Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3405

EXTRACTION, CHARACTERIZATION, ANTIBACTERIAL AND ANALGESIC ACTIVITY OF ESSENTIAL OILS OBTAINED FROM MICROCEPHALA LAMELLATA AND ALHAGI MAURORUM

Amjad Hussain[1], Sultan Ayaz[1*], Abid Rashid[1], Syed Muhammad Ali Shah[1], Muhammad Akram[1]

[1*]Department of Eastern Medicine, Government college, University Faisalabad-Pakistan

***Corresponding Author:** Sultan Ayaz
*Department of Eastern Medicine, Government college, University Faisalabad-Pakistan,
E-mail: sultan.ayaz@gcuf.edu.pk

Abstract
The primary and secondary metabolites of medicinal plants possess pharmacological activities such as anti-bacterial, anthelmintic, analgesic, anti-rheumatic etc. The current study evaluated essential oils of *Microcephala lamellata* (ML) and *Alhagi maurorum* (AM) for their primary and secondary metabolites, antibacterial and analgesic activities. The phytochemical screening of essential oils of both plants showed the presences of phenolic compounds, terpenes and terpenoids. The thin layer chromatographic studies with different mobile phases separated different compounds in both oils. The gas chromatographic studies also confirmed the presences of phenolic compounds terpenes and terpenoids. Analysis by UV-visible spectroscopy showed the absorption maxima at 230-235nm and 280-285nm, respectively. The FTIR analysis, indicated the presence of functional groups such as phenols (OH stretching vibrations), alkanes (CH stretching vibrations), carboxylic acid (C = O carboxylic acid stretching vibrations), methyl (CH3 bending vibrations), and carbonyl (C-O stretching vibrations). The antibacterial studies as evaluated by disc diffusion method have revealed that AM essential oils have antibacterial activity against *B. subtillis, E.coli, P. aeruinosa and S. aureus* whereas the ML essential oils have low antibacterial activity against these microbial strains. Both essential oils showed significant analgesic activity in the dose dependent manner as evaluated by acetic acid induced writhing test.

Keywords: *Microcephala lamellata*, *Alhagi maurorum*, Essential oil, Phytochemical analysis, FTIR, Gas chromatography.

Abbreviations: ML (*Microcephala lamellata)*, *AM* (*Alhagi maurorum*), EOs (Essential oils), FTIR (Fourier transform infrared spectroscopy), TLC (Thin layer chromatography),

Introduction
Most of the allopathic drugs are derived from the natural sources. The allopathic drugs such as Atropine (*Atropa belladonna)*, Ephedrine (*Ephedra gerardiana)*, Digoxin, Digitoxin (*Digitalis purpura*), vincristine, vinblastine & vindesine (*Catharanthus rosues)*, strychnine & brucine (*Strychnos nux-vomica*), atropine (*Atropa belladonna*), quinine (*Cinchona ledgeriana)*, caffeine (*Coffea arabica*), colchicine (*Colchicum autumale*), nicotine (*Nicotiana tabacum*) and cocaine

(*Erythroxylum coca*) are derived from plants and still widely used in the form of modern medicines (Petrovska, 2012).

According to WHO report, about 80% of population use herbal medicines in developing countries in tablets, capsules, decoction, concoction and syrup form for the treatment of different diseases. It is estimated that about 70%, 49%, 48% and 42% of population of Canada, France, Australia and United States use herbal medicines respectively for their primary health care (Cassileth & Chapman, 1996; WHO, 2002).

Alkaloids, Anthraquinones, glycosides, monoterpenes, sesquiterpenes, Tannins, Phenols, saponins, Phytosterols, Terpenoids, Triterpenoids, Phlobatanins, minerals, vitamins, saponins, flavonoids, isoflavonoids, terpenoid, isoterpenes, terpenes, polyphenols and phlobatannin are the phytochemicals /bioactive natural products of medicinal plants having various pharmacological actions against different diseases (De Silva *et al*., 2017).

The use and popularity of the herbal medicines increase day by day. The medicinal plants contain primary and secondary metabolites. These metabolites possess pharmacological activities such as antipyretic, anthelmintic, narcotics, diuretics and astringent (Petrovska, 2012). Furthermore, the popularity of the herbal medicine is due to the scientific evidences-based studies by using advanced techniques such as NMR, FTIR, UV-spectroscopy, TLC, HPLC, Mass spectroscopy and atomic absorption (Khattak *et al*., 2020).

Microcephala lamellata (Bunge) Pobed, (ML) belongs to the family "*Asteraceae*" is widely distributed in district Kalat, Noshki and Ziarat, Balochistan, Pakistan. The herb of *Microcephala lamellata* is erect up to 50 cm tall, repeated branches, with smooth surface, marked with parallel grooves, obtused angled between two successive nodes. April to August is the flowering period. Traditionally it is used for the treatment of Jaundice, colic pain, persistent fever and dysentery in children (Abbas *et al*., 2012).

Alhagi maurorum(AM) belongs to family *Fabaceae/Leguminosae* is highly branched shrub. The height of AM is about 1.5 to 4 feet. Traditionally the plant is used as expectorant, purgative, diuretic and diaphoretic. The plant is also used for rheumatism, migraine, warts and hemorrhoid (Ahmad *et al*., 2015).

