

PRELIMINARY SCREENING OF PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT, AND ANTI-BACTERIAL ACTIVITIES IN THE METHANOLIC LEAF EXTRACT OF *MELIOSMA SIMPLICIFOLIA* (L.)

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ABSTRACT

Objective: In the present study is investigated of phytochemicals and antioxidant activities of the leaf extracts from *Meliosma simplicifolia* (L.).

Methods: The seaweed sample was subjected to extraction with solvents of different polarities (hot water, methanol, acetone, chloroform, and petroleum ether) and screened for phytochemicals according to standard methods. The ability of the plant extract to act as hydrogen/electrons donor or scavenger of radicals was determined by *in vitro* antioxidant assays using 2,2-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging, reducing power assay, and superoxide radical (O₂•) scavenging activity. The ICPMS and GC-MS analysis of the methanol leaf extract of *M. simplicifolia* was revealed the presence of antibacterial.

Results: Phytochemical analysis revealed the presence of Preliminary metabolites Quantitative studies of estimated phenol, flavonoid and tannin, as for the methanol extract of stem showed the highest content of phenolic compounds (40.71±0.94 GAE mg/100). Antioxidant activities were concluded the estimation *M. simplicifolia* leaf for as followed the studies. In leaf the highest DPPH scavenging activity (132.3 µg/ml), ICP-MS analysis of the leaf extract showed the presence minerals such as Mg, Fe, Cu, Na, and Zn in excess. The leaf extract of the plant was also tested for its antibacterial activity and results confirmed that it has potential activity.

Conclusion: The preliminary studies in the methanol extract of the leaf of *M. simplicifolia* are suggestive of the antibacterial potentials of the methanol extract of leaves of *M. simplicifolia*.

Keywords: *Meliosma simplicifolia*, Antioxidant, ICPMS, GC-MS, Antibacterial.

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INTRODUCTION

Herbal medicine is a prescription as yet another foundation of around 75–80% of the total population, for essential medicinal services. In India, over 70% of aggregate population utilizes home developed medications for the treatment of illnesses. However, oxygen-center free radicals and other reactive oxygen species (ROS) which are produced *in vivo* continuously, results in cell death and tissues damage. Scientific evidence has suggested that under oxidative stress conditions, oxygen radicals such as superoxide anions (O₂⁻), hydroxyl radical (OH), and peroxy radicals (H₂O₂) are produced in a biological system [1]. When added to foods, antioxidants minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life [2]. Natural antioxidants are in high demand for application as nutraceuticals, biopharmaceuticals, as well as a food additive because of consumer preference. Some phytochemicals are known to exhibit medicinal and physiological activities which are phenols, tannins, flavonoids, phytosterols, etc. The various prominent bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds. Medicinal herb is estimated to be a biochemical laboratory as it contains an abundance of chemical compounds such as alkaloids, glycosides, saponins, resins, oleoresins, lactones, and essential oils [3]. Plant phenols products of the phenylpropanoid pathway Influential antioxidants might limit oxidative destruction to biomolecules such as DNA, lipids, and proteins which play a role in chronic diseases as cancer and cardiovascular [4]. Free radicals produced from absorption or biodegradable resources interact continuously in natural systems and their unconstrained generation associates with the molecular level of the cause by so many diseases [5]. Antioxidants are such essences in life which possess free radicals continue reaction of smashing their properties. At present, there is an improvement

of concern in the remedial potential of highly contain medicinal plants as antioxidants and decrease the oxidative stress. It has caused tissue damage of living being [6]. Based on the numerous naturally appear antioxidants such as carotenoids, Vitamin E, Vitamin D, ascorbic acid, and phenolic compounds, those are much effective of reduction [7].

METHODS

Sodium phosphate, ammonium molybdate, potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate, polyvinyl pyrrolidone (PVP), sodium nitrite, trichloroacetic acid, ethylenediaminetetraacetic acid (EDTA), aluminum chloride, ferrous sulfate, gallic acid, rutin, quercetin, safranin, and fast green were purchased from Sigma-Aldrich. Solvents used are purchased from HiMedia and all reagents were used without further purification.

Collection of plant material

Meliosma simplicifolia plant parts were collected from Nilgiris, Western Ghats region of Tamil Nadu, India. The plant material was identified as *M. simplicifolia* and authenticated by Botanical Survey of India, Southern circle, Coimbatore, and the voucher specimen has been deposited in Bharathiar University, Coimbatore (NO.BSI/SRC/5/23/2018/Tech/2910). The freshly collected plant materials were cleaned to remove dust and impurities using distilled water. Plant materials were washed with distilled water and dried at room temperature. The dried leaf was manually ground to a fine powder and the samples were used for further studies.

