

Phytochemical screening and antibacterial activity of essential oils of *Cymbopogon citratus* and *Ocimum americanum* : a comparative account

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ABSTRACT

The present study highlights the extraction of essential oils from *C. citratus* and *O. americanum* using Microwave Hydrodiffusion and Gravity method (MHG) and Hydrodistillation Method (HD), the qualitative and quantitative screening along with determination of antibacterial potential. For the study, extraction of essential oils was performed using Microwave Hydrodiffusion and Gravity method and Hydrodistillation Method. Comparative study was performed to check the total flavonoid content (TFC), total phenolic content (TPC), biochemical test, thin layer chromatography (TLC) and determination of antibacterial activity of the essential oils. The results of the study depicted that the MHG method for extraction of essential oils was much better compared to extraction by HD method as supported by the biochemical tests, TFC, TPC and TLC results. However, the essential oils did not show antibacterial activity. More study related to the antimicrobial activity needs to be performed. Essential oils form an integral part of the pharmaceutical and healthcare industry hence, detailed study regarding the isolation and application of essential oils is required. The present study suggests the use of MHG method for extraction of essential oils.

Figures : 05

References : 27

Table : 01

KEY WORDS : Antibacterial activity, *Cymbopogon citratus*, Essential oils, *Ocimum americanum*, Phytochemicals.

Introduction

Medicinal plants play a vital role in public and primary health care¹⁸. Medicinal herbs are therapeutic agents indispensable in the primary health care system in maintaining exceptional well-being and health condition⁸. In recent times, almost 10,000 medicinal herbs have been documented and about 4500 have been examined for the bioactive components and pharmacological assays¹⁷.

Essential oils obtained from medicinal plants show a wide range of pharmaceutical activities like anticancer, antimicrobial, and anti-parasitic activity. *Cymbopogon citratus* and *Ocimum americanum* are majorly used in traditional medicine. The crude extracts and essential oil extracted from *C. citratus* and *O. americanum* contain several classes of secondary metabolites which show bioactivities.

Cymbopogon citratus is a medicinal plant belonging to the family Poaceae. It is widely known as lemongrass. It has anti-inflammatory and anti-oxidant

properties hence it is used in medicine. *Cymbopogon citratus* depicts potential antimicrobial activity. The essential oils, aqueous extracts, phenolic compounds and other extracts extracted from this plant are industrially and economically important. The extracts are used in the perfumery, cosmetics and pharmaceuticals¹⁹. Additionally, it contains a considerable group of flavonoids, essential oils, phenolic compounds and other phytochemical constituents which possess pharmacological activities such as anti-obesity, anti-bacterial, anti-fungal, anti-nociceptive, anti-oxidants anti-diarrheal and anti-inflammatory properties which could enhance health²¹.

Ocimum americanum plant is widely used as a medicinal plant as traditional medicine for treating various types of diseases and lowering blood glucose, ache (tooth) antiseptic, bactericide, carminative, cold, cough, diarrhea, dysentery, respiratory, stomach tonic, tuberculosis tumor, and also to treat cold, fever, parasitic infestations on the body and inflammation of joints and headaches¹. These

TABLE-1: Phytochemical Screening of *C. citratus* and *O. americanum* essential oils extracted using MHG and HD method.

S. No.	Plant	Sample	Test Name	Reagent	MHG	HD
1.	<i>C. citratus</i> <i>O. americanum</i>	Leaf	Alkaloid	Dragendroffs	+	-
		Leaf			-	-
		Flower			+	-
2.	<i>C. citratus</i> <i>O. americanum</i>	Leaf	Tannin	Lead acetate	+	+
		Leaf			+	+
		Flower			+	+
3.	<i>C. citratus</i> <i>O. americanum</i>	Leaf	Flavonoid	Alkaline	+	-
		Leaf			-	-
		Flower			-	-
4.	<i>C. citratus</i> <i>O. americanum</i>	Leaf	Amino acid	Ninhydrin	-	-
		Leaf			-	-
		Flower			-	-
5.	<i>C. citratus</i> <i>O. americanum</i>	Leaf	Sugar	Fehling solution A & B	+	-
		Leaf			+	-
		Flower			+	-
6.	<i>C. citratus</i> <i>O. americanum</i>	Leaf	Terpenoids	Chloroform & H ₂ SO ₄	-	-
		Leaf			-	-
		Flower			+	-

