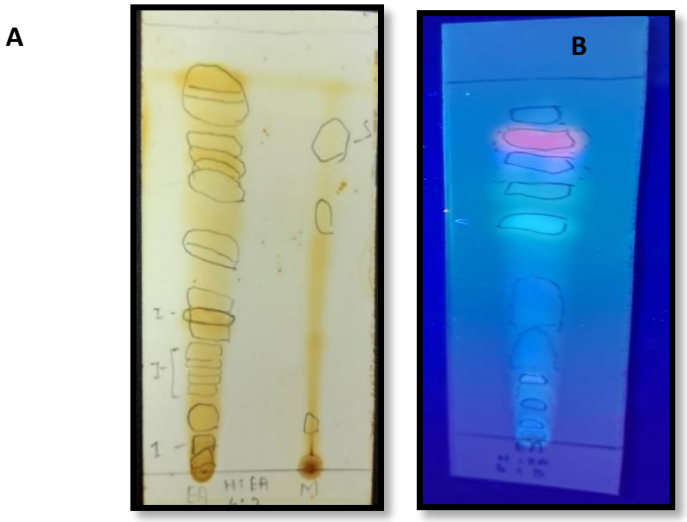

Fig.3: (A): TLC plate showing methanol and ethyl acetate extract components of *Cymbopogan citratus* **separated by developing the plate in Hexane:ethyl acetate (6:8) by giving iodine crystal tretment (B): Observing the ethyl acetate extract plate under low UV light.**

Minimum inhibitory Concentration (MIC)

Lemon grass extract in methanol showed the MIC of 31.25mg/ml against the gram-positive strain while the MIC of 15.6mg/ml was observed against the gram-negative strain after the addition of tetrazolium salt. Similarly, lemon grass extract in ethyl acetate showed the concentration MIC of 7.8mg/ml against both, gram-positive and gram-negative bacterial strain.

Thin layer chromatography

Only *Cymbopogan citratus* was found to possess the best antibacterial, antimitotic and antioxidative potential, that's why this plant was chosen for thin layer chromatography to identify the bioactive compounds present in the plant extract. About 7 spots were obtained

from TLC analysis of the plant extract in solvent system of Hexane: Ethyl acetate (6:8). All of these spots were checked for their antibacterial activity. Spot number 5 having the R_f value of 0.76 showed the maximum zone of inhibition (14mm), and was then sent for GCMS (Figure 3).

GC-MS

GCMS is a chromatographic technique for identifying, separating, and quantifying the chemicals present in a mixture. The goal of this GC-MS analysis was to identify the partly purified bioactive component with antibacterial activity in *Cymbopogan citratus* methanol extract based on its mass. The compound produced two peaks at 3.23- and 5.30-minutes retention time (Figure 4A and B). Mass analysis shown that these compounds to be amine and monoterpenoids, respectively (Table 4).