Plant extracts preparation

The powdered plant material was extracted in Soxhlet extractor successively with petroleum ether, chloroform, acetone, methanol, and hot water. Each time before extracting with the next solvent, the thimble

was dried in hot air oven below 40°C. Rotary vacuum evaporator was used to concentrate different solvent extracts and then air dried. The dried extract obtained with each solvent was weighed. The percentage yield of air dried weight of plant material was expressed in terms. The anti-inflammatory, antispasmodic, anti-analgesic can be attributed to their excessive steroids, tannin, terpenoids, and saponins. Phytochemicals are responsible for the medicinal pastime of plant life, the different extracts obtained were subjected to preliminary phytochemical screening following standard methods [8-10].

Phytochemical analysis

Dragendorff's test

To 2 mg of the extracts, 5 ml of distilled water turned into brought; 2 M hydrochloric acid became introduced until an acid reaction takes place. To this, 1 ml of Dragendorff's reagent turned into brought. Formation of orange or orange purple precipitate suggests the presence of alkaloids.

Mayer's test

To some drops of Mayer's reagent, 2 mg of extracts were delivered. Development of white or decreased yellow precipitate determines the appearance of alkaloids.

Test for carbohydrates

Molisch's test

Few drops of Molisch's reagent was added to 2 ml of the extract and mixed well. 2 ml of concentrated sulfuric acid was added to this solution. Formation of the red-violet ring at the junction of the solution indicates the presence of carbohydrates.

Fehling's test

2 mg of extracts had been shaken with 10 ml of water, filtered and the filtrate became focused. To this, 1 ml combination of the same elements of Fehling's answer, A and B were delivered and boiled for a short time. Formation of red or brick red colored precipitate suggests the presence of decreasing sugar.

Test for proteins

To 1 ml of the extract, few drops of Biuret reagent were added. The formation of the blue color indicates the presence of protein.

Test for amino acids

Amino acids inside the samples were decided as per the manner of the citrate buffer was prepared by mixing 20.5 mL of solution A and 29.5 mL of solution.

Test for flavonoids

Shinoda's test

2 mg of extracts have been dissolved in 5 ml of ethanol and to this ten drops of dilute hydrochloric acid followed by a small piece of magnesium had been added. Formation of pink, reddish, or brown color shows the presence of flavonoids.

Tannins

Potassium hydroxide test

In 10 ml of freshly prepared 10% potassium hydroxide (KOH) takes in a beaker, 0.5 g of extract modified into delivered and shaken to dissolve. A dirty precipitate indicated the presence of tannin.

Test for phenolic compounds

Ferric chloride test

A little extract was dissolved in 2 ml of distilled water. This was followed by addition of few drops of 10% ferric chloride. Formation of blue/green color indicates the presence of phenols.

Test for Saponins

Foam test

In a take, a look at tube containing approximately 5 ml of extracts, a drop of sodium bicarbonate answer turned into introduced. The check

tube becomes shaken vigorously and left for 3 min. Formation of honeycomb-like froth suggests the presence of saponins.

Test for anthraquinone

1 ml of the extract was macerated with ether, and after filtration, aqueous ammonia was added. The formations of coloring the layer after shaking red pink, and violet indicate the presence of anthraquinones.

Quantification of total phenolic and tannin

The overall phenolics approach each tannin and non-tannin phenolics. The amount of tannins turned into calculated by way of subtracting the non-tanninphenolics from overall phenolics. For the determination of non-tannin phenolics (Makkar, 2003), 500 µl of each plant extracts had been incubated with 100 mg of polyvinyl polypyrrolidone (PVPP), and 500 µl of distilled water taken in a 2 ml Eppendorf tube for 4 h at °C. After incubation the Eppendorf tubes had been centrifuged at 4000 rpm for 10 min at °C. The supernatant consists of simplest the non-tannin phenolics since the tannins would have been deposited in conjunction with PVPP. The supernatant changed into accumulated and the non-tannin phenolics were measured with the aid of the equal method described for the quantification of total phenolics. The analyses of total phenolics and free phenolics have been additionally performed in triplicates and the results had been expressed in tannic acid equivalent (TAE). From those effects, the tannin content of the plant samples has been measured as follows,

Tannins = Total phenolics–Non-tannin phenolics.