Material and Methods

Collection of plant material

Fresh leaves and flowers of *M. lamellata* and *Alhagi maurorum* were collected in the month of April from the district Kalat, Balochistan, Pakistan and identified by Dr. Sultan Ayaz assistant professor, Department of Eastern medicine Government College University Faisalabad. The samples of ML and AM were submitted in the department of Eastern medicine and the identification/Herbarium numbers are, DEMHN:300/2021 and 301/2021 respectively.

Arrangement of animals

Mice were arranged from the animal house of Dow medical University and health sciences, Karachi.

Selection of experimental Animals

Albino mice, weight 25-30 grams were used for the current study.

Housing conditions of the animals

Temperature and relative humidity of the selected animals was maintained at 23°C (±2) and 50-55% respectively. Animals were kept in polypropylene cage (5 mice per cage) and exposed to 12:12 light/dark cycle. The animals were fed the standard food pellets and free access to water during the entire period of research.

Ethical committee approval

All animal experimental protocols were approved from the animal ethical committee of GC University Faisalabad. The reference number of the approved protocols of animal study is DEMEC-320/2021.

Extraction of Essential oils (EOs) from leaves and flower of ML and AM

Fresh leaves and flowers of ML and AM were washed with distilled water. After that the both samples of the plants were air dried and subjected for further drying in an oven at 50^{o}C for two days. The dried material of ML and AM were chopped and subjected to clevenger type distillation apparatus for extraction of essential oils (EOs). The distillation rate of the apparatus was 3ml/minutes which is according to the methodology of European Pharmacopeia (European Pharmacopoeia .7.0, 2022) described as below:

200g of the both plant's material was soaked in 500ml of water in the two separate distillation flask and boiled the water. After 20 minutes of distillation, the distilled mixtures contains both oils and water. The distilled mixtures were taken in separate flasks and added chloroform in each flask. The oil and chloroform were miscible and separated from the water. Furthermore, the chloroform was evaporated from the mixtures of oil and chloroform. Then, cotton was fixed in the separate funnel and sodium sulfate powder was sprinkled on the cotton of cotton of each funnel. The oils were passed through cotton fixed funnels. The trace amount of water was absorbed by sodium sulfate. The oils were separated with the help of chloroform and dried through sodium sulfate. Dark sealed air tight glass vials were used for the collection of the oils. The vials were stored at 4^{o}C in the refrigerator.

Phytochemical screening of essential oils of ML and AM

Detection of phenolic compounds

Libermann's test

5ml essential oils of ML and *A.maurorum* were treated with 2ml sodium nitrate ($NaNO_3$) and 3ml concentrated sulfuric acid (H2SO4) in separate test tubes. Deep green colour is obtained which change into red colour when dilute with water. When sodium hydroxide was added to it, the colour of the solution turns to deep blue colour.

Detection of Terpenes

Baeyer's Test

Alkaline potassium permanganate solution was mixed to essential oils of *Microcephala lamellata* and *Alhagi maurorum*. Pink colour of potassium permanganate was disappeared and may or may not form brown precipitate of manganese oxide.

Detection of terpenoids

Salkowski's test

3-5ml essential oils of ML and AM were dissolved in 2ml of chloroform in separate test tubes. The test tubes were heated in water bath to form concentrated solutions. Furthermore, 2-3ml of concentrated sulfuric acid was added to both essential oils containing test tubes and mixed well and allowed to stand for few minutes. Golden yellow layer at the bottom of the test tubes indicated presence of terpenoids (Shaikh & Patil, 2020).

Test for Terpenoids

Salkowski Test

Methanolic solutions of EOs of ML and AM were poured in two separate test tubes, then added concentrated sulfuric acid. Red, blue or purple colour indicates presence of terpenes.

Spectroscopic Characterization of essential oil of ML and AM

Ultra Violet (UV) Visible Spectroscopy

Thermo Scientific, Switzerland model 201 UV -Visible Spectrophotometer was used for characterization of essential oils of ML and AM. Methanol was used for dilution of EOs. The diluted oils were subjected to the sample cell and scanned from 200-800nm of wavelength. Whereas methanol was used as a blank and the spectra of the oils were recorded by using "INSIGHT" software.

Fourier Transform Infrared Spectroscopy

Thermo Scientific, USA model, Nicolet iS10 ATR-IR FTIR was used for characterization of EOs of ML and AM. The scanning range of the apparatus was 4000-500c/m with a resolution of 4c/m. The obtained IR spectra of the oils were interpreted with the known wave number functional groups in the OMNIC, USA software.

