MORPHOLOGICAL, HISTOLOGICAL CHARACTERISTICS, PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF LEAVES OF *MESOSPHAERUM SUAVEOLENS* (L.) KUNTZE

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Abstract

Mesosphaerum suaveolens (L.) Kuntze known as Taw Pin Sein or Nan Saw Pin, belonging to the family Lamiaceae. The specimens were collected from Chan Mya Tharzi Township, Mandalay Region, during September 2022 to April 2023. The phytochemical results showed the presence of steroids, glycosides, phenolic compounds, tannins, saponins, α-amino acids, proteins, reducing sugars, carbohydrates and starch. Antioxidant activity was evaluated by using 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging method. The antioxidant activity of *Mesosphaerum suaveolens* (L.) Kuntze was IC_{50} = 7.87 mg/L. In antimicrobial activities, the different solvents chloroform, pet-ether, ethyl-acetates, ethanol, methanol and aqueous extracts of leaves were tested on five pathogenic microorganisms by using agar well diffusion methods. Among these extracts, activity was more effective on *Candida albicans* (32.2 mm inhibition zone) by the ethyl-acetates extract. The results of the studies indicated that the *Mesosphaerum suaveolens* (L.) Kuntze possesses phytoconstituents, antioxidant and antimicrobial activities. Thus, it can be utilized as a plant-derived natural product.

Keywords: *Mesosphaerum suaveolens*, Morphological, Histological, Phytochemical, Antioxidant, Antimicrobial Activities

Introduction

Mesosphaerum suaveolens (L.) Kuntze commonly knowns as taw pin sein or nan saw pin belongs to the family Lamiaceae (the Mint family). This family is the sixth largest angiosperm family with about 236 genera and 7173 species and has a cosmopolitan distribution. Naturally growing Lamiaceae members have been used as tea, spice and for various medicinal purposes and also used to treat fever, cough, headaches, wound healing, heart diseases and stomachaches (Ulhe and Narkhede 2013).

Mesosphaerum suaveolens (L.) Kuntze is an herbaceous plant. The word "*mesosphairon"* comes from the Greek and Latin "*mesosphaerum*," meaning "a type of tuberose with medium-sized leaves," while its specific epithet *suaveolens*, means "with a sweet fragrance" due to the aroma of essential oils exhaled by the trichomes present on its leaves. Taxonomically, *M. suaveolens* presents as botanical synonyms *Ballota suaveolens* L., *Hyptis suaveolens* (L.) Poit., and *Hyptis congesta* Leonard, with *H. suaveolens* as the most widespread synonym in scientific circles. However, the current circumscription of the genus *Mesosphaerum* P. Browne was recognized in 2012 after phylogenetic studies (Almeida 2022). This plant a shrubby, scented plant with blue or purple small flowers. The plant has been considered as a weed, distributed throughout the tropic and subtropics, common in open uncultivated areas, rocky dry substratum, on roadsides and waste grounds (Kum 2013).

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M. suaveolens (L.) Kuntze is an important source of essential oil, steroids, terpenes, alkaloids, flavonoids, phenols, and saponins. Leaves and flowers contains a wide variety of flavonoids and polyphenolic compounds and some plants species of the Lamiaceae family also contain vitamin C, vitamin E, quercetin, isorhammetin, kaempferol having the effective antioxidant property that is due to strong free radical scavenging activity. Almost all parts of this plant are being used in traditional medicine as well as it has immense of ethnomedicinal importance for treating various diseases. It was reported to be used for traditional medicine as an anticancer (Devi and Basavaiah 2023).

This plant is a very common plant in India. Indians use it the morning soup as a memory aid. Tea made from the roots of *M. suaveolens* (L.) Kuntze is used to purify the blood, and it is also used as a remedy for the "diseases" of women. It has been used as a medicinal tea in many places in Asia, and as a food and source of essential oil is South America (Sharma *et al* 2013). According to the Suganya and Sombat 2007, *M. suaveolens* (L.) Kuntze is also the edible plant which is usually applied in Thai food recipe as an appetizer because of their flavored essential oils.

In Myanmar, *Mesosphaerum suaveolens* (L.) Kuntze is an introduced species and widely distributed in roadsides and open field. This plant is one of the indigenous herbs. Its leaves are mainly used to treat respiratory diseases and diseases related to the gastrointestinal tract.