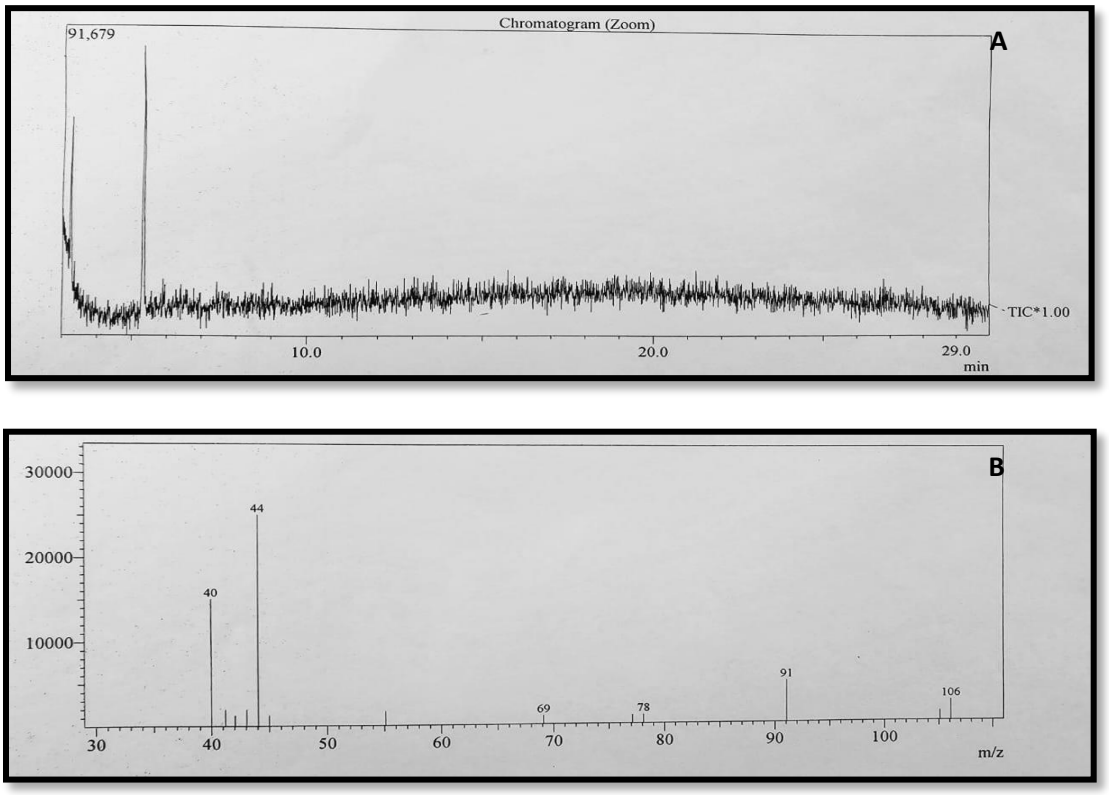

Fig. 4 (A) GCMS analysis gave two peaks of the partially purified spot at 3.23 and 5.30 minutes (B) Spectrum of the partially purified spot

Table 4: Compounds Obtained from the GCMS Analysis of Lemon Grass

Identified Compound	Formula	Molecular Weight	Retention Time	Compound Name
1-(5-bicyclo [2,2,1] heptyl) ethylamine	$C_9H_{17}N$	139	3.233	Amine
Octodrine	$C_8H_{19}N$	129	5.308	Amine
dI-phenylphrine	$C_9H_{13}NO_2$	167	3.233	Amino acid
1-adamantanemethylamine	$C_{12}H_{21}N$	179	3.233	Amine
3,7-dimethyl-2,6-octadienal.	$C_{10}H_{16}O$	152.24	3.233	Monoterpenoids

4. DISCUSSION

Plants have been the foundation of advanced traditional medical systems and to give humans novel treatments for thousands of years. Because of the presence of bioactive compounds in plants and foods they have the potential to influence metabolic processes, which can lead to improved health (Galanakis, 2017).

In the present study, medicinal plants like *Cymbopogan citratus, Piper longum, Althaea officinalis, Malva herb* and *Vasaka* were studied to identify their phytochemical elements and investigate their phytochemical properties like antibacterial, antimitotic and antioxidative. These plants were specifically utilized for the treatment of respiratory ailments. The used solvents were ethyl acetate and methanol. Methanol has the greatest degree of polarity of the chosen two solvents, whereas ethyl acetate was seen to have the lowest, which is why methanol can dissolve more of the bioactive elements of plants as opposed to other solvents (Bhebhe et al., 2016). Strong antioxidant molecules can be extracted in polar solvents, as evidenced by the polarity-dependent increase in overall antioxidant activity (Nawaz et al., 2020).

Phytochemicals like alkaloids, carbohydrates, flavonoids, reducing sugars and steroids were the components that were seen in practically all chosen plant extracts while phlobatanins, saponins and tannins were found in only a few of the extracts. Certain components have a beneficial function as antioxidants, by scavenging free radicals (Thorat et al., 2017).

The antibacterial activity of plant extracts was determined against a gram positive and gram-negative bacterium by the agar well-diffusion method. The majority of our extracts were inhibited by both of the strains, observed in the form of zones. The zones demonstrate that the majority of our plant extracts have powerful antibacterial components. Every extract of plant in methanol and ethyl acetate exhibited stronger inhibition activity contrary to gram positive bacteria strains but reduced inhibitory activity in contrast to gram negative bacteria strains. It indicated that these extracts have more capacity to inhibit gram positive

bacterium than gram negative bacterium (Subramaniam et al., 2020).

Maximum zone of inhibition against gram positive bacteria was observed by lemon grass extract in ethyl acetate (12mm) and by Khabazi extract in methanol (23mm) while all the other extracts had lesser inhibition potential than these extracts. The reason why there is a larger zone of inhibition may be because of the flavonoids or tannins found in the extract. However, reduced zone of inhibition of other extracts could be due to the lower the penetration capacity of herbal extracts, through agar, to inhibit the bacterial growth (James et al., 2007).

It is not usually preferred to estimate antibacterial potential using the disc diffusion approach since the results can vary based on the elements' levels of penetration. The broth micro-dilution method is a good solution to this issue. It provides more thorough outcomes, such as the quantitative analysis (Klančnik et al., 2010). Tetrazolium salt was used as an artificial electron acceptor that changes color from colorless to reddish pink in presence of living cells. It is utilized in this study to increase the sensitivity of bacterial growth (Romainor et al., 2014). This test examines the capacity of bacteria to stick to a micro titer plate's plastic surface under the specific temperature and nutrient requirements chosen by the investigator (De Silva et al., 2017).