Chromatographic characterization of essential oil of ML and AM

Thin Layer Chromatography

Merck silica gel pre- coated TLC plates (60 GF254, 250µm) were used for chromatographic evaluation of EOs of ML and AM. Four mobile phase solvent system such as Toluene:Ethyle acetate, Hexane:Methyl chloride, Light petroleum:chloroform and Carbon tetra chloride:Acetone: Glacial Acetic Acid at ratio of 93:7, 5:1, 70:30 and 15.2:3:1 v/v were respectively used. 10-30 µg samples of both essential oils were loaded at the start line drawn at the bottom of the plates and placed in mobile phase chamber and allow to ascent the mobile phase up to 10cm. To visualize the bands of the separated compounds, 5% sulfuric acid solution in ethanol was sprayed. After drying the plates, the plates were sprayed with 10% vanillin solution in methanol. The plates were heated at 100^{o}C for 10 minutes. All the measurement were carried at 20^{o}C and the R_f values for the band of the separated compounds were measured accordingly (Nickavar *et al*., 2014).

Gas Chromatography

The gas chromatographic characterization of both EOs was made on trace-1300 gas chromatograph (Thermo scientific, Switzerland). The procedure and chromatographic conditions were based on previous related studies. K. Hüsnü Can Başer *et al*. 2012; and Ester R. Chamorro *et al*., 2012). Helium gas was used as the carrier gas at a constant pressure of 65Kpa. The EOs was diluted 1:200 in cyclohexane and 1µl of the solution was injected to the chromatographic system in a spilt ration of 1:25 and a solvent delay of 2 minutes. The increasing oven temperature was programmed from 60- 240^{o}C with a step of 3^{o}C/min until reaching 240^{o}C. The injector temperature was kept at 230^{o}C and the FID at 280^{o}C. The fused silica capillary column of 30m length, 0.32mm ID, 0.25mm film thickness (Trace gold, TG-5MS) was used during the study. Calculation of peak area % was performed on the basis of FID signal using chromeleon TM software.

Evaluation for Biological Activities

Anti-bacterial Activity

The Kirby-Bauer test/disk diffusion method (DDM) was used for evaluation of antibacterial activity of EOs of ML and AM (CLSI, 2012). Gram positive and gram-negative bacterial cultures were grown on the Mueller Hinton Broth. With sterile saline solution, the cultures were adjusted to approximately 10^5 CFU/ml. 500 ml of bacterial suspensions were spread on the surface of the plates and a sterile cotton swab were used to achieved the homogeneous distribution of microbial growth on the test and control plates. The plates were allowed to dry for few minutes at room temperature. Essential oils of *Microcephala lamellata* and *Alhagi maurorum* were dissolved in 10% aqueous dimethyl sulfoxide with Tween 80. The solution was filtered through a size of 0.45µm membrane filter. Whatman, Japan, sterilized discs were soaked with 50µl of 1:1, 1:5, 1:10 and 1:20 concentrations of the corresponding essential oils of ML and AM and placed on the agar surface

with the help of sterilized forceps and gently press down to confirm good contact with the surface (NCCLS, 2002). As a vehicle control, a moistened paper disc containing aqueous DMSO was put on the seeded petri dish plates. As a reference control, a standard disc containing 25µg/disc of streptomycin was used. To avoid the evaporation of the test samples, all the plates were sealed with sterile laboratory parafilm. For diffusion of the oil, the plates were kept for 30 minutes at room temperature. Then, incubate the plates upside down at 37°C for 24hrs in the incubator. After 24hrs, the diameter of the zones (zone of inhibition) was measured with the help of vernier caliper.

Analgesic Activity (Acetic Acid Induced Writhing Test)

For evaluation of analgesic activity, acetic acid induced writhing test was performed for essential oils of ML and AM which was described by Siegmund *et al* in 1957. Thirty mice were divided in to six equal groups and fasted for 12 hours before starting the experiment. Animals were given free access to water ad libitum. Group-I (control group) and group II were respectively administered sweet almond oil (10mg/kg) and diclofenac sodium (50 mg/kg), whereas group III,IV,V and VI were administered essential oil (6.25 mg/kg, 12.5 mg/kg, 25 mg/kg and 50 mg/kg) respectively through intraperitoneal route before 30 minutes of administration of 1% acetic acid (10ml/kg). Diclofenac sodium was considered as positive control group. The mice were kept in observation box and counted the number of writhes for 30 min after acetic acid administration. Following formula was used for measuring of analgesic activity (El Ouahdani *et al*., 2021).

$$\% \text{ Analgesic activity} = \frac{\text{Mean writhing count (control} - \text{treated)}}{\text{Mean writhing count (control)}} \times 100$$

Results and discussion

1. **Phytochemical screening of essential oils of ML and AM**

Previous study reported that essential oils of the plants contain terpenes, terpeniods and Phenolic compounds (Šojić *et al*., 2023). For identification of phenolic compounds, terpenes and terpeniods Libermann's, Baeyer's and Salkowski tests were performed. The results of the tests are shown in table no.1.

Table No.1. Phytochemical screening of essential oils of ML and AM

Essential oil	Phenolic compound	Terpenes	Terpenoids
Microcephala lamellata	Present	Present	Present
Alhagi maurorum	Present	Present	Present

Spectroscopic Characterization of essential oils of Leaves and Flowers of ML and AM

Ultra Violet (UV) Visible Spectroscopy

Various studies of essential oils have been reported regarding characterization by UV-visible spectroscopy (Kamila *et al*., 2021). UV-visible spectroscopy was performed for essential oil of ML and AM. The spectral range of UV-visible spectroscopy was adjusted at 200-400nm. The obtained spectra of ML and AM are shown in figure number 1 and 2. The absorption maxima of *Microcephala lamellata* ranges from 230 – 235nm. Whereas, the absorption maxima of *Alhagi maurorum* ranges from 280 – 285nm. These results support the study of other essential oils (Shao *et al*., 2020).