essential oils are being used as pharmaceutical agents because of their analgesic, anticancer, anti asthmatic, antistress, antimicrobial, antioxidant, antidiabetic and insecticidal activities²⁰. Thus the medicinal plants are useful for healing as well as for curing human diseases in the presence of phytochemical constituents²¹.

The aromatic plant secretes phenolic compounds. Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups recreated by plants mainly for protection against stress. Phenolics are generally the largest group of phytochemicals that account for most of the antioxidant activity in plants or plant products¹². The organic compounds separated using thin layer chromatography because of the simplicity and rapidity. It is often used to monitor the progress of organic reactions and to check the purity of products²⁴.

Essential oils have been used from ancient times as food flavorings, food preservatives, pharmaceuticals, and non-traditional medicine²². Essential oils have proved

their activity in a wide range of pharmaceutical products like anticancer drugs, antimicrobial agents, anti-inflammatory products^{10, 14}. Hence, applying an effective extraction method is needed for the preparation of high quality and high yield volatile compounds. Conventional methods for extracting essential oils from plants are organic solvent extraction, hydro distillation, and steam distillation¹⁶. In the present study, a preliminary phytochemical investigation of essential oils of *C. citratus* and *O. americanum* was carried out. Also, the efficacy and antibacterial activity of both medicinal plants was evaluated.

Materials and Methods

Collection of Plant samples

Lemongrass (*Cymbopogon citratus*) and *Ocimum americanum* leaves were collected from medicinal plant garden of Guru Ghasidas Vishwavidyalaya. The plant materials were rinsed and washed in 400 ml of distilled