Quantification of total flavonoid

The flavonoid content of the considerable number of concentrates was evaluated as it goes about as a noteworthy antioxidant in plants diminished oxidative anxiety. Assessed according to portrayed by Zhishen *et al.* [11]. Initially, 150 µl of all the plant extracts were taken in different test tubes. To each extracts 2 ml of distilled water was added. Then, 150 µl of NaNO₂ was added to all the test tubes followed by incubation at room temperature for 6 min. After incubation, 150 µl of AlCl₃ (10%) was added to all the test tubes. The test tubes were incubated for 6 min at room temperature. Then, 2 ml of NaOH was added to all the test tubes which were made up to 5 ml using distilled water. The contents in all the test tubes were vortexes well and they were allowed to stand for 15 min at room temperature. The pink color developed due to the appearance of flavonoids was read spectrophotometrically at 510 nm. The amount of flavonoids was calculated as rutin equivalents.

In vitro antioxidant studies

DPPH radical scavenging activity

The stable radical DPPH was used to measure the free radical scavenging activity by the method of [12]. Samples at various concentrations (20, 40, 60, 80 µg/mL of extracts with respective organic solvents) were taken and the volume was adjusted to 100 µL with methanol. About 5 mL of a 0.1mM methanol solution of DPPH was added to the aliquots of different extracts of plant sample and standards (BHT and Quercetin). 100 µL of methanol in 5 mL of DPPH solution was used as a negative control. All the reaction mixtures were incubated for 20 min at 27°C. Inhibition of DPPH radical by the plant samples was measured at 517 nm against the blank (methanol).

Heavy metal analysis

Instrument: Perkin Elmer ICP-MS, Nexion 350× inductively coupled plasma mass spectrometer was used for the determination of the minerals [13].

Sample preparation

0.3 g of leaf and stem powder were weighed into the tri-acid digestion tube and then added with (9:2:1) of concentrated HNO₃, H₂SO₄, and perchloric acid. Samples were pre-digested for 15 min. Microwave absorption of the samples was performed utilizing the Mars 6 Microwave digestion method. After cooling, the whole digest were shifted into a 50 ml volumetric flask and reduced to 50 ml with double

deionized water. Reagent blanks were prepared similarly for all sample solutions.

Reagents

For sample digestion trace metals-grade concentrated HNO_3 (69–70%, Fisher Scientific) was used. Double deionized water (Milli-Q Millipore 18, 2 M cm^{-1} resistivity) was used for all dilutions. The element standard stock solutions (Sodium [Na], Magnesium [Mg], Aluminum [Al], Chromium [Cr], Manganese [Mn], Zinc [Zn], Copper [Cu], Iron [Fe], Nickel [Ni], Potassium [K], Phosphorus [P], Lead [Pb], and Selenium [Se]) in 1000 mg L⁻¹ used for calibration were supplied by Merck.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of the leaf extracts was determined using agar well diffusion method with slight modification [14]. The bacteria were cultured in nutrient broth at room temperature and kept in orbital shaking incubator (Remi, India) at 200 rpm for 2–3 days. The Muller hint on agar plates (bacteria culture) was prepared and microbial strains were swabbed. After 5 min, the well (5 mm size) was made using gel puncher and different concentrations (25 μg , 50 μg , 75 μg and 100 $\mu\text{g}/\text{ml}$) of the sample were added in the well. The positive control (10 $\mu\text{g}/\text{ml}$) (tetracycline) was prepared and poured into wells. The plants were incubated at 37°C for 24 h (bacteria). After incubation, the antibacterial activity was assessed. Each screening test was performed with three replicates and the mean values are recorded.

Statistical analysis

The results are expressed as the mean \pm S.E.M. One-way ANOVA followed by Dunnett's multiple comparison tests. Data were computed for statistical analysis using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, and compared with the vehicle control group. P values ($p < 0.05$) were considered statistically significant.

RESULTS

Phytochemical screening

Phytochemical analysis of studied plant extract suggests the presence of, carbohydrates, protein and amino acid, flavonoids, and saponins methanol extracts showed good result compared to acetone and chloroform extracts. Moreover, methanol extracts relatively slight alkaloid, tannin, and anthraquinone of absence (Table 1).

