

Phenolic, Flavonoid And Tannin Dynamics Coupled With Free Radical Quenching Ability In Leaf And Stem Of *Mesosphaerum suaveolens* (L.) Kuntze

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ABSTRACT

Mesosphaerum suaveolens (L.) Kuntze belonging to Lamiaceae family, commonly known as American Mint Weed. It is an invasive plant it was introduced to India by travellers and traders. This study aims to conduct compressive analysis of Phytochemical and antioxidant properties. Compounds like Tannin, Terpenoids, Alkaloid, Glycoside, Flavonoid, were highly present, compounds like saponins, starch, protein were slightly present. Quantitative analysis was done for Total Phenol Content which resulted in Ethanolic Leaf 28.98 ± 0.10 mg GAE/g, Methanolic Leaf 25.62 ± 3.34 mg GAE/g, Ethanolic Stem 13.61 ± 1.21 mg GAE/g, Methanolic Stem 1.455 ± 1.1 mg GAE/g. For total tannin Ethanolic Leaf 28.98 ± 0.10 mg TAE/g, Methanolic Leaf 25.62 ± 3.34 mg TAE/g, Ethanolic Stem 13.61 ± 1.21 mg TAE/g, Methanolic Stem 1.455 ± 1.1 mg TAE/g. For Flavonoid Content Ethanolic Leaf 28.768 ± 0.6715 mg QE/g, Methanolic Leaf 29.737 ± 0.107 mg QE/g, Ethanolic Stem 14.736 ± 0.7 mg QE/g, Methanolic Stem 7.737 ± 0.32 mg QE/g. Antioxidant activity performed via DPPH method reveals that Ethanol Leaf $894.81 \mu\text{g/ml}$, Ethanol Stem $575.05 \mu\text{g/ml}$, Methanol Leaf $169.73 \mu\text{g/ml}$, Methanol Stem $574.83 \mu\text{g/ml}$. It shows that only Methanolic leaf extract is showing good antioxidant activity. This study also suggests that phytogeographical changes can lead to change in concentration of bioactive compound of plant.

Keywords: *Mesosphaerum suaveolens* (L.) Kuntze, Qualitative Phytochemical analysis, Quantitative analysis and Antioxidant activity.

INTRODUCTION

The global shift toward natural pharmacotherapy has intensified the search for plant-derived bioactive compounds capable of mitigating oxidative stress a precursor to numerous chronic pathologies including cancer, diabetes, and neurodegenerative disorders. Among the vast botanical repertoire, the family Lamiaceae stands out for its high concentration of polyphenols and essential oils. *Mesosphaerum suaveolens* (L.) Kuntze (synonym *Hyptis suaveolens*), commonly known as American Mint Weed or Vilayati Tulsi, is an aromatic, quadrangular-stemmed herb that has emerged as a prolific source of secondary metabolites with significant pharmacological potential. (Mishra *et al.*, 2021).

The physical structure of *M. suaveolens* is defined by its vertical, pubescent, and lignified adult stems, which can grow to heights of 6 feet, alongside heart-

shaped, toothed leaves. These leaves produce a characteristic scent attributed to chemical components such as alpha-caryophyllene and eucalyptol (Pandey and Shrivastava, 2014). Historically, the plant has been prized for its anti-fertility, anti-rheumatic, and antispasmodic effects.

The species' adaptability is demonstrated by its dimorphic seeds, which maintain the ability to germinate under varying environmental conditions. This robustness has enabled it to establish itself as a widespread weed across Central America, Africa, and India.

While the qualitative phytochemical makeup of *M. suaveolens* is largely uniform globally—regularly exhibiting alkaloids, flavonoids, tannins, and glycosides—quantitative data show marked disparities. For instance, Riddhi *et al.* (2025) identified a total phenolic content (TPC) of 5.3 mg/ml

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in leaf samples alongside elevated flavonoid levels, yet Obiahu *et al.* (2025) recorded a TPC of 10.30 mg/100 g. These variances emphasize how external conditions, including climate and soil chemistry, modulate the plant's secondary metabolic processes. (Akharaiyi *et al.*, 2023)

The antioxidant potential of *M. suaveolens* has been substantiated through various analytical methods, specifically DPPH and ABTS assays. Existing literature suggests that extraction techniques play a critical role in determining potency; methanolic leaf extracts, for example, have exhibited IC₅₀ values ranging from 0.28 mg/ml (Soumahoro *et al.*, 2023) to as low as 14.04 µg/m (Gavani and Prakash, 2008). Despite the extensive scrutiny of the leaves, there remains a notable deficiency in comparative research concerning the stem components. While certain investigations have identified the presence of proteins, starch, and tannins in the stem (Dhamale *et al.*, 2023), a comprehensive side-by-side quantitative assessment of the antioxidant capacity between the leaf and stem represents a significant research gap.