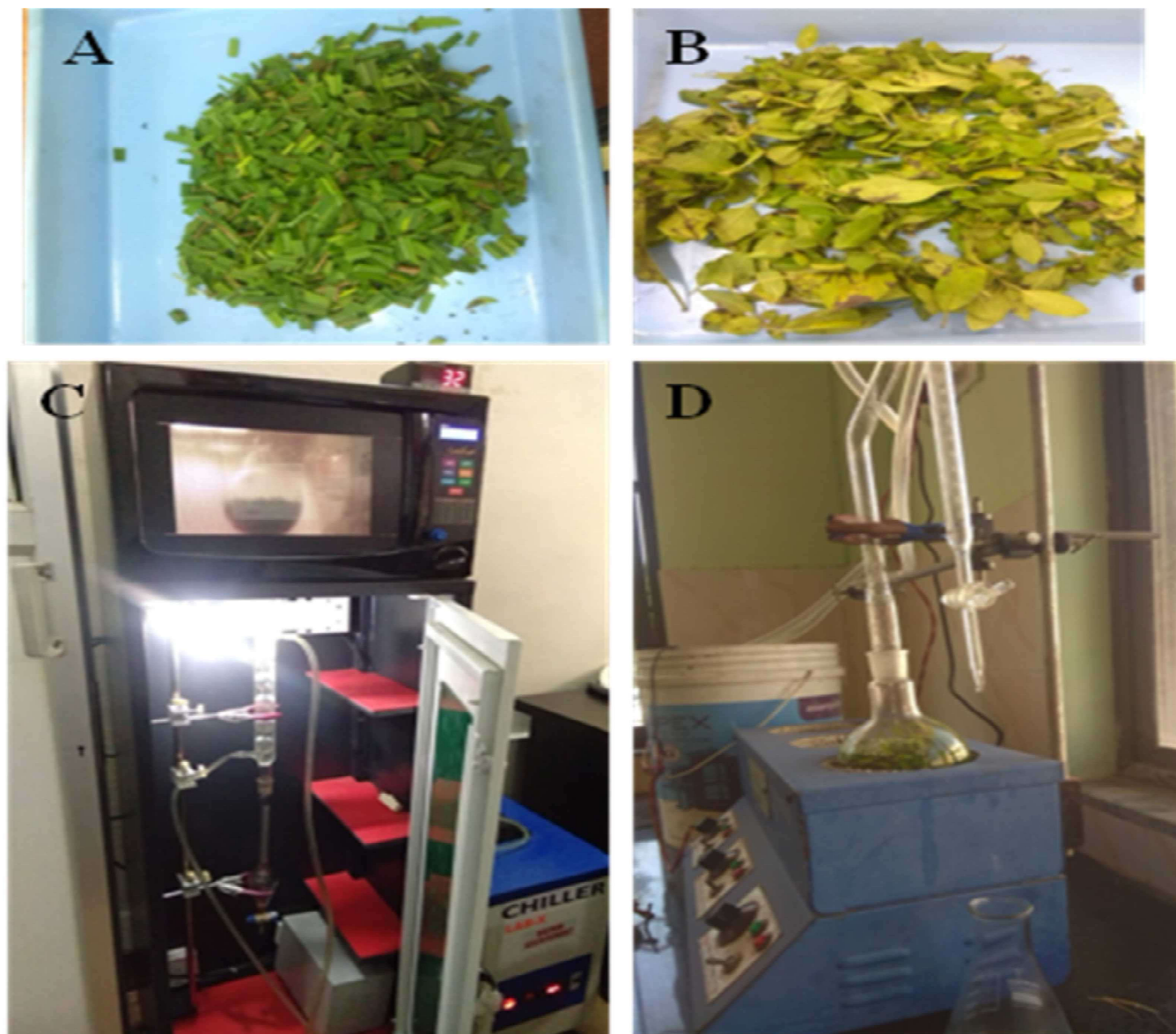


Fig. 1: A: Leaves of *Cymbopogon citratus*; B: Leaves of *Ocimum americanum*; C: Extraction of essential oil using MHG method; D: Extraction of essential oil using HD method.

water to remove dirt on surface of leaves. The plant materials were dried at room temperature for a week then kept in a sealed plastic bag at ambient temperature and protected from the light. The leaves were homogenized using mortar and pestle.

Extraction

Solvent-free Extraction, Microwave Hydrodiffusion and Gravity (MHG)

The fresh leaves, cut into small pieces with the help of scissors were accurately weighed 80 g and kept it in the extraction vessel without the addition of any solvent. An immediate surge of high Microwave power 60% (510W) was applied for 2 minutes followed by natural cooling of the system and then the subsequent surge of 40% (340 W) microwave power for 4 min was applied.

The basic idea was to help quick rupture of the oil gland. The system was allowed to cool down naturally which was followed by sustained microwave firing at 20% power level (170 W) for 10 min with 2 minutes time interval. The oil was collected at the bottom of the apparatus. The extracted oil was dehydrated by passing through anhydrous sodium sulphate and then stored at 4 °C in sealed centrifuge tubes for further analysis.

Conventional extraction

Clevenger apparatus

The Clevenger apparatus used for extracting the essential oil with steam that circulates through plant material. The plant material was boiled in 2 to 3 liters of water. The vapor moved to the condenser where vapor turned to droplets and collected in a graduated burette.