Mishra (2021) stated that *M. suaveolens* (L.) Kuntze possesses the ursolic acid which can inhibit on trascriptase-replicase and assembly of corona viral particles. And thus, this plant can be used as potent therapeutic drug against COVID-19 virus and other life- threatening RNA viruses.

Therefore, this study was aimed to study the morphological, histological characteristics, phytochemical analysis, antioxidant and antimicrobial activities of leaves of *Mesosphaerum suaveolens* (L.) Kuntze. The objectives were to observe the morphological and histological characteristics as the authentic botanical identification and to investigate the bioactive compound of this plant species.

Materials and Methods

Plant collection and identification

The specimens were collected from Chan Mya Tharzi Township, Mandalay Region during the flowering and fruiting periods from September 2022 to April 2023. After the collection, the specimens were studied and identified by using literatures such as Hooker (1875), Backer & Brink (1946) and Dassanayake (1987).The morphological and histological characteristics of *Mesosphaerum suaveolens* (L.) Kuntze were studied at the Department of Botany, University of Mandalay. For histological study, killing and fixation, dehydration, infiltration, embedding, staining and mounting were made according to Johansen's method (1940). The parts of specimens were macerated by warming in equal volumes of 50% hydrogen peroxide solution and 50% glacial acetic acid solution according to Berlyn & Miksche (1976). Stomatal index were calculated by using the following equation (Kokate 2000).

$$S.I = \frac{S}{E+S} \times 100$$

Where,

S.I = Stomatal Index

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

Phytochemical analysis

The preliminary phytochemical analysis on the powder of *M. suaveolens* was carried out to determine the presence or absence of alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α-amino acids, proteins, reducing sugars, starch, carbohydrates and cyanogenic glycosides by Trease and Evans (1980) and Harbone (1984) methods. All the qualitative analysis had been undertaken at the Department of Botany, University Research Center, University of Mandalay.

Measurement of antioxidant activity by DPPH radical scavenging assay

The antioxidant activity of the *M. suaveolens* was determined by using different concentration and ascorbic acid was used as a standard. This activity was carried out at the U Kar Kar Company Limited (Head office) in Mandalay.

Antimicrobial activity analysis

The antimicrobial activity analysis of *Mesosphaerum suaveolens* (L.) Kuntze leaves were performed by agar well diffusion method reported by Balouiri *et al*. (2016) at the microbiology laboratory in Department of Botany, University of Mandalay. Six extracts of dried powdered leaves, such as chloroform, pet-ether, ethyl-acetate, methanol, ethanol and aqueous extracts were used. Five pathogenic microorganisms such as *Candida albicans, Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa* and *Escherichia coli* were used to investigate the antimicrobial activities of six different extracts of *M. suaveolens* (L.) Kuntze leaves.

Results

Morphological characteristics

Scientific name : *Mesosphaerum suaveolens* (L.) Kuntze, Revis 2:525.1891

Ballota suaveolens L., Syst. Not. 10.1100.1759

Family name : Lamiaceae

Myanmar name : Taw Pin Sein or Nan Saw Pin

English name : Pignut

Flowering period : September to April

Annual, erect herbs to undershrubs; stems and branches quadrangular, strongly aromatic, fistular and woody at the base, densely patently pilose with glandular, shallowly grooved, branches wide-spreading above, pubescent. Leaves simple, opposite and decussate, exstipulate.

Inflorescences axillary, capitate cyme with 1 to 5 flowers on the tops. Flowers bisexual, zygomorphic, tetramerous, hypogynous, blue. Stamens 4, didynamous, epipetalous; anthers dithecous. Ovary superior, ovoid, bilocular; style gynobasic; stigma bifid, minute. Fruits 2-nutlets. Seeds flattened, dark brown (Figure 1. A – H).