The microenvironment frequently modulates the chemical profiles of invasive species. In particular, the canal areas of Gandhinagar constitute a distinct ecological region where anthropogenic variables and specific soil moisture conditions potentially affect the sequestration of secondary metabolites.

Research Objective: This investigation is designed to address current gaps in literature through a comparative assessment of the antioxidant and phytochemical profiles of *M. suaveolens* stems and leaves, specifically those sourced from the canal regions of Gandhinagar. Through the application of rigorous quantification and extraction protocols, the study endeavours to substantiate the therapeutic viability of this common weed, establishing a scientific framework for its integration into advanced medicinal formulations.

MATERIAL AND METHODS

Sample Collection

Mature plant was collected from Road side plantations of Koba Gandhinagar; India The plant was collected in winter season in mid of January. The plant

was authenticated from sources like e Flora of Gandhinagar, e Flora of India and Gujrat university.

Sample Preparation

The plant material was initially washed 2 to 3 times by distilled water. Leaves and Stem were separated afterwards, using a paper towel excess water was removed and the material was kept to shade dry for 4 days. Stem was kept till 7 days due to excess water content. The leaves were directly grinded by a mechanical grinder but the stem was broken down into piece using mortar and pastel due to their large size and flexibility. Leaf powder was obtained directly. Again the exception stands with the stem, after grinding it multiple times the powder was subjected to a fine sieve to get fine powder and the leftovers was grinded again.

Extraction

The powdered plant material was extracted by macerating it in Ethanol and Methanol. 200ml of Ethanol and Methanol was added in 10 gram of plant material. The beaker was covered with a foil paper to prevent evaporation and it was kept inside a cupboard for 3 days in room temperature without disturbance. After 3 days a setup containing funnel, filter paper, test tube stands and a petri dish was built for filtering out the extract. Solution was poured into petri dish via this setup till half full then kept at room temperature till it dries out. Yield was obtained by repeating the process. Extract obtained by this process is called Cold Extract and this method is called cold extraction method.

Qualitative Phytochemical analysis

Alkaloid: Dragendroff's Test extract up to 1ml + Few drops of Reagent alongside the test tube which leads to formation of Reddish Brown ppt. (De Silva, G. O., *et al.*, 2017; Kumar, M. K., *et al.*, 2011).

Tannin: Ferric Chloride test extract 1ml+ 2ml Ferric chloride (5%) formation of bluish black colour formed (Audu, S. A. *et al.*, 2007; Sayma *et al.*, 2025)

Glycoside: Keller-Killiani test. Extract 1ml+ 3ml Glacial acetic acid+ few drops of Ferric Chloride (0.5%)+ Few drops of concentrated H₂SO₄ alongside the test tube. Brown ring at the junction and the acetic acid layer appears bluish (Singh and Kumar, 2017)

Flavonoids: Lead Acetate test extract up to 1ml+ Lead acetate leads to formation of yellow ppt (Singh and Kumar, 2017).

Phenol: Ferric Chloride test 1ml Extract+ 5% Ferric chloride few drops Dark green or bluish black colour (Raaman N, 2006; Tiwari *et al.*, 2011)

Saponins: Persistent Foam Test. Extract up to 1 ml and add 5ml of distilled water and shake it.

Terpenoids: Salkonski's Test. Filtrate + Few drops of H₂SO₄ concentrated Golden yellow colour layer (Shaikh, J. R., *et al.*, 2020).

Protein: Millon's Test. 2ml extract and add Millon's reagent whit ppt will form (Silva *et al.*, 2017)

Carbohydrate (Starch): Iodine test. Plant extract than few drops of iodine blue black colour will appear (Rathod *et al.*, 2022).

Quantitative Analysis

Formula for Total Yield

Total Yield percentage calculation.

$$\text{Yield}\% = \frac{(\text{Weight of Dry Extract})}{(\text{Weight of Plant Powder Taken})} \times 100$$

Total Phenol Content (TPC)

Phenol is a group of compounds and usually present in almost every plant in a good amount. To determine Total Phenol Content, I used Folin-Catechu Reagent technique. 1ml Plant extract/Gallic acid solution (1mg/ml in distilled water). Than make series of gallic from 10µl to 50µl add distilled water to make it up to 1ml. Now in a test tube the put 0.5ml Folin-Catechu (Prepared in 1:1 ratio with distilled water). Add 10ml distilled water. Now make 20% Sodium Carbonate solution and add 1ml directly after adding distilled water. Again add Distilled water till the solution is of 25ml. Now incubate it in dark for 30 minutes. Observe its Optical density at 765nm (Madhu *et al.*, 2016)