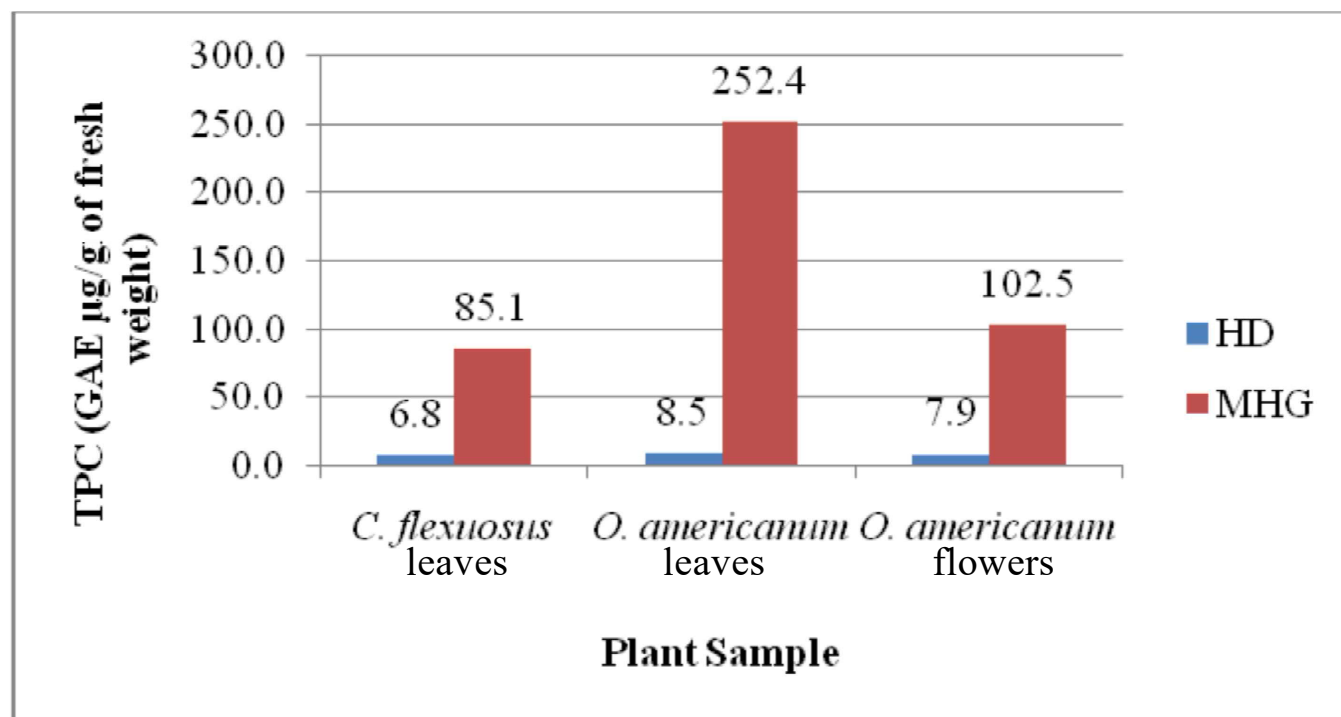


Fig. 2: Determination of Total Phenolic Content.

Hydrodiffusion (HD)

The 25 g of fresh leaves were subjected to HD in a classical apparatus and extracted with 200 ml of water for 3 h (unit on essential oil was obtained). The oil so collected at the end of the extraction was dehydrated by passing through anhydrous sodium sulphate and then stored at 4 °C in sealed centrifuge tubes for further analysis.

Biochemical Test

Biochemical tests were carried out. The test for Alkaloids carried out using Dragendorff's reagent (solution of Potassium Bismuth Iodide), test for tannins by using basic lead acetate and distilled water, test for flavonoids using sodium hydroxide solution, test for amino acids by using Ninhydrin reagent, test for Sugar Fehling solution A and B and distilled water and Test for Terpenoids Chloroform and concentrated sulphuric acid.

Thin Layer Chromatography

TLC – 0.2 mm thickened TLC Aluminum sheets silica gel 60 F254 was purchased from local

Vender of S D fine Chemical Limited.

Thin-layer chromatography (TLC) was used in this study. The total phenolics of the extracts were determined using 1ml ethanolic extract; the test sample (0.2 ml) was mixed with 0.6 ml of water and 5ml of Folin- Ciocalteu's phenol reagent. After 5 ml, 4 ml of a saturated sodium carbonate solution was added to the mixture and the volume was made upto 9ml with distilled water. The reaction was kept at room temperature in the dark for 45 minutes and after centrifuging the absorbance of blue color

from UV- spectrophotometer different samples were measured at 765 nm. The phenolic content was calculated as gallic acid equivalents GAE/g of extract based on a standard curve of gallic acid. All determinations were done in triplicate.