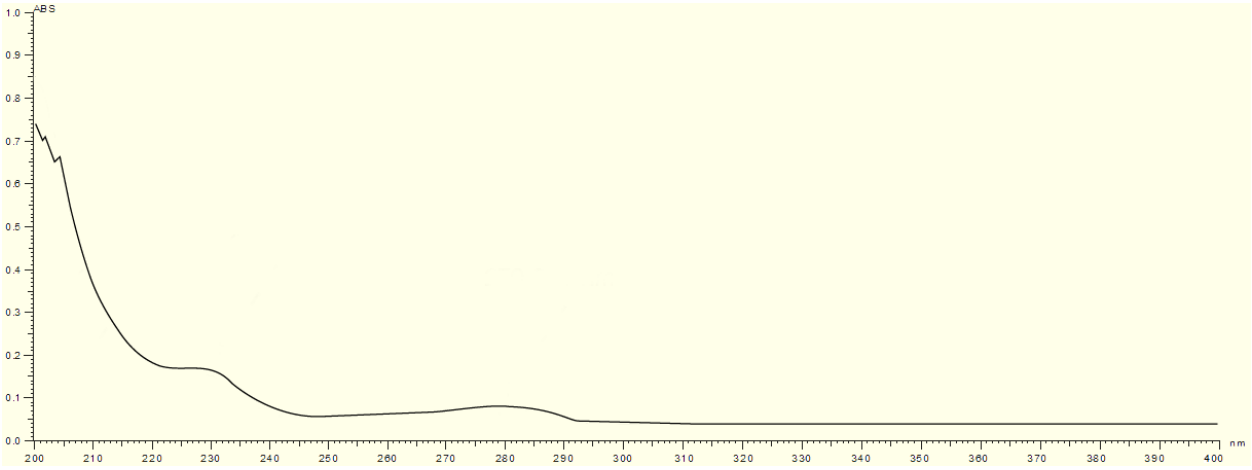

Figure No.1. UV-visible spectrum of essential oil of *Microcephala lamellata*

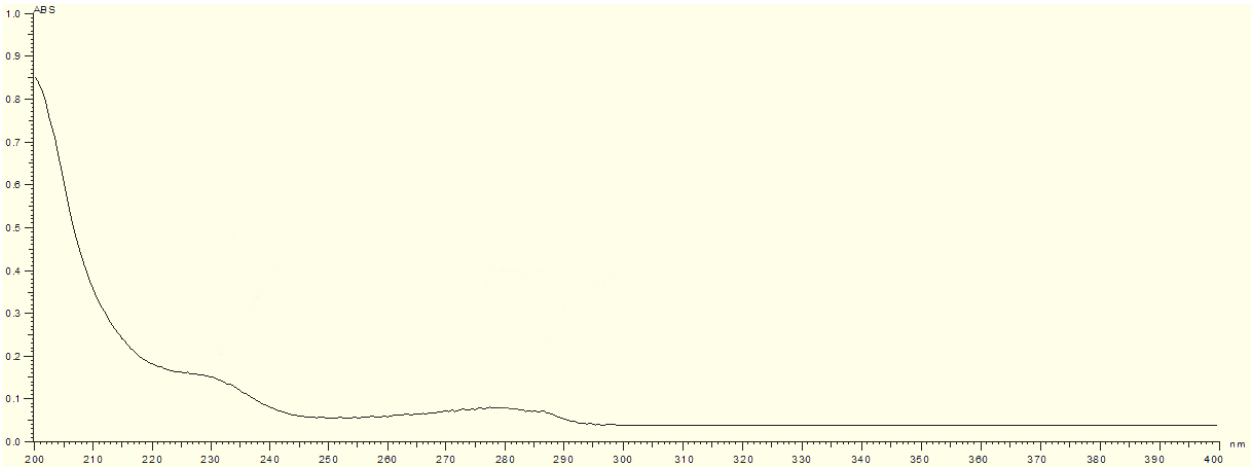

Figure No.2. UV-visible spectrum of essential oil of *Alhagi maurorum*

Fourier Transform Infrared Spectroscopy

Many researchers have been worked on the characterization of essential oils by using FTIR spectroscopy (Sufriadi *et al*., 2021). The spectra of essential oil of ML and AM in the range 500-4000cm^{-1} were mentioned the figure no.3 and 4, whereas the detail of FTIR spectroscopy is mentioned in the table no.2.