Calculation

$$C = \frac{X \times V}{M}$$

C → Total Phenol content

X → Concentration of Phenolic (mg/ml)

V → Volume taken

M → Plant powder per ml

Now formula to find concentration of Phenol

$$y = mx + c$$

$$x = \frac{y - c}{m}$$

y → Absorbance of sample

m → Slope of Calibration curve

c → Concentration of Phenol (Siddiqui *et al.*, 2017)

Total Flavonoid Content

Total Flavonoid Content (TFC)

Flavonoids are obtained from phenol. this phytochemical some time possess antioxidant potential. To determine the Total Flavonoid Content, I used Aluminium Chloride Colorimetric method. It starts with adding 1ml plant extract/ Quercetin solution (1mg/ml in methanol). Then make the series of Quercetin from 50µl to 500µl add distilled water up to 1ml and then add 4ml of distilled water. Then add 0.3ml of 55 Sodium nitrate incubate it in dark for 5 minutes. After 5 minute add 0.3ml of 10% Aluminium Chloride. At 6th minute add 2ml of 1M Sodium Hydroxide. At last add distilled water to make total solution up to 10ml (Madhu *et al.*, 2016). Observe its optical density at 510nm.

Calculation

$$C = \frac{X \times V}{M}$$

C → Total Flavonoid content

x → Concentration of Flavonoids(mg/ml)

V → Volume taken

M → Plant powder per ml

Now formula to find concentration of Flavonoids

$$y = mx + c$$

$$x = \frac{y - c}{m}$$

y → Absorbance of sample

M → Slope of Calibration curve

c → Concentration of Flavonoid (Siddiqui *et al.*, 2017)

Total Tannin Content (TTC)

These phytochemical is renowned for protecting the plant in its juvenile stage by producing very strong taste. To calculate total tannin content, I used Potassium Iodate method. It starts from adding 1 ml of plant extract/ Tannic Acid solution (1mg/ml in distilled water) in test tube. Make a series of tannic acid from 100µl to 500µl and add distilled water to make 1ml. Prepare a cold water bath and keep those test tubes in it for 5minutes. Then add 5 ml of 2.5% KIO₃. Take out the test tube and incubate it at room temperature for 25-30 minutes. Observe its optical density at 550nm (Willis, R.B, 1998)

Calculation

$$C = \frac{X \times V}{M}$$

C → Total Tannin content

x → Concentration of Tannin(mg/ml)

V → Volume taken

M → Plant powder per ml

Now formula to find concentration of Tannin

$$y = mx + c$$

$$x = \frac{y - c}{M}$$

y → Absorbance of sample

m → Slope of Calibration curve

c → Concentration of Tannin (Siddiqui *et al.*, 2017)

Antioxidant essay (DPPH)

This is a popular and common method used to check the presence of free radical scavenging activity of a given plant material. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a dark crystalline powder having stable free radical molecules. 4mg (0.004%) of DPPH was dissolved in 100 ml methanol. 3ml of prepared DPPH was added to various concentration of sample (200, 400, 600, 800, and 1000 µg/ml) and incubate for 30 minutes at room temperature in dark, the absorbance was read at 517nm in duplicates for each concentration in spectrophotometer. To prepare the standard make the ascorbic acid solution in 0.2mg/ml then add methanol in series of 200µl to 800µl. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants, thus a low value corresponds to a good scavenging ability. Ascorbic acid solution of same concentration is used as standard for the free radical scavenging assay. The antioxidant activity is given terms of mg/g Ascorbic acid equivalent was used as standard (He and Venant 2004; Sayma *et al.*, 2025).

Calculation

$$I\% = \frac{(A \text{ Control} - A \text{ Sample})}{A \text{ Control}} \times 100$$

For IC₅₀, Take y=50

$$y = mx + c$$

$$x = \frac{y - c}{M}$$

y → Absorbance of sample

m → Slope of Calibration curve

c → Concentration of Ascorbic acid

Result and Discussion

Qualitative analysis result

Table 1

Sr.No	Phytochemical	Preferred Test	Solvent and Plant Part	Present (+)/Absent (-)
1	Alkaloid	Dragendroff's Test	Ethanol leaf	+
			Ethanol Stem	+
			Methanol Leaf	++