Total Flavonoid content (TFC)

Total Flavonoid content was determined by the aluminum chloride method using quercetin as a standard. 1 ml of water was added to a volumetric flask (10 ml volume). 1 ml of ethanolic extract solution added in 4 ml of distilled water with 5% Sodium nitrite solution, 0.3 ml of 10%, incubated at room temperature for 5 minutes. After incubation added 10% aluminum chloride solution then again incubated for 6 min at room temperature, 2 ml of 1M sodium hydroxide was added to the reaction mixture. The final volume was made upto 10 ml with distilled water. It was incubated for 15 min. The absorbance of the sample was measured against the blank at 510 nm using a spectrophotometer.

Antibacterial Activity

The nutrient growth medium *i.e.* nutrient agar was obtained from Hi-media, Mumbai and was used for the growth of bacteria. The bacteria *E. coli* was used for the antimicrobial study. The pour plate method was used for antibacterial activity. The nutrient agar was poured in petri plates and wells were formed using sterile cork. The extract (0.5ml concentration) was loaded into the wells. Antibacterial activity of extracts and essential oils were evaluated against *E. coli*. Essential oils containing

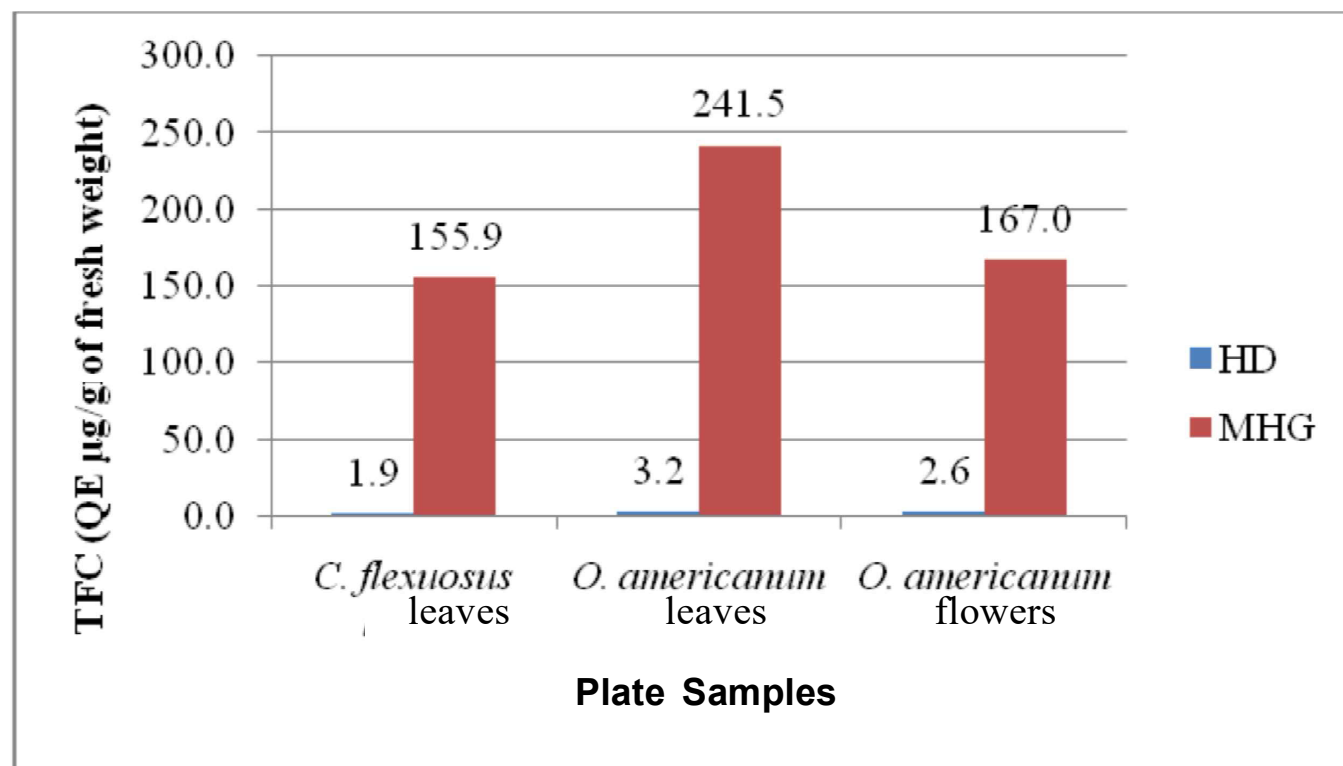


Fig. 3: Determination of Total Flavonoid Content

phenolics and flavonoids were extracted from *Cymbopogon citratus* and *Ocimum americanum* and were assessed for their antimicrobial activities against *E.coli*.

Result and Discussion

Traditional methods like Hydrodistillation (HD) are generally used for the extraction of essential oils however, these traditional techniques are expensive, consume time and energy and also require use of solvents¹¹. Microwave Hydrodiffusion and Gravity (MHG) method is a recent system for the extraction of essential oil which provides high yield, short extraction period and low energy requirement, and these advantages make it a cost effective and environment friendly process⁵.