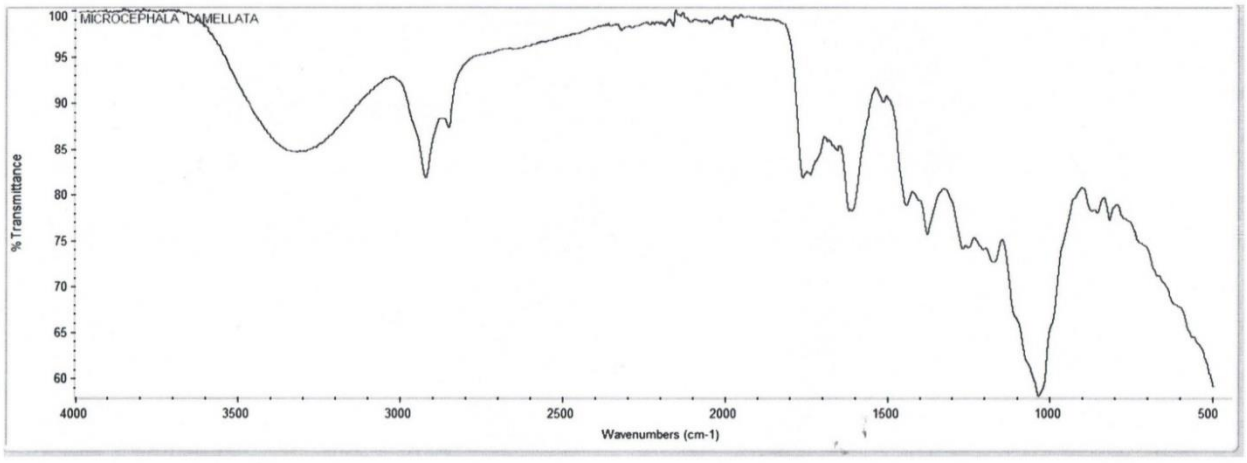

Figure No.3. FTIR spectrum of essential oil of *Microcephala lamellata*

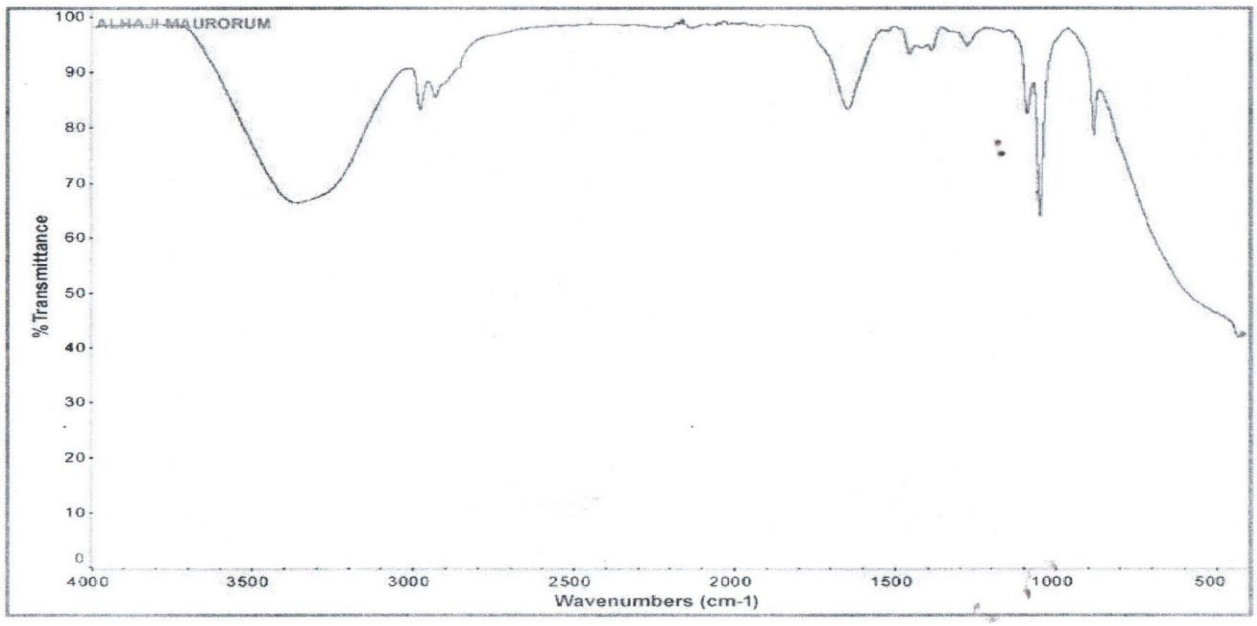

Figure No.4. FTIR spectrum of essential oil of *Alhagi maurorum*

Table 2. Functional groups identified by FTIR spectroscopy in ML and AM EOs.

