RESEARCH ARTICLE

Phytochemical, Antimicrobial and Antioxidant Studies on the Leaf Extract of an Endemic Medicinal Plant *Canthiumera neilgherrensis* (Wight) K. M. Wong (Rubiaceae)

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Abstract

Canthiumera neilgherrensis (Wight) K.M. Wong is a rare plant that is endemic to Southern Western Ghats. Phytochemical analysis was done using standardized biochemical tests on *C. neilgherrensis viz.,* the quantitative analysis includes total phenol, flavonoids, terpenoids, alkaloids, and estimation of steroids. Antioxidant activity is determined by FRAP (Ferric Reducing Antioxidant Power Assay) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Antibacterial analysis by agar well gel diffusion method was done against a gram-positive bacterium*, Staphylococcus aureus,* and a gram-negative bacterium*, Pseudomonas aeruginosa*. Phytochemicals are mostly concentrated in chloroform and methanol extracts. Phytochemical analysis of extracts showed that phenol, saponin, and alkaloid are present in all extracts. The phytochemicals such as flavonoids, alkaloids, and terpenoids were highly concentrated in chloroform extract. Out of the five quantified phytochemicals, terpenoids showed the highest quantity (227.14 mg/ml) in the chloroform extract. From the result of the antibacterial analysis, it was clear that the highest inhibition was found in water extract against *Pseudomonas aeruginosa*, a gram-negative bacterium. The DPPH radical scavenging and FRAP analysis of an effective extract of *C. neilgherrensis* (Wight) K.M. Wong showed appreciable antioxidant activity. For the DPPH assay, the highest antioxidant activity was observed at 1000 mg/ml concentration with inhibition of 41.54%. For the FRAP assay, the highest antioxidant activity was observed at 100 mg/mL with inhibition of 12.65%. From the result of the assays, it was clear that chloroform and methanolic extracts show significant inhibitory properties. Higher levels of flavonoids and phenols were the reason behind both the antioxidant and antibacterial activities.

Keywords: Phytochemical, Antimicrobial, Antioxidant, *Canthiumera neilgherrensis* (Wight) K.M. Wong

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Introduction

Nowadays, Ayurveda, Unani, and Siddha are in the mainstream of medicine systems and which is to complement biomedicine. Traditional medicinal procedures are n
ow governed by modern medicine's medical standards. However, the tribal people of different continents provide effective raw medicines using all biological resources around them. Plant-derived secondary metabolites are the reason for the therapeutic effects of many modern drugs as well as medicinal plants (Teoh, 2015). Secondary metabolites such as alkaloids, phenols, flavonoids, terpenoids, tannins, and other metabolites naturally occur in plants (Wong *et al.*, 2006). Antioxidant compounds have the ability to inhibit both free radicals and reactive oxygen and nitrogen species (ROS/ RNS). Antioxidants can also delay the onset of aging, cardiovascular disease, and cancer that are associated with free radicals (Halliwell & Gutteridge, 2000). Various phytochemicals found in medicinal plants, including flavonoids, alkaloids, tannins, and terpenoids, have antimicrobial and antioxidant qualities (Talib & Mahasneh, 2010)

Canthiumera neilgherrensis (Wight) K.M. Wong is a species coming under the genus *Canthiumera* K.M. Wong & Mahyuni (Family: Rubiaceae) (Wong *et al.,* 2018). It is previously treated under the genus *Canthium* Lam. as *Canthium neilgherrense* Wight. It is endemic to the Southern Western Ghats and has a rare distribution. The IUCN status of the taxa is vulnerable (IUCN Red List, 2023). The general characters of *Canthiumera neilgherrensis* (Wight) K. M. Wong include large unarmed shrubs, leaves to 6 x 3 cm, elliptic to obovate, sub coriaceous; domatia glandular; petiole to 5 mm long; stipules ovate, acute, flowers 3-12 together in axillary fascicles; pedicels 3 mm long; calyx 2mm long, obconical, lobes triangular, glabrous; corolla tube 4 mm long, campanulate, densely reflexed hairy below the stamens and erect hairy above the stamens inside; lobes 3 x 1.5 mm, oblong; style hispid, drupe 13 x 7 mm, obovoid (Sasidharan, 2012)

The major aim of the present study is the phytochemical analysis of *Canthiumera neilgherrensis* (Wight) K.M. Wong. Phytochemical analysis includes qualitative and quantitative tests of components that are in different concentrations. In addition to that, the present study also assesses the antioxidant

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and antimicrobial properties of the selected plant. The concentration of the active components in plants varies from structure to structure. It is distributed in different parts of the plant, such as the stem, root, leaf, flowers, fruits, and seeds (Rangari, 2009).

Materials And Methods

Collection of Plant Material

Fresh and healthy leaves of *Canthiumera neilgherrensis* (Weight) K.M. Wong were collected from Agasthyamala, Thiruvananthapuram, and the specimen was deposited in KUBH (Acc. number: KUBH 11152). The plant was identified by comparing it with the prologue, herbarium, and several local Floras [(Manilal, 1988), (Mohanan *& Sivadasan,* 2002), (Sasidharan, 2012)]. (Fig. 1)

Preparation of Leaf Extract

About 100 mg of the powdered leaf was subjected to successive Soxhlet extraction using four solvents such as petroleum ether, chloroform, methanol, and distilled water, depending on their increasing polarity (Snyder *& Kirkland,* 1979). Polarity was thus determined based on boiling points and the ability to evaporate. The above process was repeated until the solvent got colorless in the extracting chamber. Finally, the desired compound came out as a concentrated mass which was then collected in the distillation flask.