In the present a comparative study was conducted for the extraction of essential oils of *Cymbopogon citratus* and *Ocimum americanum* using Hydrodiffusion (HD) and Microwave Hydrodiffusion and Gravity (MHG) method (Fig.1). A comparative analysis was performed to conclude the advantages of MHG method over HD method. Phytochemical Tests prove useful to identify and isolate pharmacologically distinct compounds like flavonoids, phenols, alkaloids and glycosides that allow the use of plants for different ailments²³. The essential oils of *C. citratus* and *O. americanum* were used to perform biochemical tests (Alkaloid, Tannin, Flavonoid, Amino acids, Sugar and Terpenoids) to detect the presence of phytochemicals in the essential oils (Table-1).

The results of the study depicted that the essential oils extracted using MHG method showed presence of alkaloids and tannins in both the plant samples, however, it was found absent in the essential oils extracted using HD method. Similarly, in the sugar test, sugar was found present in test samples of MHG method and absent in HD methods. While, amino acid was found absent in both the plant samples. Thus, it could be concluded that the essential oils extracted using MHG methods showed presence of phyto-compounds with minimum degradation while in the HD method it was observed that the phytochemicals got degraded during the extraction presence. The phytochemical screening helps in identification of therapeutically important compounds like alkaloids are mainly responsible for protection and work as an antimicrobial agent bacteria and fungi. Alkaloids are also used as analgesic and inhibitor of acetylcholinesterase⁷. Flavonoids serve as nutrients and provide antioxidant and anti-inflammatory properties. Reducing sugars aids in weight loss, digestion and improves mental health. Glycosides are used as an active ingredient in drugs associated with heart problem due to its ability to dilate coronary arteries. Phenolic compounds protect against pathogenic infections. Tannins possess anticarcinogenic, antioxidant and antimutagenic properties^{2,3,7}. Workers⁷ studied the phytochemical study of *Cymbopogon citratus* oil and confirmed presence of phytochemicals like tannins, saponins and terpenoids.