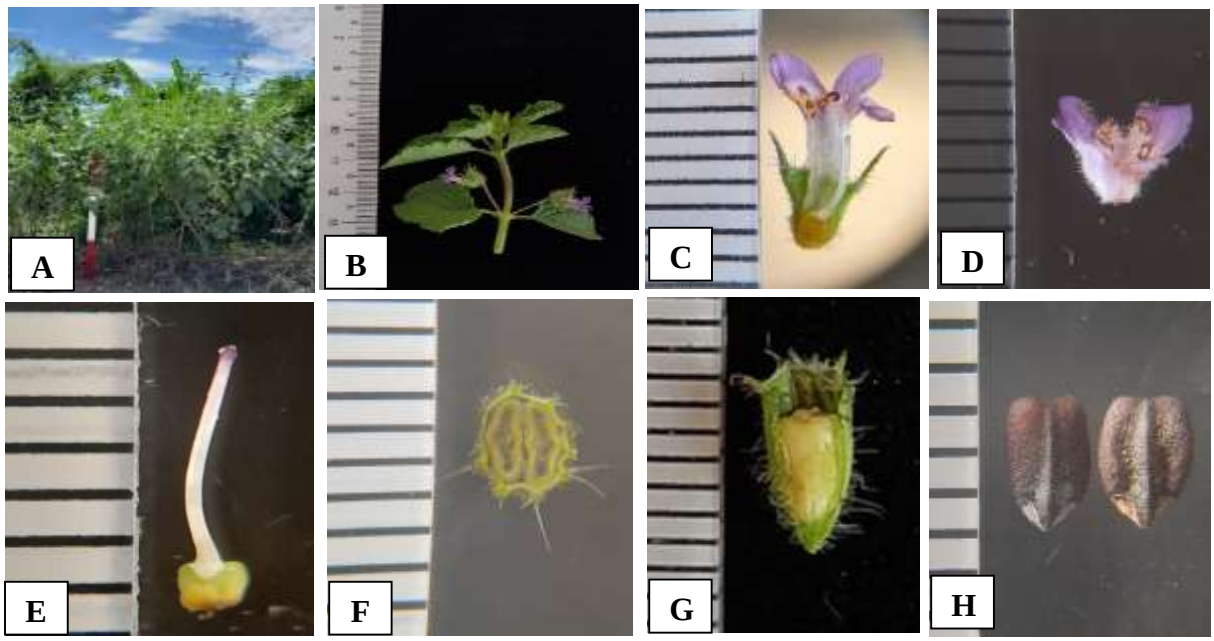

Figure 1. Morphological characteristics of *Mesosphaerum suaveolens* (L.) Kuntze

A. Habit B. Inflorescence C. L.S of flower D. Androecium
E. Gynoecium F. T.S of ovary G. Fruits H. seeds

Histological characteristics

In transverse sections, the petioles of *M. suaveolens* (L.) Kuntze were shield-shaped in outline, prominent wing at the adaxial side with capitate glandular and non-glandular trichomes. Vascular bundles were arranged as a crescentic, discontinuous, collateral type, accompanied by 3-accessory bundles present in adaxial 2-lateral prominent wings, each bundle circular in shape. In the lamina, *M. suaveolens* (L.) Kuntze. was dorsiventral with reticulate venation. Stomata types were diacytic and epidermal cell walls were wavy. The highest stomatal index were 75.00 and the lowest stomatal index were 28.58 in adaxial surfaces and the highest stomatal index were 42.86 and the lowest stomatal index were 40.00 in abaxial surfaces. The midribs were flat at the abaxial sides and convex at the adaxial sides. The stems of *M. suaveolens* (L.) Kuntze were quadrangular-shaped in outline with prominent ribs at 4-corners with deep notches in between. Vascular cambium formed a continuous ring, 1- or 2-layered. Xylem composed of vessel elements, tracheids, xylem fibres and xylem parenchyma. In transverse section of roots, they were circular-shaped in outline and periderm formation was found. Fibers, tracheids and vessel elements (thick-walled, lateral walls straight, end walls oblique or transverse, thickenings spiral, scalariform or pitted, perforation plates simple) were found in *M. suaveolens* (L.) Kuntze (Figure 3). Capitate glandular trichome, unicellular uniseriate, multicellular uniseriate and multicellular multiseriate trichomes were found through the plant (Figure 2).