Qualitative and quantitative phytochemical analysis

Quantification of total phenolic, tannins, and flavonoid

In the Soxhlet method for extraction, the bound phenol compounds from plant materials can be released using heat treatment [15]. Estimation of *M. simplicifolia* leaf and revealed the highest phenolic content in methanol extract (40.71 \pm 0.94 GAE/100 g). From the above results, the tannin content of the sample was calculated as follow: Tannin (%) = Total phenolics (%) - Non-tannin phenolics (%) methanolic extract of leaf and stem (7.82 \pm 3.23 GAE/100 g). Estimation of the *M. simplicifolia* leaf found to be in maximum amount of flavonoid content in methanol extract (20.61 \pm 0.28 RU/100 g) (Table 2).

In vitro antioxidant assays

DPPH radical scavenging activity

The free radical-scavenging activities in stem and leaf of the plants *M. simplicifolia* samples along with standards Vitamin C was determined by the DPPH radical scavenging assay. The reduction in absorbance of the DPPH radical caused by antioxidant was because of the rummaging of the radical by hydrogen ability. The color change from purple to yellow after reduction can be quantified decrease in absorbance at wavelength 517 nm. A lower value of IC_{50} (inhibitory concentration at 50%) indicates a higher antioxidant activity. In leaf, petroleum ether and chloroform extracts show the higher inhibitory IC_{50} value was (132.1 $\mu\text{g}/\text{ml}$), whereas acetone (64.69 $\mu\text{g}/\text{ml}$) and methanol (11.23 $\mu\text{g}/\text{ml}$) show the minimum inhibitory concentration activity. It has been generally used to test the capacity of mixes as free-radical scavengers or hydrogen contributors and to assess the ant oxidative action of plant concentrates and nourishments [16] (Fig. 1).

Minerals analysis of *M. simplicifolia*

Inductively Coupled Plasma Mass Spectroscopy (ICPMS) result

In the investigation the physiochemical parameters of the sample were analyzed the determination of essential elements and heavy metals were studied using ICPMS equipment. The results shows the stem sample of *M. simplicifolia* contains macro element such as magnesium, potassium, calcium, phosphorus, iron, manganese, zinc, copper, molybdenum, and selenium and some heavy metals such as nickel and chromium were also present. The element found in this plant are included in the nine elements that are common in living beings and in humans constitute around 4% of the body weight [17]. Minerals are part of human organ systems, proteins, enzymes, hormones, etc., they regulate the metabolism and cause disease if not found in adequate amounts or are absent (Table 3).

GC-MS chromatogram of *M. simplicifolia* methanol leaf extract

The GC-MS study of the methanolic extract of the leaf *M. simplicifolia* had shown the presence of many photochemical which might contribute

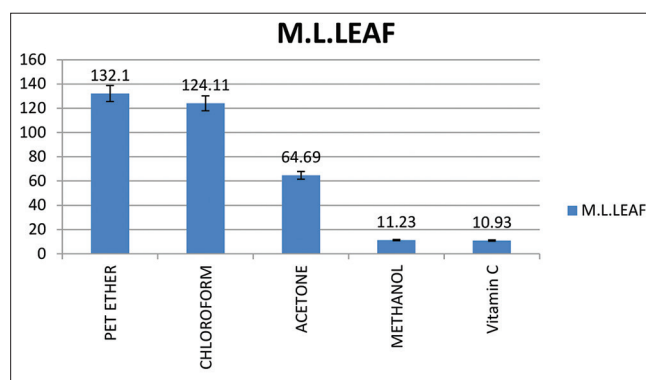


Fig. 1: DPPH radical scavenging activity of *Meliosma simplicifolia* leaf, values are mean of three replicate determinations ($n=3$) \pm standard deviation Bars having different letters are significantly different ($p < 0.05$)

Table 1: Phytochemical screening of *Meliosma simplicifolia* leaf extracts

S. No.	Phytochemical test	Pet ether	Chloroform	Acetone	Methanol	Water
1	Alkaloid	-	-	-	-	-
2	Carbohydrates	+	+	+	+	+
3	Protein and amino acid	++	++	+	++	+
4	Flavonoid	++	+	+	++	++
5	Tannin	-	-	-	-	-
6.	Phenolic compound	++	+	++	++	++
7	Saponin test	+	+	+	+	+
8	anthraquinone	++	-	-	-	-

+++ : Relatively a strong presence, ++ : Relatively moderate presence, + : Relatively slight presence, - : Absence

Table 2: Total phenolic, flavonoid and tannin contents of *Meliosma simplicifolia*