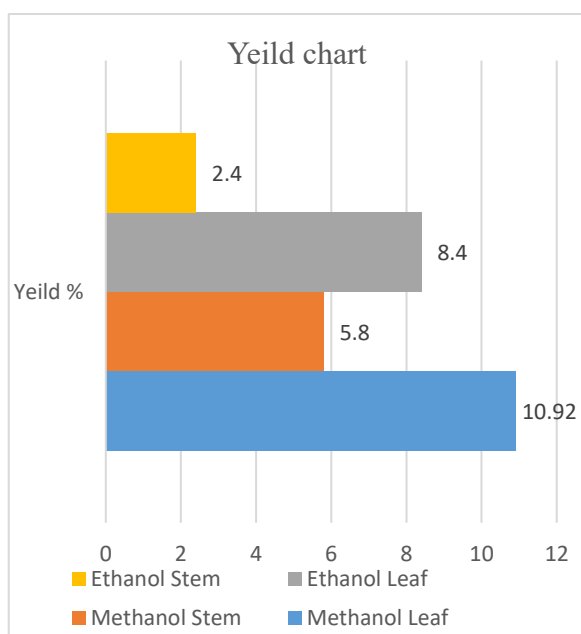
			Methanol stem	++
2	Tannin	Ferric Chloride test	Ethanol leaf	+
			Ethanol Stem	+
			Methanol Leaf	++
			Methanol Stem	-
3	Glycoside	Keller Killiani test	Ethanol leaf	++
			Ethanol Stem	+
			Methanol Leaf	++
			Methanol Stem	+
4	Flavonoids	Lead Acetate test	Ethanol leaf	++
			Ethanol Stem	+
			Methanol Leaf	++
			Methanol Stem	++
5	Phenol	Lead Acetate test	Ethanol leaf	+
			Ethanol Stem	+
			Methanol Leaf	+
			Methanol Stem	+
6	Saponins	Persistent Foam Test	Ethanol leaf	++
			Ethanol Stem	-
			Methanol Leaf	++
			Methanol Stem	+
7	Terpenoids	Salkonski's Test	Ethanol leaf	++
			Ethanol Stem	++
			Methanol Leaf	++
			Methanol Stem	++
8	Protein	Millon's Test	Ethanol leaf	+
			Ethanol Stem	-
			Methanol Leaf	+
			Methanol Stem	+
9	Carbohydrate (Starch)	Iodine test	Ethanol leaf	+
			Ethanol Stem	-
			Methanol Leaf	+
			Methanol Stem	-

(Present= +, Absent= -, Highly Present= ++)

Quantitative analysis result

Table 2 showing Total Yield

Sr No.	Plant Part	Solvent	Yield percentage
1	Leaf	Ethanol	8.4%
2	Leaf	Methanol	10.92%
3	Stem	Ethanol	2.4%
4	Stem	Methanol	5.8%

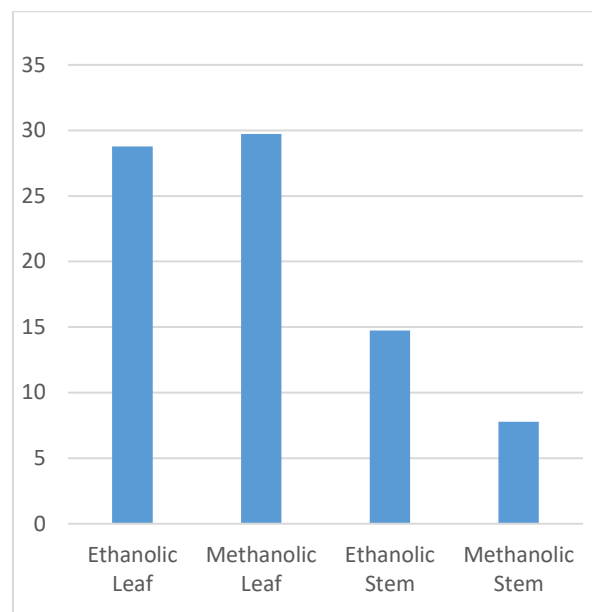
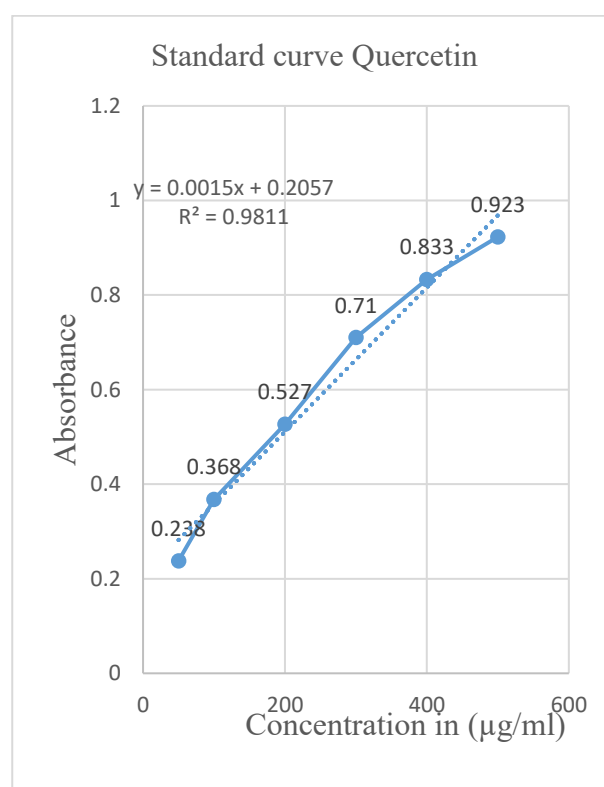
Graph 1 for Total Yield