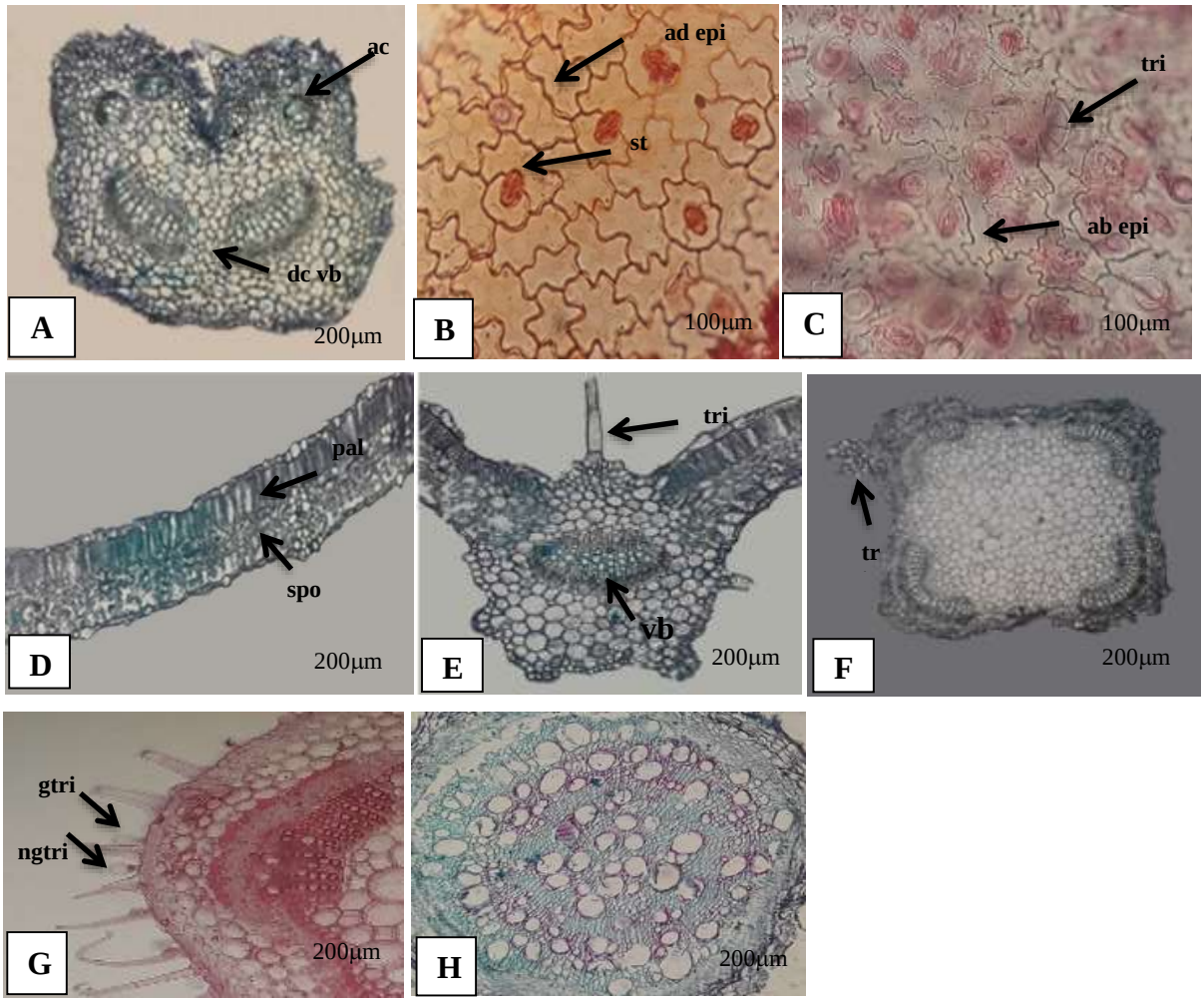

Figure 2. Internal structure of of *Mesosphaerum suaveolens,*(L.) Kuntze

A. T.S of petiole B. Adaxial surface view of lamina
C. Abaxial surface view of lamina showing diacytic types of stomata
D. T.S of lamina showing palisade and spongy mesophyll cells
E. T.S of midrib
F. T.S of stem
G. T.S of stem showing vascular bundle and trichome
H. Close up view of root

(ab epi = abaxial epidermal cell, ad epi = adaxial epidermal cell, pal = palisade parenchyma cell, spo = spongy parenchyma cell, st = stoma, tri = trichome, dc vb= discontinuous vascular bundle, vb= vascular bundle)