To test the presence of antioxidants in the extracts, DPPH (2,2-diphenyl 1-1 picrylhydrazyl) was utilized as a free radical. Both methanol and the ethyl acetate had ability for anti-oxidation. But when matched to methanolic extracts, ethyl acetate extracts were significantly less effective radical scavengers. This anti-oxidation potential of methanol and ethyl acetate can be due to the presence of phenolic compounds such as flavonoids (Balakrishnan et al., 2014). The antioxidant performance of lemongrass leaves extract is caused by phenolic chemicals being present and can be evaluated by the free radical scavenging method (DPPH). These compounds serve as radical scavengers as well as reducing agents (Lawrence et al., 2015).

Certain chemicals, known as anti-mitotic agents, have the ability to disrupt mitosis in cells by disturbing mitosis at any time in the cell cycle (Akrayi and Abdulrahman, 2013). Antimitotic action is the arrest of cell proliferation during mitosis. One of the earliest, simplest, most trustworthy, and least expensive methods for examining the induction of chromosomal abnormality uses plant tissue, particularly root tips to explore the chromosomal aberrations (Timothy et al., 2014). *Allium cepa* was the test organism employed in this work because Allium test has a good correlation with mammalian test systems, it may be helpful in determining the antimitotic effect of herb extracts (Parmar et al., 2021).

In this study, only lemon grass extracts in methanol and ethyl acetate were used and both demonstrated notable antimitotic activity. The highest antimitotic activity was experienced by methanol extracts of lemon grass i.e., *Cymbopogan* citratus. The potential to exhibit strong anti -mitotic activity by the extracts can be due to the presence of glycosides. Lemon grass extract in methanol showed the mitotic index of 44%, while that in ethyl acetate showed the mitotic index of 36%, having a difference of

8%. This plant extract in both methanol and ethyl acetate displayed an important decline in mitotic index when matched to control; that was methanol, ethyl acetate and water (Jose et al., 2020). A considerable drop in the mitotic index and a high number of chromosome abnormalities were found in the current investigation, which indicates that aqueous extracts of lemon grass leaves had cytotoxic and genotoxic effects.

Thin layer chromatography was conducted in order to separate the various components contained in the extract. It is a low-cost, simple, and remarkably successful method for separating and analyzing the distinct biochemical pigments in tissue extract. It seeks to acquire as many bands of biochemical and pigments as possible (Anand et al., 2017). Bands were observed in each spot of methanol and ethyl acetate extracts when they were developed in hexane: ethyl acetate solvent system (6:8). The best solvent to employ is determined by the type of the substance and the adsorbent used on the plate. A development solvent must be chosen in such a way that it doesn't undergo a chemical reaction with the ingredients of the combination under investigation (Kumar et al., 2013).

In order to separate a mixture's components into sequentially eluting individual pure compounds, a gas chromatograph (GC) was used. A mass spectrometer was then used to identify pure unknowns (Grayson, 2016). By contrasting the retention times and peak areas of the different compounds and by deciphering the mass spectra, the compounds were identified. Numerous of them are employed in industry for a number of purposes, including flavor, antioxidant, anti-inflammatory, antibacterial, pesticide, and cancer prevention. The bioactive component in *Cymbopogan citratus* that gives this plant its antibacterial properties was found via GCMS analysis. 1 major component was identified via GCMS analysis 3, 7-dimethyl-2, 6-octadienal i.e., citral, a bioactive ingredient that is reported to be existing in *Cymbopogan citratus* and is a mono-terpenoid (Dangol et al., 2023).

Some other components were also found that were not present in lemon grass but were found in other plants. Like 1-(5 bicyclo [2, 2, 1] heptyl) ethylamine was found in Betel (Sarkar and Sawardekar, 2022), dI-phenylphrine was observed in papaya (Sundaram and Prabhakaran, 2017), 1-adamantanemethylamine in Asofeyeje (Okereke et al., 2017) and lastly Octodrine was observed in the leaves of N. officinale (Shaheen and Ahmad, 2020).

CONCLUSION:

In conclusion, these locally used medicinal plants such as *Cymbopogan citratus, Piper longum, Althaea officinalis, Malva herb* and *Vasaka* were found to exhibit antibacterial properties, antioxidant and antimitotic potential because of presence of certain phytochemicals in them. These selected plants have demonstrated pharmacological benefits, particularly in the treatment of colds, coughs, and as a daily pain reliever. Moreover, future research should concentrate on analyzing the phytochemical components at molecular level so these herbs can be effectively used in pharmaceutical industry in place of synthetic compounds.

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