1.1 Total Flavonoid content

The total Flavonoid content present in Ethanolic leaf extract is 28.768 ± 0.6715 mg QE/g of extract, in Methanolic leaf it is 29.737 ± 0.107 mg QE/g of extract. On the other hand, Ethanolic extract of stem shows 14.736 ± 0.7 mg QE/g of extract and Methanolic stem extract shows 7.737 ± 0.32 mg QE/g of extract.

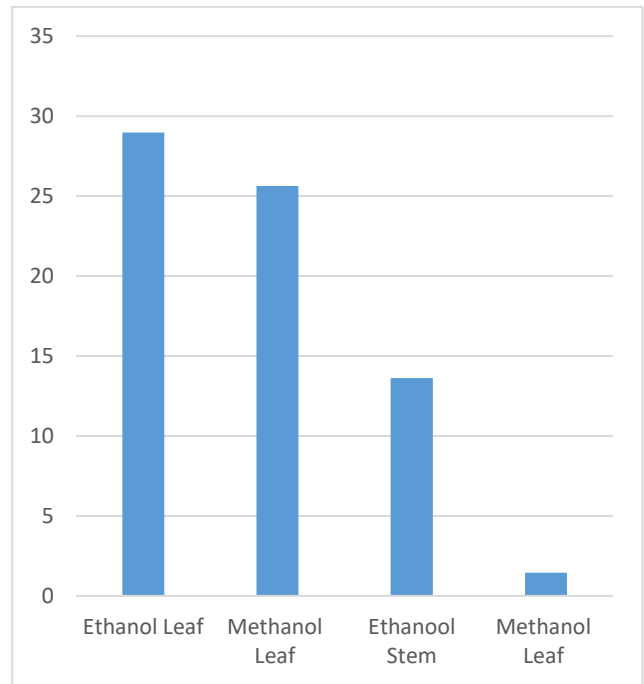
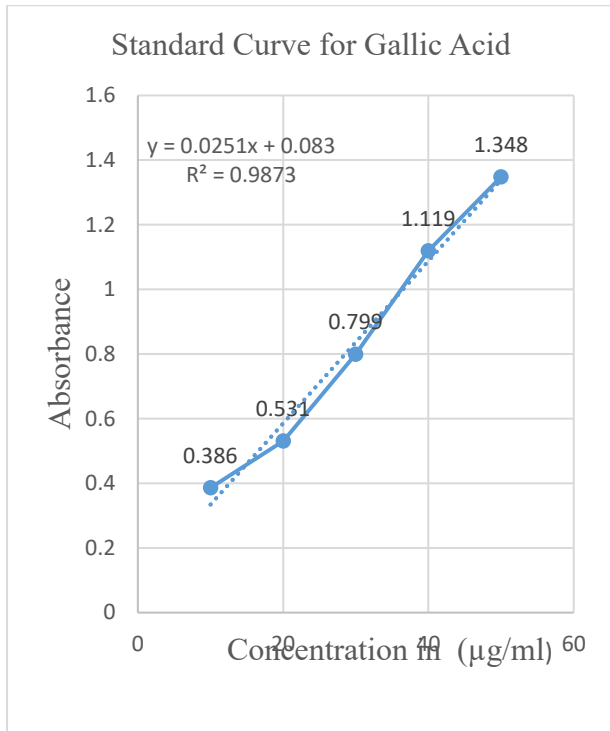
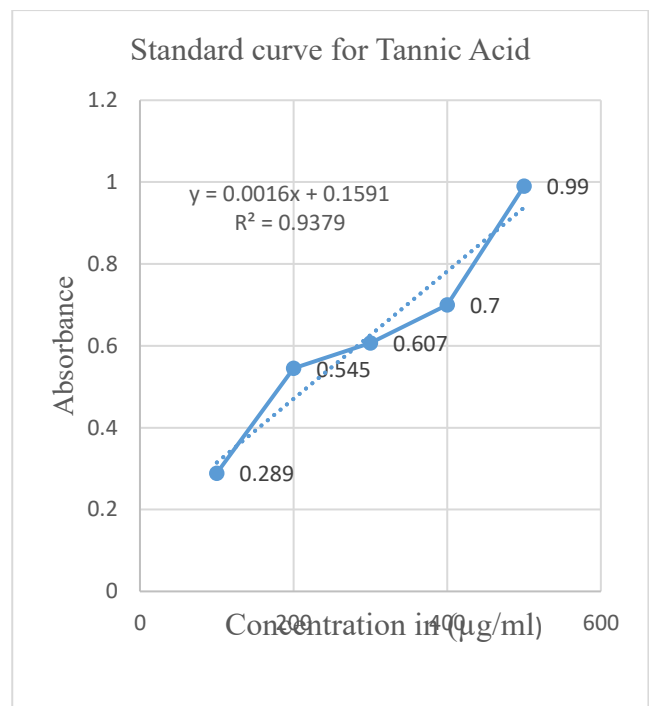
Table 3