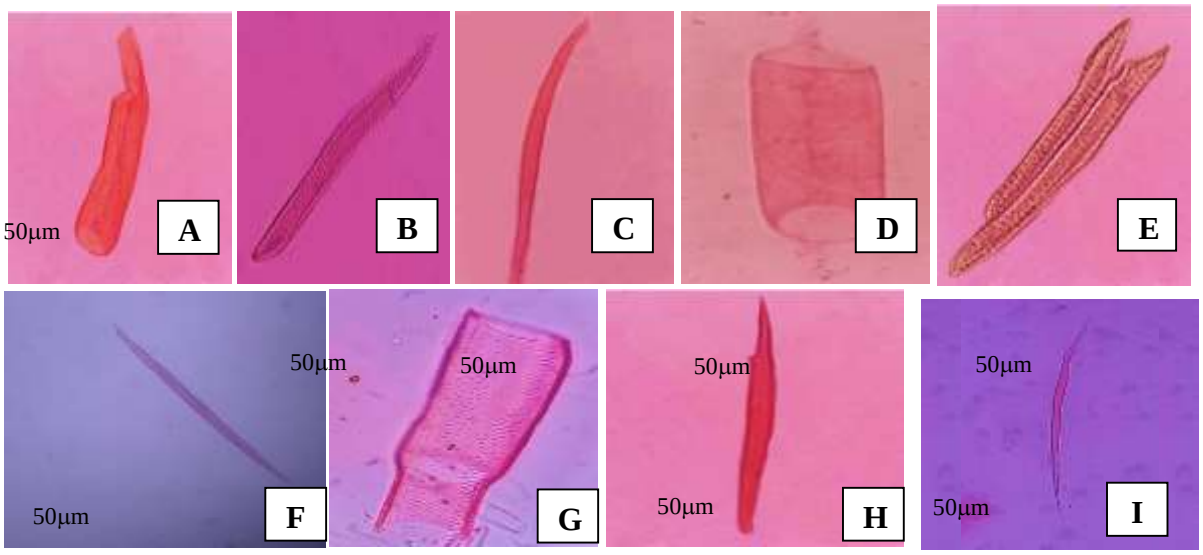

Figure 3. Macerated elements in leaves, stems and roots of *Mesosphaerum suaveolens* (L.) Kuntze
A-C Vessel, tracheids and fibers in leaves of *Mesosphaerum suaveolens* (L.) Kuntze
D-F Vessel, tracheids and fibers in stems of *Mesosphaerum suaveolens* (L.) Kuntze
G-I Vessel, tracheids and fibers in roots of *Mesosphaerum suaveolens* (L.) Kuntze

Phytochemical analysis on the leaves of *Mesosphaerum suaveolens* (L.) Kuntze

The preliminary phytochemical investigation on the powder of *M. suaveolens* (L.) Kuntze leaves was carried out to determine the presence or absence of alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α-amino acids, proteins, reducing sugars, starch, carbohydrates and cyanogenic glycosides. Carbohydrates and cyanogenic glycosides were not detected in all extracts. The results of the preliminary phytochemical analysis were shown in Table 1.

Table 1. Phytochemical analysis on the leaves of *Mesosphaerum suaveolens* (L.) Kuntze

No.	Phytochemicals	Reagent Names	Aqueous	Ethanol	Methanol	Observation
1.	Alkaloids	Mayer's reagent	-	+	+	Cream ppt.
		Wagner's reagent	-	+	+	Reddish brown ppt
		Hager's reagent	-	+	+	Yellow ppt
2.	Steroids	$CHCL_3$, conc:H_2SO_4	+	+	-	Upper reddish
3.	Terpenoids	$CHCL_3$, conc:H_2SO_4	-	+	+	Lower greenish yellow
4.	Glycosides	Sodium hydroxide	+	+	+	Yellow
5.	Phenolic compounds	10% Ferric chloride	+	+	+	Dark green
6.	Flavonoids	Sodium hydroxide	+	+	+	Pale green
7.	Tannins	10% Ferric chloride	+	+	+	Black
8.	Saponins	Distilled water	+	-	+	Frothing

No.	Phytochemicals	Reagent Names	Aqueous	Ethanol	Methanol	Observation
9.	α-amino acids	Ninhydrin	+	+	+	Purple spot
10.	Proteins	Millon's	+	+	+	Yellow ppt
11.	Reducing sugars	Benedict's	+	+	+	Green
12.	Starch	Iodine	+	+	+	Black
13.	Carbohydrates	Molish's	+	+	+	Purple ring
14.	Cyanogenic glycosides	Sodium picric	-	-	-	No brickish color