Essential Oil	Peak	Bond	Functional Group
Microcephala lamellata	3354.21 cm^{-1} - 3334.92 cm^{-1}	OH Stretching vibrations	Phenols
	2956.87 cm^{-1} – 2872.01 cm^{-1}	CH Stretching vibrations alkanes	Alkanes
	1722.43 cm^{-1}	C = 0 carboxylic acid stretching vibrations	Carboxylic acid
	1459.76 cm^{-1}	CH_3 bending vibrations	Methyl
	1384.89 cm^{-1} – 1367.53 cm^{-1}	C-O Stretching vibrations	Carbonyls
	1037.7 cm^{-1}	C-F Stretching vibrations	Carbon fluorine
Alhagi maurorum	3354.21 cm^{-1} - 3334.92 cm^{-1}	OH Stretching vibrations	Phenols
	2956.87 cm^{-1} – 2872.01 cm^{-1}	CH Stretching vibrations alkanes	Alkanes
	1722.43 cm^{-1}	C = 0 carboxylic acid stretching vibrations	Carboxylic acid
	1459.76 cm^{-1}	CH_3 bending vibrations	Methyl
	1384.89 cm^{-1} – 1367.53 cm^{-1}	C-O Stretching vibrations	Carbonyls
	1037.7 cm^{-1}	C-F Stretching vibrations	Carbon fluorine

Table No.2 explain that there are many functional groups such as carboxylic acid, Phenols, Alkanes, carbonyl, Carbon fluorine and Methyl group which were present in the EOs of ML and AM. Presence of these functional groups indicates that essential oil of ML and AM contain numerous compounds. The results are similar to the previous studies regarding essential oils (Elzey *et al*., 2016).

Chromatographic Characterization of essential oil of ML and AM

Thin Layer Chromatography

Thin layer chromatography technique was conducted for characterization of essential oil of ML and AM. In the current study, four mobile phases that had previously been employed by researchers in their studies were used (Soran *et al*., 2009). The results are given in table no.3.

Table No.3.Retardation or retention factor value (R_f values) of components of *Microcephala lamellata* and *Alhagi maurorum* essential oils

Mobile Phase	***Microcephala lamellata***	***Alhagi maurorum***
	R_f value	**R_f value**
Toluene:Ethyl Acetate(93:7 v/v) (Mobile phase-A)	3.9,4.1,5.9	2.7,3.8,6.2,6.8
Haxane:Methylene chloride(5:1 v/v) (Mobile phase-B)	4.1,5.9,6.2	2.9,6.1
Light petroleum:chloroform(70:30 v/v) (Mobile phase-C)	4.3,6.1	3.9,6.3
Carbon tetrachloride:Acetone:Glacial Acetic Acid (15.2:3:1v/v/v) (Mobile phase-D)	4.1,4.4,6.0,6.3	2.8,4.0,6.2

The result of table no.3 explained that there are different compounds in the essential oil of *Microcephala lamellata* and *Alhagi maurorum*. Three separated compounds of ML are same for the mobile phase A, B and D with the R_f value 4.1, whereas two separated compounds are same for the mobile phase A and B with the R_f value 5.9. Moreover, various compounds are different in all the mobile phase. For *Alhagi maurorum*, two compounds are separated with the help of mobile phase A and D with the R_f value 6.2, whereas, rest of the compounds are different with the different R_f values. Mobile phase A and D have highest separating affinity for separating compounds from essential oil of ML and AM. Both mobile phases were able to separate four compounds each for essential oil of ML and AM.

Gas Chromatography for essential oils of *Microcephala lamellata* and *Alhagi maurorum*

Gas chromatography was performed for characterization of essential oil of ML and AM. The previously described chromatographic conditions by Novi *et al*., 2019 and were used for the current study. The chromatograms of ML and AM EOs are given in figure no.5 and 6.

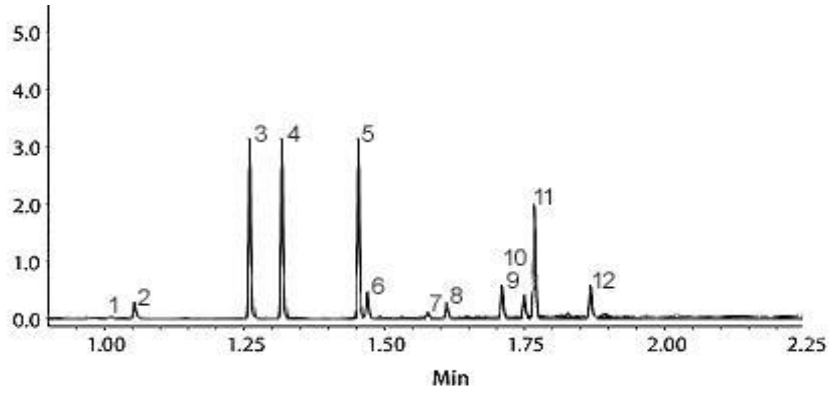

Figure No.5.Gas Chromatography chromatogram of *Microcephala lamellata* essential oil

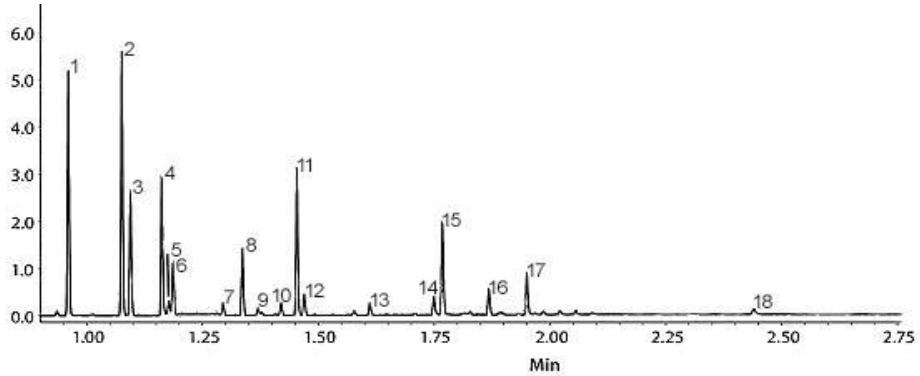

Figure No.6.Gas Chromatography chromatogram of *Alhagi maurorum* essential oil.