S. No.	Plant material	Extract	Total phenol	Flavonoid	Tannin
			(Ggae/100g Extract)	(gRE/100gExtract)	(gGAE/100Extract)
1	M.S. Leaves	Pet ether	8.67±0.12	8.26±0.08	3.15±0.17
2		Chloroform	8.89±0.07	11.75±1.22	2.96±0.50
3		Acetone	23.46±0.78	17.43±0.86	6.30±0.79
4		Methanol	40.71±0.94	20.61±0.28	7.82±3.23
5		Water	19.54±0.15	15.03±0.72	3.83±0.36

*Values are mean of replicate determination (n=3)±standard deviation. GAE (p<0.05 where a>b > c)

Table 3: Minerals analysis of *Meliosma simplicifolia*

S. No.	Parameters	Leaf
1	Potassium	50292 ppm
2	Magnesium	5871.17 ppm
3	Sodium	1006.03 ppm
4	Chromium	2.32 ppm
5	Manganese	34.56 ppm
6	Nickel	8.04 ppm
7	Copper	113.54 ppm
8	Zinc	20.26 ppm
9	Selenium	28.62 ppm

ppm: parts per million

to the medicinal activity of that plant. The comparison of the mass spectrums with the data base gave more than 90% match as well as confirmatory compound structure match. The GC-MS analysis of the methanol extract resulted many compound which have diverse use. These compound are having so mainly medicinal purpose as such as anti-inflammatory, antibacterial, antifungal, acute toxicity, and anti-cancer, skin conditioning properties have been identified (Fig. 2).

Antibacterial activity

The antibacterial activity of the *M. simplicifolia* methanol extract. The zone of inhibition was maximum at the concentration (100 µg/ml) of given sample the highest zone of inhibition was observed in *staphylococcus pyogenes* (36±0.06 mm) at concentration of 100 µg/ml. Similarly, the lowest zone of inhibition was observed in *Enterococcus aerogenes* (15±0.05 mm) at concentration of 25 µg/ml. The results confirm that the given sample shows excellent antibacterial activity (Table 4 and Fig. 3). Antibacterial activity of *M. simplicifolia* leaf methanol extract.

DISCUSSION

The phytochemical test showed that a positive result for the primary metabolites such as proteins, carbohydrate, and amino acids are present in the appreciable amount in all the parts. Among the leaf is used to seem therapeutically important part which possesses a higher amount of secondary metabolites, such as phenolic compounds, flavonoids, tannins, saponins, steroid, and terpenoid. Quantitative studies of estimated phenol, flavonoid and tannin, as for the methanol extract of leaf showed the highest content of phenolic compounds (40.71±0.94 GAE mg/100). The methanolic extract of stem showed highest amount of tannin content (7.82±3.23 GAE mg/100). The flavonoid content is presented of methanol extract of *M. simplicifolia* stem revealed maximum amount of flavonoid content (20.61±0.28 RE/100 g). The stem of *M. simplicifolia* was collected, separated, and then the powdered material was extracted with various solvents using such as petroleum ether, chloroform, acetone, and methanol using maceration method. Extract yield percentage of methanol shows most recovery percentage (leaf-10.4%) compared with other solvents. Antioxidant activities were concluded the estimation *M. simplicifolia* leaf for as followed the studies in stem the methanol extract showed the highest DPPH scavenging activity (132.1 µg/ml). In this present study, the analysis was carried out with dried stem sample of *M. simplicifolia*. From the ICP-MS, we can see clearly that each band represent characteristic absorption peaks of