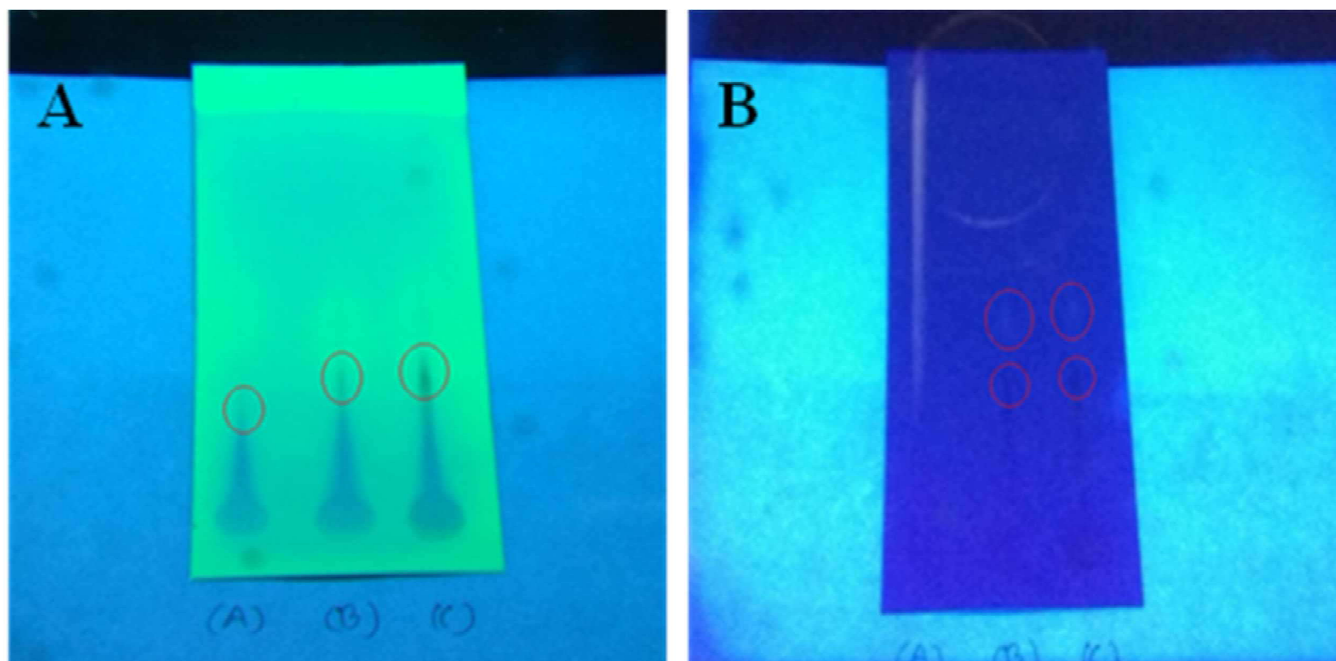


Fig. 4: TLC visualization A: Fluroscent Light; B: UV Light

There was report of phytochemical analysis of *Ocimum sanctum* leaf extract and presence of alkaloids, glycosides, phenols and tannin⁹. Similarly, there were studies on phytochemical screening of *Ocimum basilicum* and reported the presence of pharmaceutically active secondary metabolites like phenolic, flavonoids, carbohydrates, glycosides and tannins²⁴.

The essential oils were also checked for Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). In the TPC study, it was observed that the essential oils extract using MHG method showed highest yield. The highest phenolic content was observed in leaves extract of *O. americanum* (252.4 µg/g) followed by *O. americanum* flower extract (102.5 µg/g) and *C. citratus* leaves extract (Fig.2). Similarly, in the TFC study also essential oils extracted using MHG activity showed higher yield. *O. americanum* leaves showed highest yield (241.5 µg/g), followed by *O. americanum* flowers (167.0 µg/g) and *C. citratus* leaves extract (155.9 µg/g) (Fig. 3). Phenolic components like flavonoids, terpenoids are major antioxidants that show scavenging effect on free radicals and reactive oxygen species¹⁵. It was reported that phenolic compounds possess the capacity to destroy radicals as they consist of hydroxyl groups⁶. Phenolic compounds provide hydrogen atoms from the hydroxyl groups to radicals leading to formation of stabilized phenoxy radicals. Thus, phenols play a vital role in antioxidant activity and determination of total phenolic compounds is important for the determination of antioxidant potential of the plant extract. Flavonoids show many health promoting properties like antioxidant, anti-

allergic, anticancer, anti-inflammatory and anti-viral activity⁷.

Separation of phytochemicals can be achieved used Thin Layer Chromatography (TLC). TLC majorly depends on the solvent system and the migration of active compound is detected by the formation of the spot. Further various Light impressions are used to visualize the spots and the quantification of the sports is done through Retention factor (Rf)². Thin layer Chromatography was also performed for the *O. americanum* leaves and flowers extract, and *C. citratus* leaves extract. The TLC plates were observed under UV and Fluorescent light in UV-visible chamber. The Rf value for *O. americanum* leaves and flowers extract was found to be 0.58 and 0.47 while the Rf value of *C. citratus* leaves extract was found to be 0.35 (Fig. 4). During the TLC analysis of *Ocimum gratissimum* extract, there was observed presence of various phytochemicals in the extract like alkaloids, tannins and terpenoids¹⁴. Similarly, there was studied the TLC profiling of *Cymbopogon citratus* to isolate and identify the biomolecules of the essential oil⁴.