Extract Type	Content (mg QE/g)
Ethanolic Leaf	28.768 ± 0.6715
Methanolic Leaf	29.737 ± 0.107
Ethanolic Stem	14.736 ± 0.7
Methanolic Stem	7.737 ± 0.32

Graph 2 Showing Total Flavonoid**Graph 3 Showing Standard Curve for Quercetin**

Total Phenol Content

Total phenol content in Ethanolic leaf extract is 1.29 ± 0.01 mg GAE/g of plant extract, Methanolic leaf extract shows 1.26 ± 0.05 mg GAE/g of plant extract. On the other hand, the stem extract shows less phenol content Ethanolic stem extract is 0.8 ± 0.015 mg GAE/g of plant extract and methanolic extract is 0.83 ± 0.015 mg GAE/g of plant extract.

Graph 4 Showing Standard curve for Gallic Acid**Graph 6 Showing Standard Curve for Tannin Acid****Total Tannin Content**

Total Tannin content in ethanolic leaf extract is 28.98 ± 0.10 mg TAE/g of plant extract, Methanolic leaf extract is 25.62 ± 3.34 mg TAE/g of plant extract. I can see the same pattern in extract of stem i.e. less tannin content in stem of the plant. Ethanolic stem extract is 13.61 ± 1.21 mg TAE/g of plant extract and methanolic extract is 1.455 ± 1.1 mg TAE/g of plant extract.

Table 4

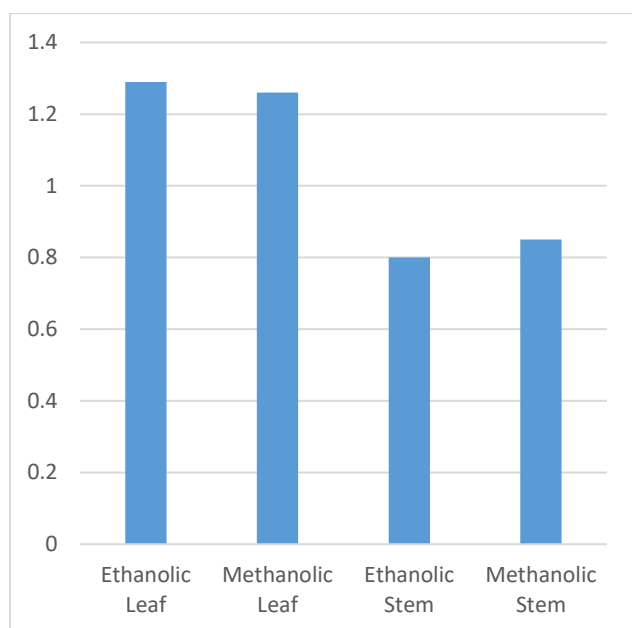
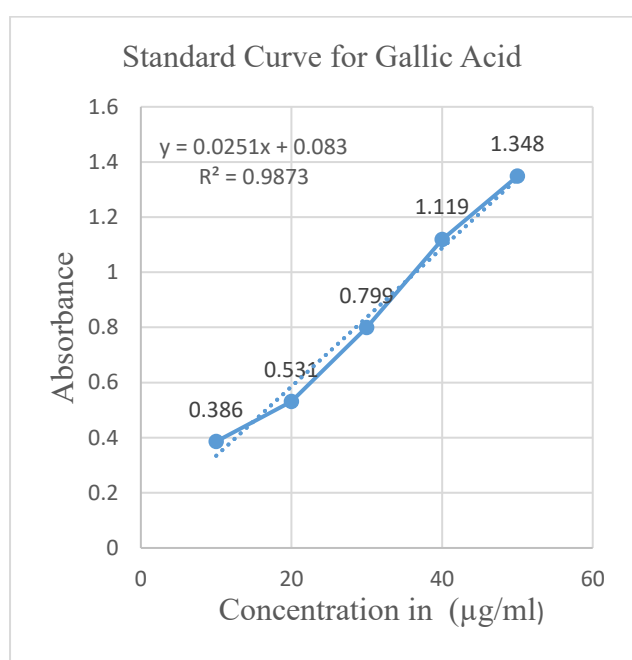
Extract Type	Content (mg TAE/g)
Ethanolic Leaf	28.98 ± 0.10
Methanolic Leaf	25.62 ± 3.34
Ethanolic Stem	13.61 ± 1.21
Methanolic Stem	1.455 ± 1.1