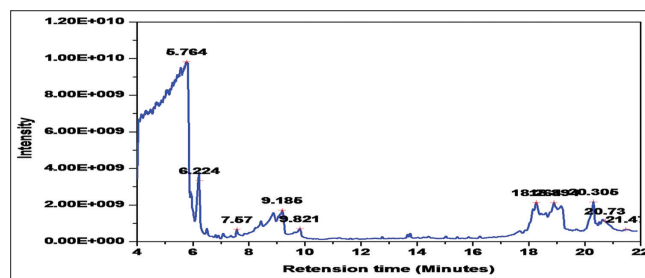


Fig. 2: GC-MS of the plant *Meliosma simplicifolia* leaf of methanol extract

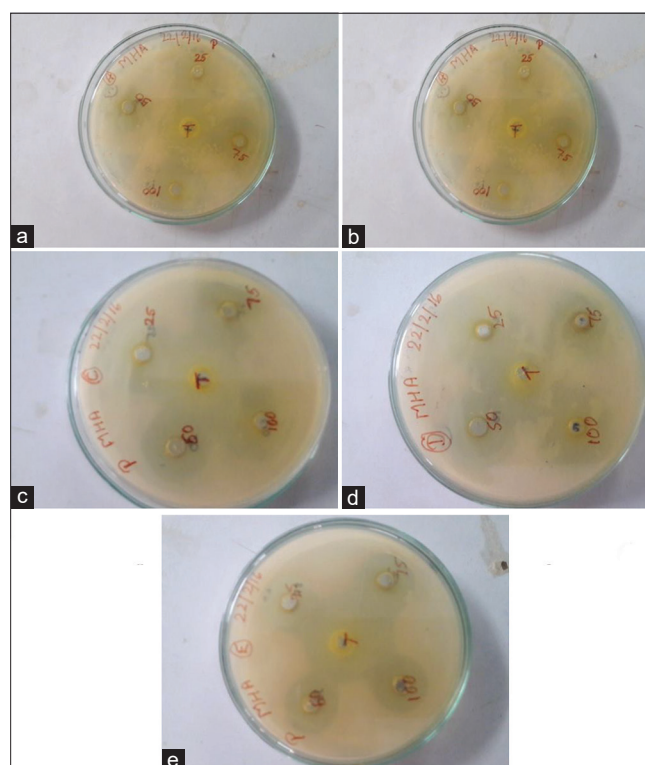


Fig. 3: Plates for Antibacterial activity of *Meliosma simplicifolia* leaf methanol extract. (a) *Staphylococcus aureus*, (b) *Staphylococcus pyogenes*, (c) *Klebsiella pneumonia*, (d) *Pseudomonas aeruginosa*, (e) *Enterococcus aerogenes*

functional groups present in the sample. Thus, the presence of phenolic in any plant sample indicates it as a potent source of therapeutic agents for different human disorders. GC-MS are a commonly used method for identifying the bioactive compounds, which is one of the most important analytical techniques used in quality control of herbal medicine. The present study identified several compounds, which were not describing in *M. simplicifolia* earlier. In the present study, *M. simplicifolia* extracts have great potential as antibacterial

Table 4: Antibacterial activity of *Meliosma simplicifolia* leaf methanol extract

Organisms	Control (100µg/ml)	Zone of inhibition in diameter (mm)			
		25 (µg/ml)	50 (µg/ml)	75 (µg/ml)	100 (µg/ml)
<i>Staphylococcus aureus</i>	38 ± 0.5	28 ± 0.05	30 ± 1.03	33 ± 0.05	34 ± 0.07
<i>Staphylococcus pyogens</i>	35 ± 0.5	25 ± 0.01	27 ± 0.03	32 ± 0.07	36 ± 0.06
<i>Klebsiella pneumonia</i>	36 ± 0.4	22 ± 0.05	25 ± 0.05	32 ± 0.03	35 ± 0.01
<i>Pseudomonas aeruginosa</i>	34 ± 0.1	26 ± 0.5	25 ± 0.03	30 ± 1.02	31 ± 0.06
<i>Enterococcus aerogenes</i>	25 ± 1.03	15 ± 0.05	17 ± 0.04	19 ± 0.6	22 ± 0.03

compounds against microorganisms. In addition to this, the results of the antioxidant profile can be used as pharmacological tool for the medicinal purposes of this plant.

CONCLUSION

The phytochemical extraction was done as per the protocol of leaf. The results confirmed the presence of phytochemicals such as phenols, flavonoids, and tannins in the higher in proposed plant. ICP-MS analysis of the leaf extract showed the presence minerals such as Mg, Fe, Cu, Na, and Zn in excess. Moreover, antioxidant studies were performed for the plant extracts. Various assays were used to screen these particular compounds and they were further confirmed by GC-MS the results showed that the stem extract has more antioxidant activity compared to leaf. The leaf extract of the plant was also tested for its anti-therapeutic potentials and results confirmed that it has potential activity. The study will also gear up the effort to protect, conserve, and cultivate such medicinal plants for the disease-free future of human.

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AUTHORS CONTRIBUTIONS

Pavithra designed the experiments performed in laboratory analysis, experiments, data analysis, and participated in the writing of the manuscript. Dr.T.Sekar helped in paper writing and correction. Authors discussed the results and commented on the manuscript.

CONFLICT OF INTERESTS

Declared none.

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