(+) = present, (-) = absent, ppt = precipitate, H_2O = Aqueous, EtoH = Ethanol, MeOH = Methanol

Determination of Antioxidant Activity by DPPH Assay

In this experiment, five different concentrations (100, 50, 25, 12.5 and 6.25 mg/L) were used for aqueous extracts. Ascorbic acid was used as standard. According to the analysis of antioxidant activity, IC_{50} value for standard ascorbic acid is 6.75 and IC_{50} value for aqueous extract of *Mesosphaerum suaveolens* (L.) Kuntze is 7.87 as shown in Table 2, 3 and Figure 3, 4.

Table 2. % inhibition of various concentration of standard ascorbic acid

Standard Concentration	Absorbance	% Inhibition	IC_{50} (mg/L)
50	0.264	89.41459503	6.75
25	0.724	70.97032879	
12.5	1.123	54.97193264	
6.25	1.390	44.26623897	
3.125	1.511	39.41459503	

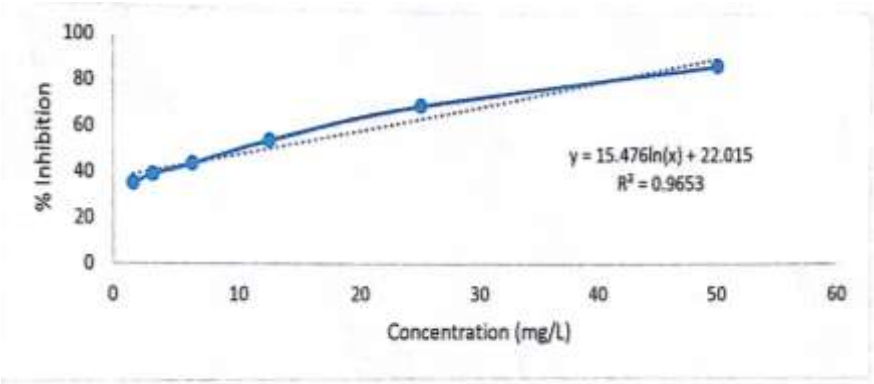

Figure 3. % Inhibition and Different Concentration of Ascorbic Acid (mg/L)

Table 3. % inhibition of various concentration of aqueous extract of *Mesosphaerum suaveolens* (L.) Kuntze

Sample Concentration (mg/L)	Absorbance	% Inhibition	IC_{50} (mg/L)
100	0.531	75.56374	7.87
50	0.826	61.98803	
25	0.954	56.09756	
12.5	1.025	52.83019	
6.25	1.056	51.40359	

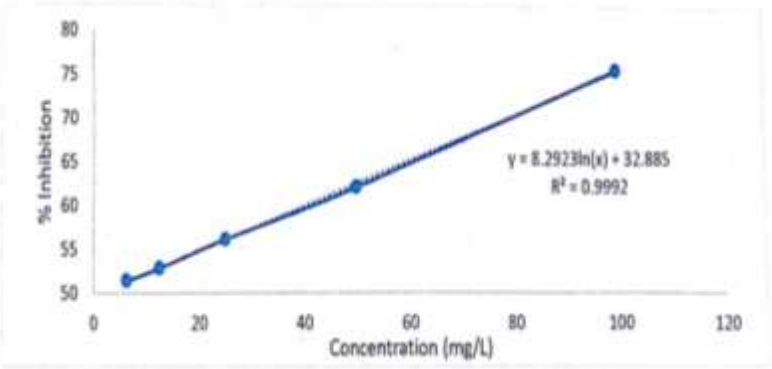

Figure 4. % Inhibition and Different Concentration of Leaves of *Mesosphaerum suaveolens* (L.) Kuntze (mg/L)

Analysis of antimicrobial activity

The antimicrobial activity analysis of *Mesosphaerum suaveolens* (L.) Kuntze leaves were performed by agar well diffusion method. Six extracts of dried powdered leaves, such as chloroform, pet-ether, ethyl-acetate, methanol, ethanol and aqueous extracts were used. Ethyl-acetate extracts of leaves indicated more effective activities on all five test microorganisms than the other extracts. Aqueous extracts of leaves did not show activities on all the five test microorganisms. The results were shown in Table 4 and Figure 5.

