PHYTOCHEMICAL SCREENING AND ANALYSIS OF ANTIOXIDANT ACTIVITY FROM THE BOTANICAL EXTRACT OF THE PLANT LEONOTIS NEPETIFOLIA

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Abstract

Background and Aim: Medicinal plants, enriched by nature with potent compounds, hold great promise in developing natural drugs with minimal side effects. This study focuses on *Leonotis nepetifolia* a plant species renowned for its medicinal potential. We aimed to identify and quantify phytochemicals in its leaf extracts and evaluate their in vitro antioxidant activity.

Methods: Methanol, hexane, and water were used as solvents for extraction.Qualitative and quantitative tests were carried out to determine the presence and amount of compounds present in the extracts.To determine the antioxidant activity various assays like DPPH,ABTS,FRAP and Ion chelating assays were carried out

Results: Qualitative analysis revealed a range of pharmacologically active phytochemicals, including alkaloids, carbohydrates, glycosides, saponins, proteins, amino acids, phenols, fixed oils, and terpenoids. Methanolic and aqueous extract exhibited the highest diversity, with six distinct compounds. Quantitative analysis showed that aqueous extract contained the most phenols (0.52 mg/g) and carbohydrates (11mg/g), while hexane had the highest flavonoid content (329 mg/g), and aqueous extract displayed the most protein (56.8 mg/g). Antioxidant assays demonstrated the superior performance of aqueous extract, with maximum inhibitory percentages of 73.96% (DPPH), 65.71% (FRAP), 85.07% (ion chelating), and 45.73% (ABTS) indicating its remarkable antioxidant potential.

Conclusion: These findings underscore *Leonotis nepetifolia* as a valuable source for phytochemicals with significant antioxidant properties, particularly in the aqueous leaf extract, holding promise for future therapeutic developments.

Keywords – *Leonotis nepetifolia*, drug, DPPH, antioxidant.

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INTRODUCTION

Leonotis nepetifolia commonly known as Lions ear is a herb found in tropical Asia, Southern India and Africa. The trade name of the plant is Barchi buti and its called in Ayurveda as Granthiparni. The plant is called in different common names namely Matijer (Gujarati), Dipmal (Marathi), Knod grass (English), Gathiyan (Hindi), Ranabheri (Telugu).This plant can be found in overgrazed pastures, roadsides, floodplains,waste areas, disturbed sites. This plant possess the inherent ability to cure various diseases like bronchial asthma, dysmenorrhea, rheumatism, diarrhea, fever and the medicinal properties of this plant were reported by many countries like Madagascar, Brazil, Canada, Kenya and many African countries [1]. The reproduction of Leonotis nepetifolia takes place by water and to help in dispersal there is no special adapatations. The flowers of this plant is visited by honey bees and it the major pollinator [2]. The plant is tall and grows to a height of 1-2 meters. This plant grows in places with rainfall of 1600-2000mm and temperature of more than 300°C. The aromatic stem is deeply sulcate, stout, grows upto 8 feet tall and is a obtusely tetragonous,pubescent and straight. Petioles are 2.5-10cm long and winged in the upper part. Nutlets are trigonous, oblong and four in number. Basal is capitellate, style gynobasic, stigma bifid. Ovary bicarpellary, tetralocular ovule one per locule. Filaments minutely bearded and disc copular.Stamen are exserted, four, and didynamous hooded by the upper lip. Calyx mouth tubular, obligues,spinescent coriaceous, valuate and 8-10 toothed. Flowers are orange, globose, spinous whorls of verticils, scarlet and axillary. Leaves are ovate, base truncate to cuneate, deeply crenate to serrate and acute. This plant prefers to grow in soil having humus content with Ph of 4.6-6.5.Seeds are also oblong [3-5].

The plant is rich in tannina, vitamins,oil, flavonoids(quercetin, rutin, apigenin, hyperoxide), iridoid glycosides(leonuride, leonurin, leonuridine),diterpenoids (leocardin) and alkaloids (stachydrene and leonurine).

Stem is used to treat jaundice and skeletal muscle stimulant. The leaves contain 6-octadecadienoic acid, neptaefolinol, neptaefolin, neptaefuran, neptaefuranol. The leaves are used in rheumatism, hepatitis, diabetes and hernia. The root contains Beta-sotosterol, campesterol, coumarin 4,6,7-trimethoxy-5-methylchromene-2-one,n-octacosanoic acid,n-octacosanol and quercetin. The seed oil contains stearic acid, oleic acid, lioleic acid and palmitic acid. Seeds are used in antimalarial and diuretic. Flowers are used to cure cut wounds, burns,and jaundice [6-9].

Scientific classification of *Leonotis nepetifolia*

Domain : Eukaryota
Kingdom : Plantae
Phylum : Spermatophyta
Subphylum : Angiospermae
Class: Dicotyledonae
Order : Lamiales
Family : Lamiaceae
Genus : *Leonotis*
Species : *Leonatis. Nepetifolia*

Fig.1: *Leonotis nepetifolia*

For treating burns, inflorescence(paste and ash) and seed flower of the plants are used. External application of the whole plant of Leonotis nepetifolia cures paralysis [10-13]. For wound healing the ground nut oil is mixed with paste of inflorescence.To treat eczema the leaf paste is also

applied externally.Mustard oil can be mixed with the ash of the whole plant and applied externally to treat breast pain during post natal period. The crushed leaves of Leonotis nepetifolia can be applied on the affected area to deaden burning sensation [14-15].To treat jaundice, decoction from stem,flowers and leaves can be administered. In the formulations of Himasagam taila, Nakula taila, Mritasanjeevanisura , Brihat guduchi taila the roots of this plant is added and is considered as an ayurvedic herb [16].