The plant samples were checked for their antibacterial activity against *E.coli*. For the study, three test samples were selected and tested for their antimicrobial potential. All the extracts did not show potential antibacterial activity. Much study regarding the antimicrobial activity of the plant extracts needs to be performed. Workers⁷ checked antimicrobial activity of leaf extract of *Ocimum sanctum* against gram positive and gram negative bacteria and reported that the antibacterial activity of the plant was due to the presence of

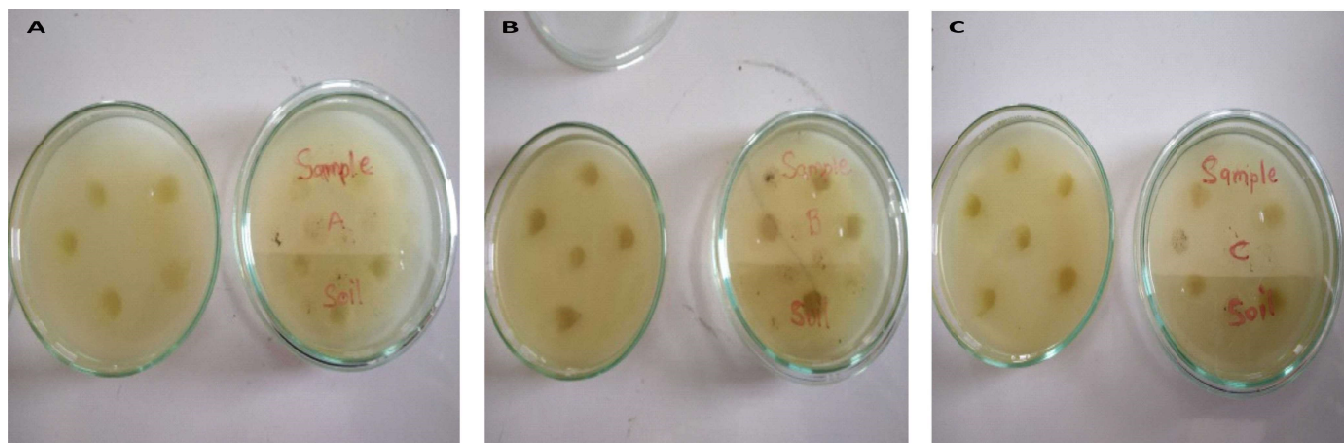


Fig. 5: Sample (A) test, Sample (B) test, Sample (C) test treat in Nutrient agar.

phytochemicals. Others²⁷ tested the antibacterial potential of *Cymbopogon citratus* extracts (aqueous and methanolic and essential oil) against multidrug resistant bacteria species and found that the essential oil of *C. citratus* showed highest antibacterial activity followed by the methanolic extract while, the aqueous extract did not show any activity (Fig. 5).

Conclusion

The present study deals with the extraction of essential oils of *C. citratus* and *O. americanum* using Microwave Hydrodiffusion and Gravity Method and Hydrodistillation method. The study focuses on the use of traditional and recently developed techniques for extraction of essential oils and its effect on the

phytochemicals of the plant was undertaken. The phytochemical screening of the essential oils revealed that the phytochemicals like alkaloids, phenols, flavonoids, terpenoids and sugars were more profoundly present in essential oils extracted using MHG method compared to the HD method. Similar results were obtained in the Total Phenolic content and Total Flavonoid Content study. The TLC studies of the essential oils demonstrated the presence of active phytochemicals which could be used as efficient antimicrobial agents. The antimicrobial study of the plant extracts against *E.coli* confirmed the same. Thus, in the above study the authors strongly support the use of MHG method for the extraction of essential oils using medicinal plants.

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