Chart 5 Showing Total Tannin Content**Total Phenol Content**

Total phenol content in Ethanolic leaf extract is 1.29 ± 0.01 mg GAE/g of plant extract, Methanolic leaf extract shows 1.26 ± 0.05 mg GAE/g of plant extract. On the other hand, the stem extract shows less phenol content Ethanolic stem extract is 0.8 ± 0.015 mg GAE/g of plant extract and methanolic extract is 0.83 ± 0.015 mg GAE/g of plant extract.

Table 5

Extract Type	Content (mg GAE/g)
Ethanolic Leaf	1.29±0.01
Methanolic Leaf	1.26±0.05
Ethanolic Stem	0.8±0.015
Methanolic Stem	0.83±0.015

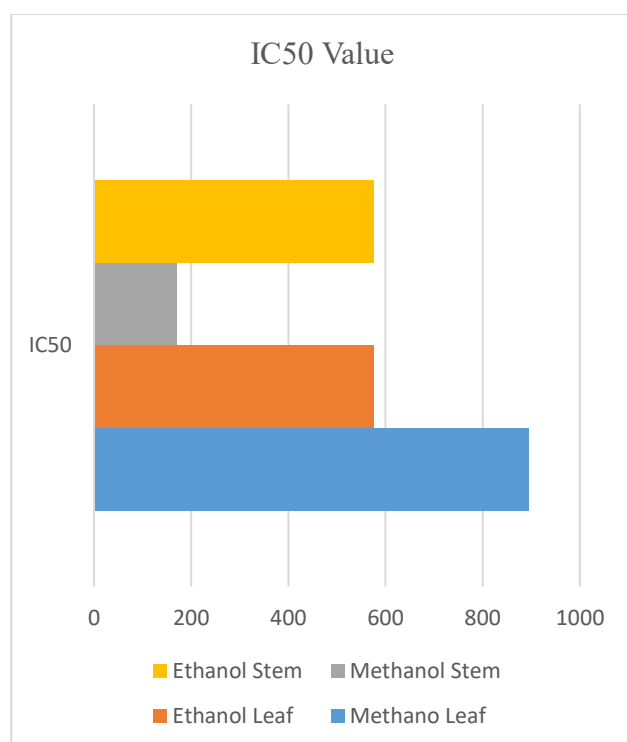
Chart 7 Showing Total Phenol Content**Graph 8 Showing Standard curve for Gallic Acid**

Antioxidant activity of *Mesosphaerum suaveolens* (L.) Kuntz

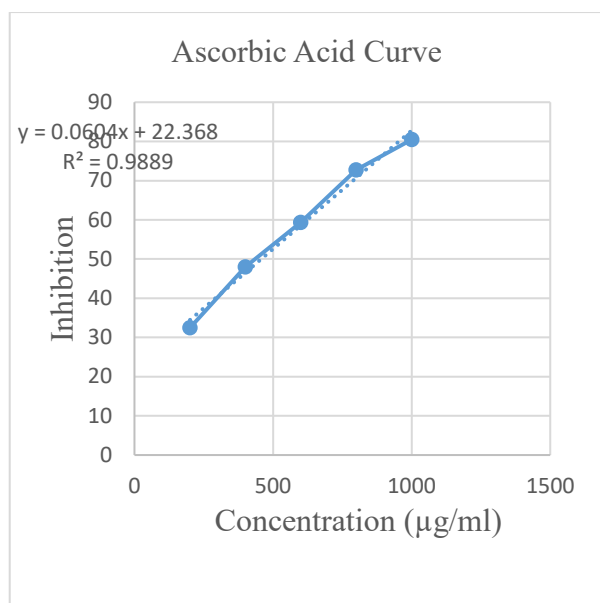
Mesosphaerum suaveolens (L.) Kuntz's antioxidant activity was evaluated by DPPH method. Ethanolic leaf extract shows very low radical oxygen scavenging activity with IC₅₀ value of 894.81 µg/ml and same goes with stem IC₅₀=575.051 µg/ml. On the other hand, the IC₅₀ value of Methanolic leaf extract is 169.734 µg/ml which shows that this extract possesses high antioxidant potential, on the contrary methanolic stem extract shows IC₅₀=574.83 µg/ml nearly negligible.