MATERIALS AND METHODS

Collection and Preparation of plant material

During the rainy season, the entire plant material of Leonotis nepetifolia was collected and completely washed in Padappai, Kancheepuram district, Tamilnadu. The plant material was shade-dried for three weeks before being coarsely powdered with an electrical blender. For four days, the finely powdered plant material (25g) was exposed to Soxhlet extraction in ascending order of polarity, namely hexane, methanol, and water. The extract was then concentrated using a rotary evaporator and kept at 4 C for future use [17].

QUALITATIVE PHYTOCHEMICALS ANALYSIS

The plant extracts obtained via Soxhlet extraction were submitted to preliminary phytochemical screening to evaluate the presence or absence of several phytoconstituents such as saponins, flavonoids, terpenoids, glycosides, alkaloids, proteins, carbohydrates, and phenols. The extract was subjected to preliminary phytochemical screening using conventional procedures [18].

Test for saponin

In a test tube, 5.0 ml of distilled water was combined forcefully with the aqueous plant extract. The presence of saponin was determined by the production of foam.

Test for flavanoid:

When aqueous plant extract was combined with 2ml of 2.0% NaOH mixture, a concentrated yellow color was formed. The presence of flavonoids was detected in the results**.**

Test for Terpenoids

5ml of aqueous plant extract was added with 2.0 ml of chloroform and evaporated in waterbath and then it was subjected to boiling with 3ml of concentrated sulphuric acid. Formation of grey colour indicated the presence of terpenoids.

Test for glycosides

To 10ml of aqueous plant extract and 1 ml of concentrated sulphuric acid, 4ml of glacial acetic acid with 1 drop of 2.0% $FeCl_3$ was mixed. Cardiac steroidal glycoside was confirmed by the formation of brown ring between the layers.

Test for Alkaloids

Dragendorff test- Presence of alkaloid was confirmed by the presence of orange-red colour precipitate when one drop of dragendorff reagent was added to 5mg extract taken in tube.

Test for Protein

5mg of plant extract was combined with a few drops of biurets reagent and left to boil for 1-5 minutes. The production of red or violet color confirmed the presence of protein.

Test for Aminoacid

Ninhydrin test- 2ml of 0.2% ninhydrin solution was added to 5mg of plant extract and boiled in a water bath for 2 minutes. The appearance of violet color confirmed the presence of aminoacid.

Test for Carbohydrate

Fehling's test- A few drops of Benedict's reagent were added to 5mg of botanical extract and left to boil. The presence of carbohydrates was suggested by the development of a reddish brown precipitate.

Test for Phenols

Powdered leaf samples were boiled with 20ml of distilled water and filtered. Occurrence of brownish green or blue colour was observed when 3-4 drops of 0.1% ferric chloride was added to the filtrate. It indicated the presence of phenols.

QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Hexane, methanol and aqueous extracts of *Martynia annua* were subjected to quantitative analysis inorder to determine the amount of metabolites present in them.

Determination of total phenolic content

bsorbance was measured. 212g of botanical powder was steeped in methanol, water, and hexane. It was shaken in an orbital shaker for 24 hours. The filtrate was evaporated after sifting the leftovers. The extracts were centrifuged for 15 minutes at 10,000 rpm. 20 ml of extract was made from the supernatant, and it was diluted to 3ml with distilled water. After adding 0.5 ml of FolinCiocalteu's phenol reagent to all of the tubes, the tubes were placed in the incubator for 3 minutes at 45 C. After

3 minutes, 2ml of 20% Na2CO3 was added to each tube and incubation was continued [19]. At 650nm, the absorbance was measued.The total phenol content wascalculated using the formula,

C(GAE)=C×V/M

Where C=Concentration of sample from the curve obtained (mg/ml)

V= Volume used during the assay (ml) and

M= mass of the sample used during the assay (g)

Determination of total flavonoid

Flavonoid content was estimated by slightly modifying Elin Novia Sembiring's spectrophotometry approach. One gram of dry powder was weighed and pulverized in a mortar and pestle with 200 ml of 80% aqueous methanol. After filtering the pulverized sample, a clear filtrate was obtained. To the test tube containing an aliquot of the sample (0.5 ml), 3 ml of distilled water and 0.3 ml of 5% sodium nitrite were added. After vortexing, the solution was allowed to rest at room temperature for 5 minutes before adding 0.6 ml of 10% aluminum chloride. After 6 minutes with distilled water, 2ml of 1M sodium hydroxide was added to the test tube, and the solution was built up to 10 ml. Finally, the absorbance was determined at 510nm [20]. The total flavonoid content was calculated as quercetin equivalent (mg QE/g) using the formula,

$X=(A.M_0/ A.M_0)$

Where, A= absorption of sample,

A_0= absorption of standard (quercetin),

M= weight of sample(mg/ml) and

M_0 = weight of the quercetin in solution (mg/ml)

Protein estimation assay

The assay is based on the finding that when a coomassie brilliant blue G-250 acidic solution binds to protein, the absorbance maximum moves from 465nm to 595nm. When the anionic form of the dye is stabilized by hydrophobic and ionic interactions, a noticeable color change occurs. The extinction coefficient of a dye-albumin complex solution remains constant over a 10-fold concentration range, making the experiment useful [21].