Essential oils of ML and AM shows various chromatographic peaks. Cecilia *et al*., conducted a study for essential oils under similar chromatographic conditions. The results of current study explain that EOs of ML and AM comprises of terpenoids, terpenes and phenolic compound (Cagliero *et al*., 2022).

Evaluation for Biological Activities

Anti-bacterial Activity

The results of antibacterial activity for EOs of ML and AM are shown in table no.4. The results exhibit that EO of ML have not antibacterial activity against gram positive (*B.Subtillis &S. Aureus*) and gram negative (*E.coli &P.aeruginosa*) bacteria. Whereas, EO of AM have antibacterial effects against all the strains of bacteria as shown in table no.4. It is reported from the previous studies that essential oil of plants has antibacterial activity against gram positive and gram-negative bacteria (Amin *et al*., 2023).

Table No.4.Antibacterial effect of ML & AM EOs on *B.subtillis, E.coli, P.aeruginosa& S. Aureus*

Oil Name	B. Subtillis				E. Coli				P. Aeruginosa				S. Aureus			
	1:1	1:5	1:10	1:20	1:1	1:5	1:10	1:20	1:1	1:5	1:10	1:20	1:1	1:5	1:10	1:20
ML oil	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AM oil	14.8	12.3	11.7	11.7	17.5	15.9	13.6	11.8	16.4	15.1	13.2	11.0	14.4	12.9	11.7	10.9
STM(2 µg) disc	27±0.6	–	–	–	20.5±0.4	–	–	–	17.7±10.8	–	–	–	19.9±0.4	–	–	–
Vehicle control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

ML: *Microcephala lamellata*, AM: *Alhagi maurorum*, STM: Streptomycin

Analgesic activity of *Microcephala lamellata* and *Alhagi maurorum* essential oil

Evidence of the previous studies shows that medicinal plants have analgesic activity (Ahmed, 2021). The results of analgesic activity for ML and AM EOs are shows in table no.5. The results explain that essential oils of ML and AM have analgesic activity in the mice model by acetic acid induced writhing test when compared with the standard drug (Diclofenac sodium). Essential oil of AM has more potent analgesic effect as compared to ML EO. Moreover, analgesic effect of ML EO and AM EO are in dose dependent manner as shown in table no.5.

Table No.5.Effect of ML and AM EOs on acetic acid induced writhing (n=5, mean ± SEM).

Groups	Doses (mg/kg)	Numbers of Writhing's	Inhibition (%)
Control (Swe*et al*mond Oil)	10	82±5	-
Stanard Drug (Diclofenac Sodium)	50	26±4	68.3
ML EOs	6.25	51±6	37.8
	12.5	40±6	51.2
	25	35±4	57.3
	50	30±3	63.4
AM EOs	6.25	48±6	41.5
	12.5	37±5	54.9
	25	32±4	61.0
	50	26±6	68.3

Conclusion

The screening tests showed that essential oils of *Microcephala lamellata* and *Alhagi maurorum* contain phenolic compounds, terpenes and terpenoids. The spectroscopic techniques such as UV-visible spectroscopy and fourier transform infrared spectroscopy and chromatographic techniques such as thin layer chromatography and gas chromatography, confirmed the presence of these compounds. The disc diffusion method indicated that essential oils of *Alhagi maurorum* have high anti-bacterial activity against *B. Subtillis, E. Coli, P. Aeruginosa and S. Aureus* whereas the essential oils of *Microcephala lamellata* have low antibacterial activity against these microorganisms. The oils were also effective in pain as evidenced by the acetic acid induced writhing test.

Conflict of interest

The authors declare no conflict interest in this study.

Acknowledgement

I am very thankful to Dr. Sultan Ayaz and Dr. Abid Rashid for their valuable suggestions and kind support. I am grateful to Dr. Saif Ur Rehman Khattak for his valuable guidance, technical support and provision of laboratory equipment's. I am also thankful to Dr. Aman Ullah khan for his valuable suggestions.