Table 6 Showing IC₅₀ value of different plant parts

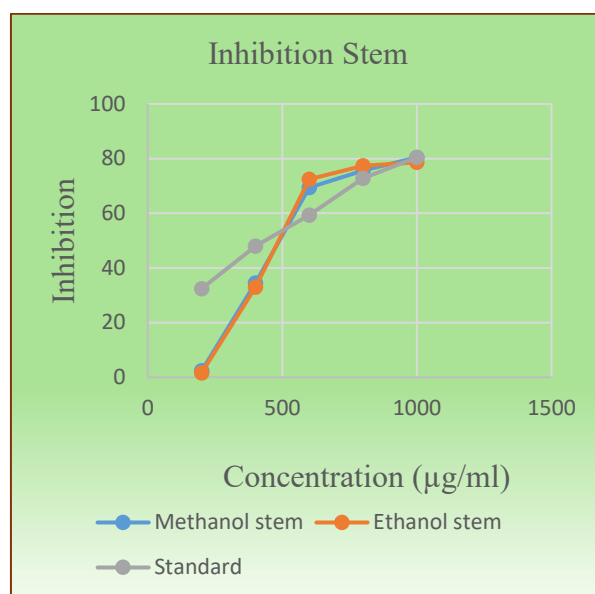
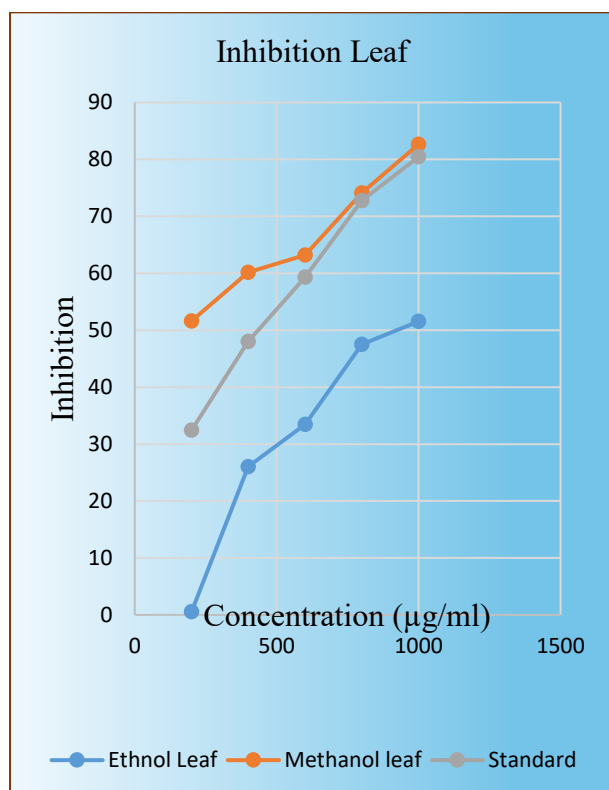
Sr.No	Plant part	IC ₅₀ (µg/ml)
1	Ethanol Leaf	894.81 µg/ml
2	Ethanol Stem	575.05 µg/ml
3	Methanol Leaf	169.73 µg/ml
4	Methanol Stem	574.83 µg/ml

Chart 9 showing IC₅₀ value of different extract

Graph 10 Showing Standard Curve for Ascorbic Acid



Graph 11 Showing Inhibition of Methanol and Ethanol Leaf extract against Standard inhibition



Graph 12 Showing Inhibition of Methanol and Ethanol Stem extract against Standard inhibition

CONCLUSION

This study focuses on the qualitative and quantitative analysis of Phytochemical and antioxidant activity present in *Mesosphaerum suaveolens* (L.) Kuntze. In qualitative analysis I found the high concentration of flavonoid, tannin, terpenoids and glycoside but the phenols, proteins and saponins shows weak presence. Quantitative analysis shows that Methanolic leaf extract and ethanolic leaf extracts contain similar flavonoid content competitively higher than their stem extracts specially the methanol stem extract shows the lowest total flavonoid content. Phenol content is apparently less concentrated. In stem extracts shows negligible amount of the phenol content. In leaf it is competitively higher. When it comes to tannin content both ethanolic and methanolic leaf extract shows abundance and stem is again competitively lower. Antioxidant activity done via DPPH method the findings suggest except the methanolic extract of leaf every other extracts are not suitable as an antioxidant agent. The relevance of this study is not only limited to the data but it also relates the phytogeographical changes in the same species can leads to changes in its phytochemistry.

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