Unknowns can be diluted to obtain between 5 and 100g protein in at least one assay tube containing 100µl sample. Each sample can be treated with an equal volume of 1M NaOH before vortexing.NaOH can also be applied to standards. Standard protein (albumin or gamma globulin) can be manufactured in 100µl volumes ranging from 5 to 100 g. After adding 5 ml of dye reagent, absorbance can be measured at 595 nm.

Total Carbohydrates

1 ml of phenol and 5ml of concentrated sulphuric acid was added to 1ml of the sample and the mixture was mixed thoroughly. The OD readings can be taken at 490nm after allowing the solution to stand in boiling water bath for 15 minutes. Using the standard graph prepared by D-glucose, the amount of total carbohydrates was calculated and the values are expressed as µg/ml [22].

ANTIOXIDANT ACTIVITY

DPPH Assay :

The percentage of antioxidant activity of any drug can be estimated by evaluating the DPPH free radical assay. The DPPH radical scavenging activity was evaluated using the approach outlined by Thasneem [23]. The sample was exposed to a methanol solution containing a stable DPPH radical. The reaction mixture contained 0.5 ml of sample, 1 ml of methanol, and 1 ml of DPPHradical solution (0.5 mm in methanol). When DPPH reacts with an antioxidant molecule capable of donating hydrogen, it is decreased.The change in color from deep violet to pale yellow was measured at 517 mm using a UV-VIS Spectrophotometer. 1 ml of methanol was used as a blank, and the control solution was made by combining DPPH radical solution (1.0 ml) with methanol.The scavenging activity percentage was determined according to, Percentage of inhibition = control OD - sample O.D / control O.D x 100

Ferric reducing antioxidant power assay

The antioxidant capacity of medicinal plants was estimated using spectrometric methods, as described by Anoob Kumar [24].

The approach is based on the fact that, at low PH, the reduction of Fe 3+ TPTZ complex (colored complex) to Fe2+ tripyridyltriazine (blue coloured complex) happens due to the action of electron donating antioxidants. Monitoring reactions can be accomplished by measuring the change in absorbance at 593 mm.

The ferric reducing antioxidants power (FRAP) reagent was made at 37 degrees Celsius by combining 300 mm acetate buffer, 10 mL TPTZ in 40 mm HCL, and 20 mm FeCl3-6H2O in a 10:1:1 ratio using a 1-5 ml variable micropipette (3.995 ml). Pipetted FRAP reagent was combined with diluted plant sample of 5 microlitre.

After 20 minutes at 37 degrees Celsius, the ferric tripyridyltriazine (Fe3+ TPTZ) complex was reduced to (fe2+) form, followed by the formation of an intense blue color complex, and the absorbance at 593 nm was measured against a

reagent blank (3.995 ml) FRAP reagent + 5 microlitre distilled water.
The calibration curve was created by graphing the absorbance at 593 nm for various concentrations of FeSo4. in comparison to the levels of the standard antioxidant trolon. The concentrations of FeSo4 were then shown It was obtained that the FRAP values were given as mg of trolon equivalent per gram of material. when the absorbance change in the test mixture was compared to those obtained from increasing Fe3+ concentrations.

ABTS radical scavenging assay
The free radical scavenging activity of plant materials was evaluated using the ABTS radical cation decolorization assay [25].
After storage in dash at room temperature for 12-16 hours, a reaction occurs between 7mm ABTS in water and 2.45mm potassium persulfate (1:1), resulting in the creation of a + cation radical.
Methanol was used to dilute the ABTS. + solution to achieve an absorbance of 0-70 degrees at 734 nm. Absorbance was determined 30 minutes after mixing 3.995 ml of diluted ABT. + solution with 5 microlitre of plant extract. An suitable amount of solvent blank was run in each test.
The percentage inhibition of absorbance at 734 nm was computed using the formula, ABTS + scavenging effect (%) = (AB-AA)/AB X100; where AB is absorbance of ABTS radical + methanol and AA is the absorbance at 734 nm. Trolox was used as the standard.

Ion chelating assay
By the method of Ilhami Gulcin al [26] the extracts chelatin of ferrous ion can be estimated .To 1ml of varying concentration of the extract (0.2 ,0.4 , 0.8 , 1.6 and 3.2 mg/ml),50 microlitre of 20 mm FeCl2 was added .By adding 0.2 ml of 5mm feroxine solution , the reactions can be initiated . after shaking vigrorously the mixture can be allowed to stand for 10 minutes at room temperature . the absorbance was thereafter measured at 562 nm . The percentage inhibition of ferrozine -Fe2+ complex formation was calculated as $[(A_0- As)/ As] \times 100$
Where A_o was the absorbance of the control as was the absorbance of the extract/standard . As positive control ,Na2EDTA was used .