Table 4. Antimicrobial activities of different solvent extracts of *Mesosphaerum suaveolens* (L.) Kuntze against five pathogenic microorganisms

Samples	Inhibition Zone Diameter (mm)				
	Escherichia coli	***Micrococcus luteus***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Candida albicans***
Chlorofrom	**9.5**	16	17.3	17.3	14.5
Pet-ether	15	15.6	16.5	17.2	13.6
Ethy-lacetate	**22.3**	**30.2**	**31.3**	**26.7**	**32.2**
Ethanol	14.4	17.6	15	14.4	17.7
Methanol	15.1	14.8	15.1	14.6	16.5
Aqueous	-	-	-	-	-

Agar well diameter = 6 mm

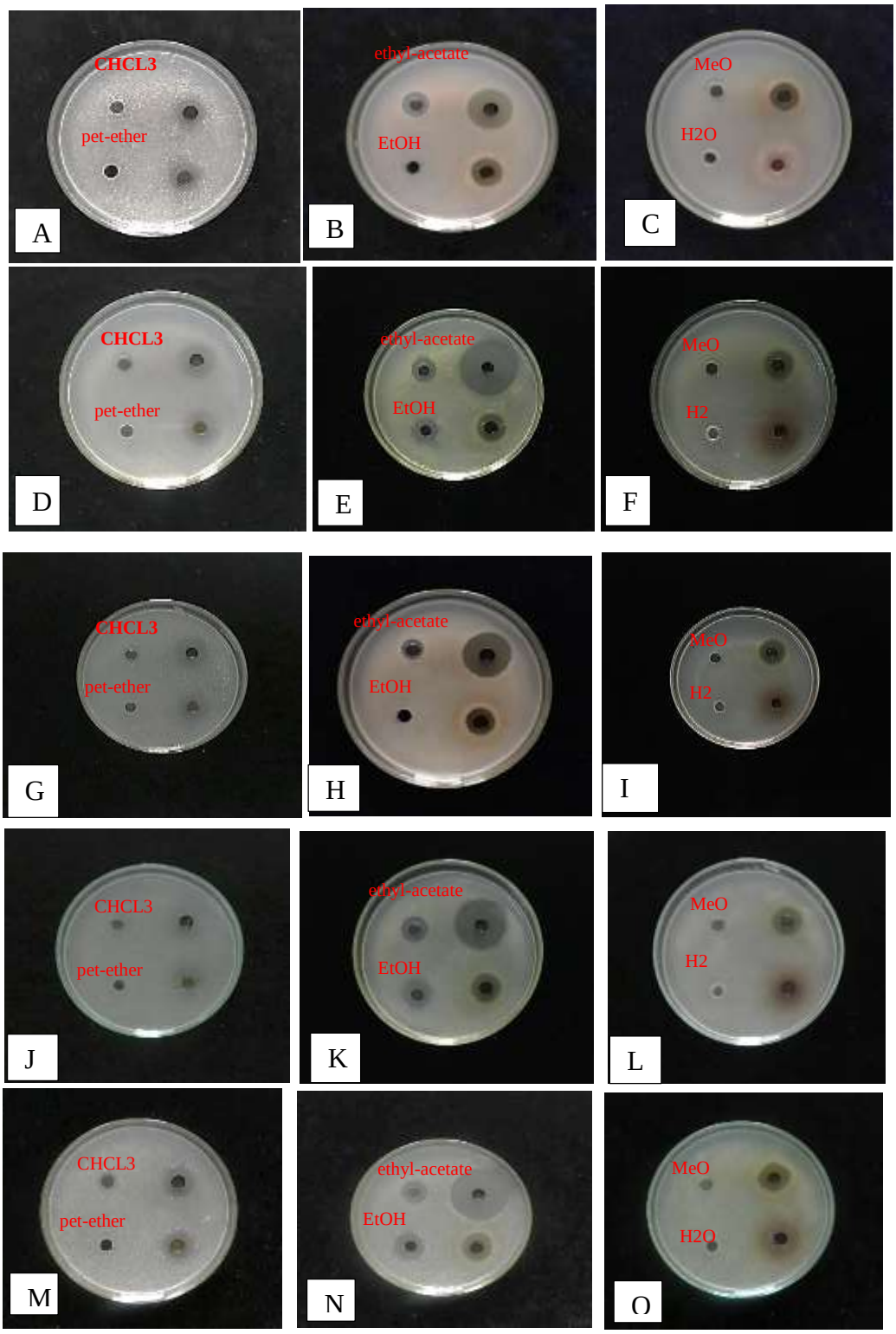

Figure 5. Antimicrobial activities of different solvent extracts of *Mesosphaerum suaveolens* (L.) Kuntze leaves against on test microorganisms