References

1. Petrovska, B. B. (2012). Historical review of medicinal plants' usage. *Pharmacognosy reviews*, *6*(11), 1.
2. Cassileth, B. R., & Chapman, C. C. (1996). Alternative and complementary cancer therapies. *Cancer: Interdisciplinary International Journal of the American Cancer Society*, *77*(6), 1026-1034.
3. World Health Organization. (2002). *WHO traditional medicine strategy 2002-2005* (No. WHO/EDM/TRM/2002.1). Geneva: World Health Organization.
4. De Silva, G. O., Abeysundara, A. T., & Aponso, M. M. W. (2017). Extraction methods, qualitative and quantitative techniques for screening of phytochemicals from plants. *American Journal of Essential Oils and Natural Products*, *5*(2), 29-32.
5. Khattak, S. R., Hussain, A., Ali, A. A., Ahmad, T., Ayaz, S., Akram, M., & MR, M. I. (2020). Comparative studies of leaves and bark of Moringa oleifera originated from Khyber Pakhtoon Khwa and Punjab, Pakistan. *Journal of Pharmacognosy and Phytochemistry*, *9*(3), 1505-1509.
6. Abbas, G., Shahzad, M., Hassan, M. J., Tareen, R. B., & Choudhary, M. I. (2012). Antiglycation, antioxidant and antilipid peroxidation activities of microcephalalamelatta with low cytotoxic effects in vitro. *Middle-East J of Sci Res*, *11*(6), 814-818.
7. Ahmad, N., Bibi, Y., Raza, I., Zahara, K., Khalid, N., Bashir, T., & Tabassum, S. (2015). Traditional uses and pharmacological properties of Alhagi maurorum: A review. *Asian Pacific Journal of Tropical Disease*, *5*(11), 856-861.
8. European Pharmacopoeia .7.0 (2022). Determination Of EOs In Herbal Drugs; *General Notices*.
9. Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, *8*(2), 603-608.
10. Nickavar, B., Adeli, A., & Nickavar, A. (2014). TLC-bioautography and GC-MS analyses for detection and identification of antioxidant constituents of Trachyspermum copticum essential oil. *Iranian journal of pharmaceutical research: IJPR*, *13*(1), 127.
11. Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012.

12. National Committee for Clinical Laboratory Standards. (2002). Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. In *Approved Standard M100-S12* Wayne. NCCLS.
13. Siegmund E, Cadmus R, Lu G. (1957). A method for evaluating both non-narcotic and narcotic analgesics. *Proceedings of the Society for Experimental Biology and Medicine*, 95, 729–731.
14. El Ouahdani, K., Es-Safi, I., Mechchate, H., Al-Zahrani, M., Qurtam, A. A., Aleissa, M., ... & Bousta, D. (2021). Thymus algeriensis and Artemisia herba-alba essential oils: chemical analysis, antioxidant potential and in vivo anti-inflammatory, analgesic activities, and acute toxicity. *Molecules*, *26*(22), 6780.
15. Šojić, B., Ikonić, P., Kocić-Tanackov, S., Peulić, T., Teslić, N., Županjac, M., ... & Pavlić, B. (2023). Antibacterial Activity of Selected Essential Oils against Foodborne Pathogens and Their Application in Fresh Turkey Sausages. *Antibiotics*, *12*(1), 182.
16. Kamila Kucharska-Ambrozej., Agnieszka Martyna., Joanna Karpinska., Anna Kiełtyka-Dadasiewicz., Aleksandra Kubat-Sikorska. (2021). Quality control of mint species based on UV-VIS and FTIR spectral data supported by chemometric tools, *Food Control*, 129.
17. Shao, H., Jiang, Y., Pan, F., Xie, J., Qi, J., Xiao, H., & Chen, Y. (2020). Chemical composition, UV/vis absorptivity, and antioxidant activity of essential oils from bark and leaf of phoebe zhennan SK Lee & FN Wei. *Natural product research*, *34*(6), 876-879.
18. Sufriadi, E., Meilina, H., Munawar, A. A., & Idroes, R. (2021, February). Fourier Transformed Infrared (FTIR) spectroscopy analysis of patchouli essential oils based on different geographical area in Aceh. In *IOP Conference Series: Materials Science and Engineering* (Vol. 1087, No. 1, p. 012067). IOP Publishing.
19. Elzey, B., Norman, V., Stephenson, J., Pollard, D., & Fakayode, S. O. (2016). Purity analysis of adulterated essential oils by FT-IR spectroscopy and partial-least-squares regression. *Spectroscopy*, *31*(8), 26-37.
20. Soran, M. L., Cobzac, S. C., Varodi, C., Lung, I., Surducan, E., & Surducan, V. (2009, August). The extraction and chromatographic determination of the essentials oils from Ocimum basilicum L. by different techniques. In *Journal of Physics: Conference Series* (Vol. 182, No. 1, p. 012016). IOP Publishing.
21. Aidha, N. N., Yunilawati, R., & Rumondang, I. (2020). Method Development for Analysis of Essential Oils Authenticity Using Gas Chromatography-Mass Spectrometry (GC-MS). In *2nd International Conference of Essential Oil Indonesia (ICEO)* (pp. 41-46).
22. Cagliero, C., Bicchi, C., Marengo, A., Rubiolo, P., & Sgorbini, B. (2022). Gas chromatography of essential oil: State-of-the-art, recent advances, and perspectives. *Journal of Separation Science*, *45*(1), 94-112.
23. Amin, M., Akrami, S., Haghparasty, F., & Hakimi, A. (2023). In vitro antibacterial activities of essential oils and extracts of six herbals against gram-positive and gram-negative bacteria. *Toxicology and Environmental Health Sciences*, *15*(1), 53-60.
24. Ahmed, S. S. (2021). Overview of various medicinal plants having potent analgesic activity.