RESULTS AND DISCUSSION

Phytochemical screening
The phytochemical analysis of the various extracts of *Leonotis nepetifolia* is shown in Table 1.

Table 1. Qualitative Phytochemical analysis

S.No.	Phytocomponents	*Leonotis nepetifolia L.*		
		Methanol Extract	**Hexane Extract**	**Aqueous Extract**
1.	Alkaloids	+	+	+
2.	Carbohydrates	+	---	+
3.	Glycosides	+	---	+
4.	Saponins	+	+	+
5.	Proteins	---	---	---
6.	Aminoacids	---	+	---
7.	Phenols	+	---	+
8.	Fixed Oils	+	---	+
9.	Terpenoids	---	+	---

(+) - Presence (---) – Absence

From the qualitative findings presented in Table 1, it has been observed that the extracts of *Leonotis nepetifolia* contained alkaloids, carbohydrates, glycosides,saponins, phenols, amionoacids,fixed oils, and terpenoids. However most of the secondary metabolites were present in the methanolic and aqueous extracts. Aminoacid was found to be present only in the hexane extract.

Quantitative analysis
Quantitative phytochemical analysis results for total phenols, flavonoids, proteins and carbohydrates were obtained using three botanical extracts and The results of quantitative phytochemical analysis is presented in Table 2.

Total phenols
The standard used for the determination of phenolic content was gallic acid. The total phenolic

content of the methanolic extract were 0.38mg/g and 0.43mg/g for hexane and 0.52mg/g for aqueous extract. The result indicated that the aqueous extract of *Leonotis nepetifolia* possess high amount of phenol when compared to methanolic and hexane extract.

Table 2. Quantitative phytochemical analysis

S.No.	Parameters	Absorbance		
		LNM	**LNH**	**LNW**
1.	Total Phenols	0.38 mg/g	0.43 mg/g	0.52 mg/g
2.	Total Flavonoids	202 mg/g	329 mg/g	106 mg/g
3.	Total Proteins	47 mg/g	43 mg/g	56.8 mg/g
4.	Total Carbohydrates	4 mg/g	6 mg/g	11 mg/g

Total flavonoid

The standard used for the determination of flavonoid content was quercetin. The total flavonoid content of the methanolic extract were 202 mg/g and 329 mg/g for hexane and 106mg/g for aqueous extract. The result indicated that the hexane extract of *Martynia annua* possess high amount of flavanoid when compared to methanolic and aqueous extract.

Estimation of protein

The standard used for the determination of protein content was bovine serum albumin. The total protein content of the methanolic extract were 47mg/g and 43 mg/g for hexane and56.8mg/g for aqueous extract. The result indicated that the aqueous extract of *Leonotis nepetifolia* possess high amount of protein when compared to methanolic and hexane extract.

Estimation of carbohydrate

The standard used for the determination of carbohydrate content was glucose. The total carbohydrate content of the methanolic extract were 4mg/g and 6 mg/g for hexane and 11mg/g for aqueous extract. The result indicated that the aqueous extract of *Martynia annua* possess high amount of carbohydrate when compared to methanolic and hexane extract.

ANTIOXIDANT ACTIVITY

In the present study extracts of *Leonotis nepetifolia* were tested using three different solvents (methanol,hexane and aqueous) for their free radical scavenging activity using DPPH assay, ABTS assay,Ion chelating assay and FRAP assay. It was observed that the plant extracts showed good potency for scavenging free radicals. The extracts were tested on a concentration range (20-100µg/ml) and it was also observed that as the concentration of botanical extract increases, the activity also increases. In all the cases the methanolic extracts proved to be a better antioxidant than the hexane and aqueous extracts.

Figure 2.DPPH scavenging activity (%) of plant extract at different concentration