A - C Antimicrobial activities against on *Escherichia coli*
D - F Antimicrobial activities against on *Micrococcus luteus*
G - I Antimicrobial activities against on *Staphylococcus aureus*
J - L Antimicrobial activities against on *Pseudomonas aeruginosa*
M - O Antimicrobial activities against on *Candida albicans*

Discussion

The morphological, histological, phytochemical analysis, antioxidant and antimicrobial activities of leaves of *Mesosphaerum suaveolens* (L.) Kuntze were studied in this research. This studied species *M. suaveolens* (L.) Kuntze belonging to family Lamiaceae.

According to this research, the habit of *M. suaveolens* (L.) Kuntze was annual erect herbs to undershrubs. Stems were quandrangular in shape. The flowers were blue, bisexual, zygomorphic, tetramerous and hypogynous. The stamens were didynamous. The ovary was superior and tetralocular with one ovule in each locule with axile placentation and fruit was nutlets in this species. These characters were in agreement with those mentioned by Hooker (1875), Backer (1946) and Dassanayake (1987).

In histological characters, the petiole of *M. suaveolens* (L.) Kuntze were sheid-shaped in outline with prominent wing at the adaxial side. Diacytic type of stomata are present on both surfaces of lamina. The stomatal index were 28.58 -75.00 on adaxial surfaces and 40.00 - 42.86 on abaxial surfaces. Glandular and non-glandular trichomes were present on both surfaces. Cresentic-shaped, discontinuous and collateral types vascular bundles were found in petiole. These characters were agreement with those described by Metcalfe and Chalk (1972). Metcalfe and Chalk (1957) pointed out that stem in many genera and species of labiatae are quadrangular in transverse section with well-defined groups of collenchyma in the four angles. *M. suaveolens* (L) Kuntze was densely covered with hairs of various kinds, one of the most characteristics being the short-stalked glands with head of 1-16 or more cells. In this studies, capitate glandular trichome, unicellular uniseriate, multicellular uniseriate and multicellular multiseriate trichomes were found through the plant. Adedeji (2012) stated that qualitative foliar histological characteristics such as stomata type, trichomes type, anticlinal cell wall pattern were the most useful in species delimitation.

In this research, phytochemical analysis from three different solvent extracts such as aqueous, ethanol and methanol indicated that the leaves of *M. suaveolens* (L.) Kuntze contain various phyto-constituents alkaloids, steroids, terpenoids, glycosides, phenolic compounds, flavonoids, tannins, saponins, α-amino acids, proteins, reducing sugars, carbohydrates and starch whereas alkaloids and terpenoids in aqueous extracts, steroids in methanol extracts and saponins in ethanol extracts were absent. Cyanogenic glycosides did not indicate in all extracts of the leaves of *M. suaveolens* (L.) Kuntze.

In the determination of antioxidant activities, the standard ascorbic acid exhibited antioxidant activity with IC_{50} value of 6.75mg/ L in DPPH assay. The aqueous extract of *M. suaveolens* (L.) Kuntze leaves showed 7.87 mg/L % inhibition in DPPH radical. Therefore, the IC_{50} value of this plant is near the same with standard ascorbic acid and it has high antioxidant activity.

According to the antimicrobial activity analysis of *M. suaveolens* (L.) Kuntze leaves, ethyl-acetate extracts of leaves indicated high activities on all the five test microorganisms. Among them, it was found the most effective on *Candida albicans* (32.2 mm). However, the aqueous extracts of leaves did not show activities on all the test microorganisms.

Conclusion

In conclusion, the studied morphological and histological characters of *M. suaveolens* (L.) Kuntze**,** were valuable to identify this species. Moreover, this plant possess the essential phyto-constituent, antioxidant properties and antimicrobial activities. And as a result, this plant can contribute as a potential plant-derived drug and as a source of other bioactive compounds to be discovered in the future.

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