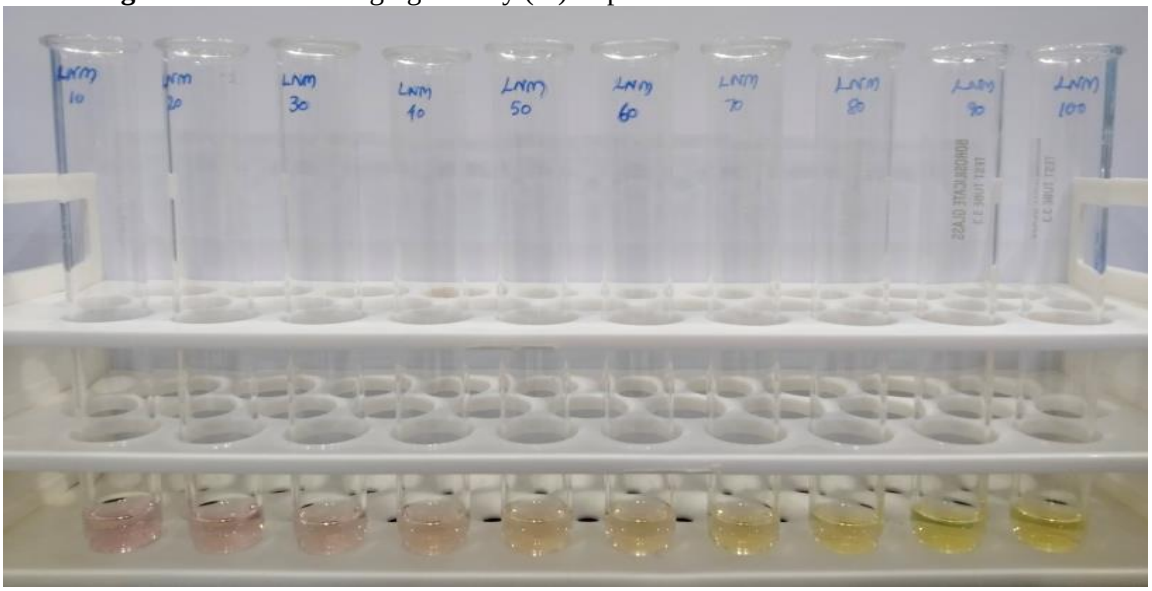

Table 3.DPPH scavenging activity (%) of plant extract at different concentration

Concentration (µg/ml)	**Methanol extract**	**Hexane extract**	**Aqueous extract**	**Quercetin**
20	16.19±0.00	27.78±0.00	11.81±0.00	13.52±0.01
40	30.85±0.00	0.31±0.00	18.81±0.00	23.53±0.01
60	46.17±0.00	33.26±0.00	40.26±0.00	41.96±0.01
80	59.95±0.00	49.89±0.00	53.61±0.00	48.82±0.02
100	73.96±0.00	68.49±0.00	64.11±0.00	72.94±0.03
IC_{50} (µg/ml)	64.97	80.17	68.95	81.93

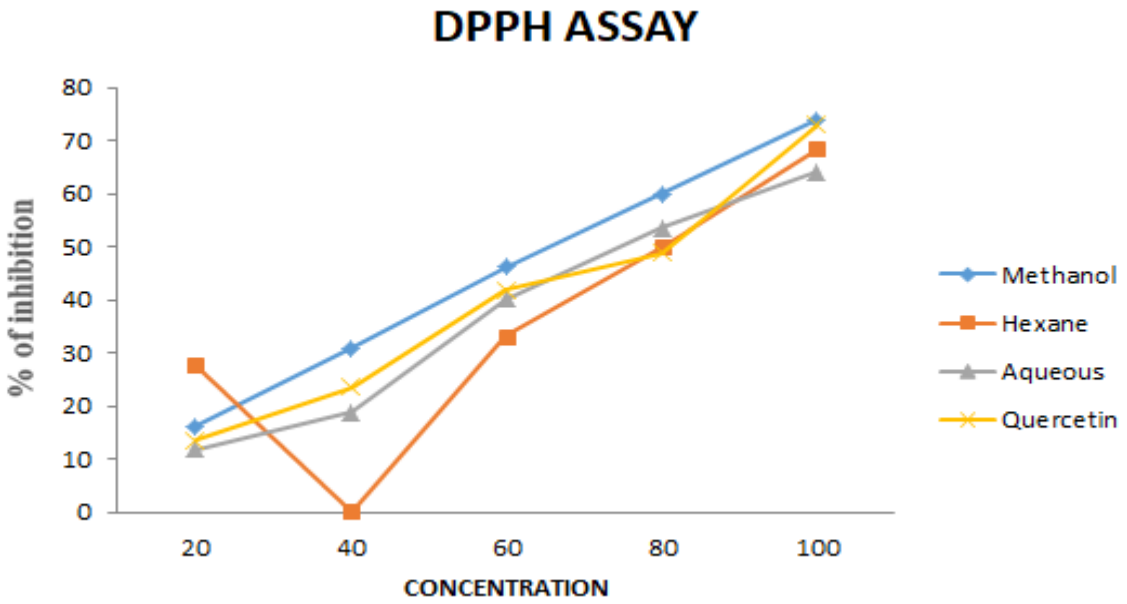

Figure 3. DPPH scavenging activity (%) of plant extract at different concentration

The IC_{50} values were calculated for all the three extracts. In DPPH assay the methanolic extract showed maximum inhibitory percentage of 73.96% at concentration of 100 µg/ml giving IC_{50} value of 64.97µg/ml. With increasing polarity the pattern of increasing antioxidant activity has been observed.

ABTS ASSAY

Figure 4. ABTS radical scavenging assay assay of plant extract different concentrations

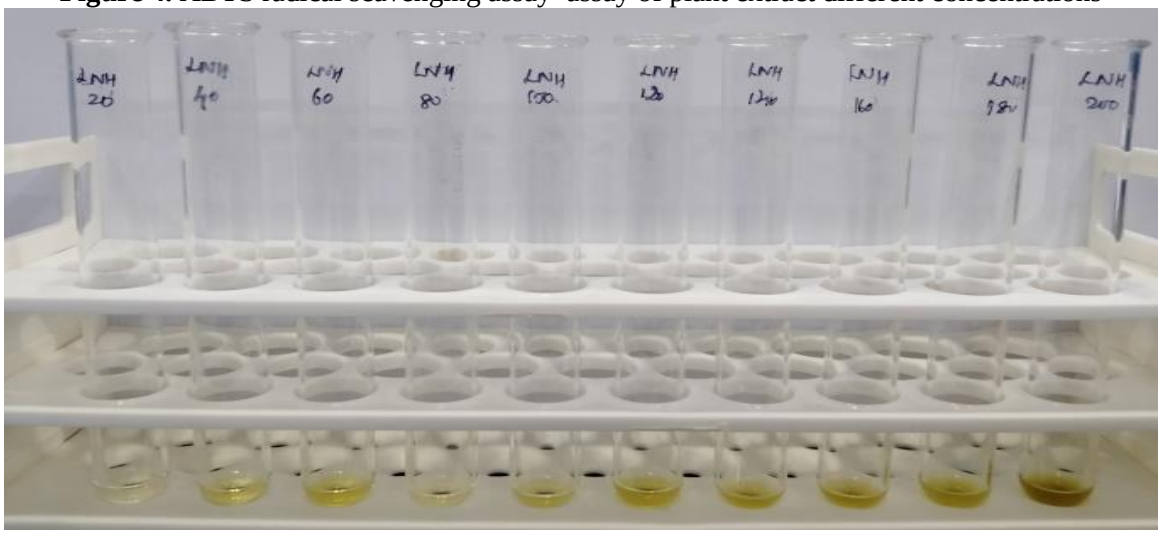

Table 4. ABTS radical scavenging assay of plant extract at different concentrations.

Concentration (µg/ml)	**Methanol extract**	**Hexane extract**	**Aqueous extract**	**Ascorbic acid**

20	7.1±0.00	9.94±0.00	7.1±0.00	33.10±1.33
40	11.64±0.04	15.62±0.00	14.77±0.00	45.94±1.48
60	17.04±0.00	27.27±0.00	32.67±0.00	48.64±1.86
80	31.25±0.00	36.36±0.00	39.48±0.00	56.08±1.29
100	45.45±0.00	42.89±0.00	45.73±0.00	71.62±1.86
IC_{50} (µg/ml)	110.01	116.57	109.33	61.67

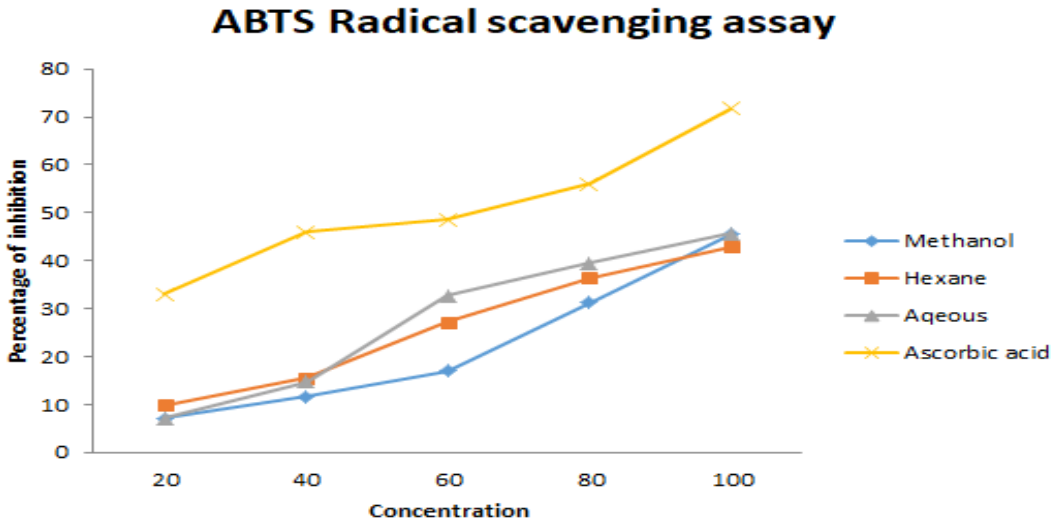

Figure 5. ABTS radical scavenging assay of plant extract at different concentrations.

Than DPPH, ABTS was considered to be more reactive and it involves the transfer of electrons. In ABTS assay the aqueous extract showed maximum inhibitory percentage of 45.73% at concentration of 100ug/ml giving IC_{50} value of 109.33 µg/ml. In this study the assay of ABTS was performed at varying concentrations and it showed good results than that of ascorbic acid whose IC_{50} value was 82.62 µg/ml.

FRAP ASSAY

Figure 6. Ferric reducing antioxidant power assay of plant extract different concentrations

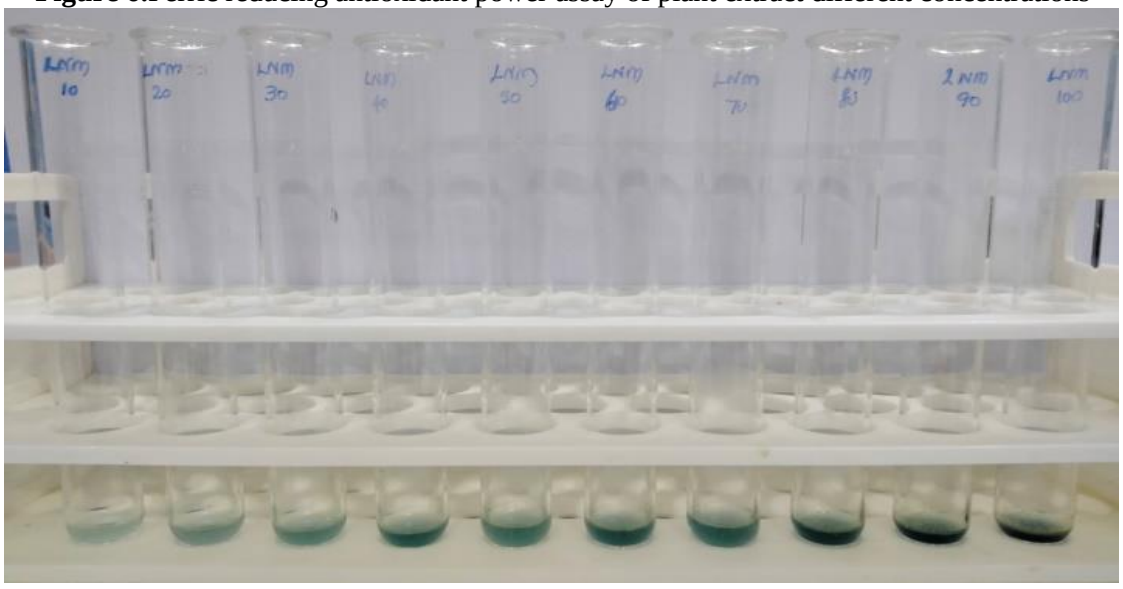

Table5. Ferric reducing antioxidant power assay of plant extract different concentrations

Concentration (µg/ml)	**Methanol extract**	**Hexane extract**	**Aqueous extract**	**Quercetin**

20	14.28±0.00	10.07±0.00	12.85±0.00	18.52±1.28
40	22.85±0.00	21.42±0.00	22.85±0.00	25.23±0.98
60	31.42±0.00	31.42±0.00	41.42±0.00	36.70±1.06
80	44.28±0.00	44.28±0.00	51.42±0.00	48.41±0.99
100	58.57±0.00	60.01±0.00	65.71±0.00	68.05±0.67
IC_{50} (µg/ml)	88.26	87.51	77.79	82.62

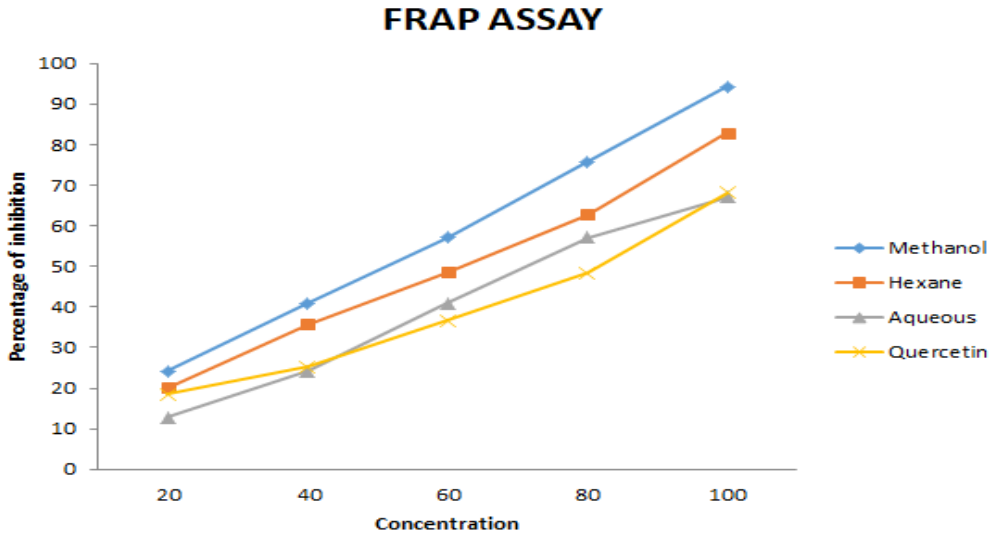

Figure 7. Ferric reducing antioxidant power assay of plant extract different concentrations

This assay is considered to be a good reflector to determine the antioxidant properties of the plant. It has also been reported that the plants possessing high reducing power is said to carry high antioxidant activity too[30].The reducing power potential of all the three plant extracts had increased with the dose, however it has been observed that the three extracts showcased low reducing power than that of Ascorbic acid. In FRAP assay the aqueous extract showed maximum inhibitory percentage of 65.71 % at concentration of 100µg/ml giving IC_{50} value of 77.79 µg/ml.

ION CHELATING ASSAY

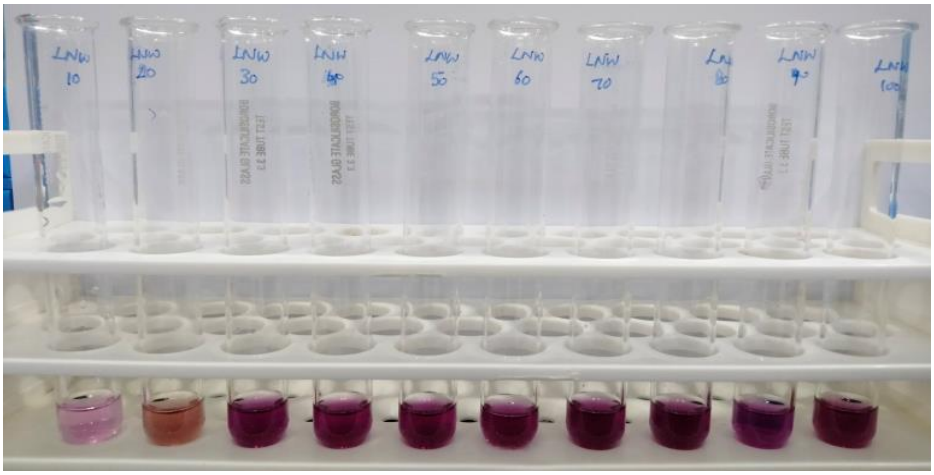

Figure 8 Ion Chelating Assay of plant extracts at different concentrations.

Table 6. Ion Chelating Assay of plant extracts at different concentrations.

Concentration (µg/ml)	**Methanol extract**	**Hexane extract**	**Aqueous extract**	**Di-Sodium EDTA**

20	16.41±0.00	23.88±0.00	19.4±0.00	32.83±0.98
40	28.35±0.00	37.31±0.00	31.34±0.00	41.79±1.04
60	38.8±0.00	46.26±0.00	50.74±0.00	46.26±1.35
80	50.74±0.00	59.7±0.00	79.1±0.00	52.23±0.98
100	70.14±0.00	70.14±0.00	85.07±0.00	59.70±0.80
IC_{50} (µg/ml)	78.83	64.85	59.12	64.85

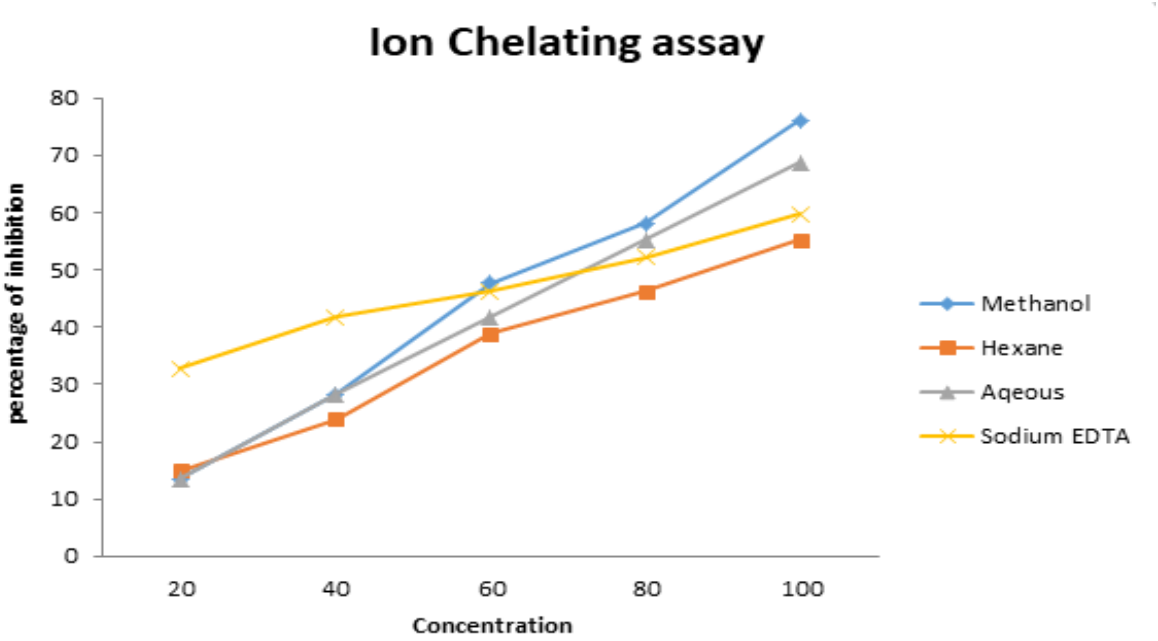

Figure 9. Ion Chelating Assay of plant extracts at different concentrations.

From the above table 4 and figure 5 it has been observed that the aqueous extract possessed more ability than the methanolic and hexane extracts. The aqueous extract showed maximum inhibitory percentage of 85.07% at concentration of 100ug/ml giving IC_{50} value of 59.12 µg/ml.

CONCLUSION

Leonotis nepetifolia although considered a weed, possesses significant therapeutic efficacies and various bioactive compounds that represent the medicinal characteristics of the plant. This plant contains various phytochemicals such as saponins, flavonoids, terpenoids, glycosides, alkaloids, proteins, carbohydrates, and phenols. The study revealed that the aqueous extract contained a higher amount of compounds. Quantitative analysis also illustrated a significant amount of phenols, flavonoids, carbohydrates, and proteins. The aqueous extract exhibited a higher level of antioxidant activity compared to other extracts, and this study paves the way for future research to isolate the bioactive compounds responsible for this activity. The curative potential of the plant inspires further research activities on this remarkable plant and the development of a novel drug for the future treatment of various diseases.

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