The Percentage of Yield

% Yield = W1/W2 x 100
W1- weight of extract residue after solvent removal
W2- Weight of powdered sample taken for extraction

Qualitative Analysis of the Phytochemicals

Several chemical tests were performed for the presence of phytochemical constituents using standard procedures. The tests for different classes of secondary metabolites are as follows:

Test for Phenolic Compounds

This test was conducted by taking 2 mL of plant extract in a test tube, and to it, 1% of lead acetate solution was added. The result was the formation of a white precipitate which indicates the presence of phenolic compounds (Trease *& Evans,* 2002).

Test for Tannin

2 ml of plant extract was taken, and 0.1% ferric chloride solution was added to it. The result was the formation of a brownish-green precipitate, which indicates the presence of tannins (Ejikeme *et al.*, 2014).

Fig. 1: The habit of *Canthiumera neilgherrensis* (Wight) K.M. Wong.

Test for Flavonoids

From 10% Lead acetate solution, 2 mL was taken and treated with 2 ml of plant extract. The result was the formation of yellowish-green color which indicates the presence of flavonoids (Trease *& Evans,* 2002).

Test for Saponins

2ml of distilled water was taken in a test tube, and to it, 1 ml of plant extract was added and shaken vigorously with a few drops of olive oil. It created a foam that persisted. This indicates the presence of saponins (Sofowora, 1993).

Test for Terpenoids

A total of 2 mL of plant extract was mixed with 2 mL of chloroform. It was allowed to evaporate, and to it, 2 mL of concentrated sulfuric acid was added and then heated for 2 minutes. The solution turned out grey. The result indicates the presence of terpenoids (Trease *& Evans,* 2002).

Test for Alkaloids

2 mL of plant extract and 2 ml of Wagner's reagent were put in a test tube. The solution showed the appearance of reddish-brown precipitate. The result indicates the presence of alkaloids [(Sofowora, 1993), (Trease *& Evans,* 2002)].

Test for Glycoside

From 5% $FeCl_3$, one drop was taken, and to it, 5ml of plant extract and 2ml glacial acetic acid and conc. H_2SO_4 was added. A brown ring appeared. The result indicates the presence of glycosides (Trease *& Evans,* 2002).

Test for Quinone

A total of 2 mL of plant extract was mixed with 3 to 4 drops of concentrated HCl. A yellow-colored precipitate was formed. The result indicates the presence of quinones (Trease *& Evans,* 2002).

Test for Fatty Acids

A total of 0.5 mL of plant extract was added to 5 mL of ether and allowed to evaporate on filter paper. Then the filter paper was dried, and there was an appearance of transparency on the filter paper. This result indicates the presence of fatty acids [(Sofowora, 1993), (Trease *& Evans,* 2002)].

Test for Steroids

A total of 1-mL of plant extract was dissolved in 10 mL of chloroform, and an equal volume of concentrated sulphuric acid was added to it. Two layers were formed. The top layer turned red, and the bottom sulphuric acid layer showed yellow with green fluorescence. This result indicates the presence of steroids (Harbone, 1973).

Quantitative Analysis of Phytoconstituents

The quantitative estimation of phytoconstituents was carried out according to the standard procedures [(Harbone, 1973) (Malik & Singh, 1980)]

Estimation of Total Phenol

Different extract of samples was pipetted out, and the volume in each tube was made up to 3 mL with distilled water. From 20% Na_2CO_3, 2 mL was taken and added to 0.5 ml of Folin-Ciocalteau reagent, and the tube was placed in a boiling water bath for exactly one minute. The tube was cooled, and the absorbance was read at 750nm in a spectrophotometer against a reagent blank. Standard gallic acid solutions (2.5-100 µg/mL) were also treated as above (Harbone, 1973).

Estimation of Total Flavonoid

The aluminum chloride colorimetric assay measured total flavonoid content. The reaction mixture (consisting of 1-mg of plant extract and 4 mL of distilled water) was taken in a 10 mL volumetric flask. To this flask, 0.30 mL of 5% sodium nitrite was added, and after 5 minutes, 0.3 mL of 10% aluminum chloride was mixed with it. Again after 5 minutes, 2 mL of 1M Sodium hydroxide was treated and diluted with 10 ml of distilled water. A set of reference standard solutions of Quercetin (20, 40, 60, 80, and 100 µg/mL) was prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with a UV/ Visible spectrophotometer. The total flavonoid content was expressed as µg of QE/ mg of extract (Harbone, 1973).

Estimation of Alkaloids

The plant extract (1-mg) was dissolved in 1-mL dimethyl sulphoxide (DMSO), and to it, 1-mL of 2N HCl was added and filtered. This solution was transferred to a separating funnel. To it, 5 mL of bromocresol green solution and 5 ml of phosphate buffer were added. This mixture was shaken with 1- 4 mL of chloroform and, by vigorous shaking collected in a 10-volumetric flask which was then diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80, and 100 µg) was prepared in the same manner as described earlier. The absorbance for test and standard solutions was then determined against the reagent blank at 470 nm with a UV/ Visible spectrophotometer (Harbone, 1973).

Estimation of Terpenoids

One mg of extract was dissolved in 1-mL of methanol was mixed with 150 µL 5% Vanillin-glacial acetic acid and 500µl of perchloric acid solution. The solution was then heated for 45 minutes at 60°C and cooled in an ice water bath to ambient temperature. After the addition of 2.25 mL of glacial acetic acid, each sample solution's absorbance was measured at 548nm using a UV-visible spectrophotometer. Linalool (100, 200, 400, 800, 1000 µg/mL) in methanol was used as standard. Results were expressed as mg Linallol equivalents (Harbone, 1973).

Estimation of Steroids

1gm of plant extract was taken in a clean test tube; cholesterol was used as standard and was taken at varying concentrations of (1-10 µg/mL) in test tubes. To the standard and test samples, 5 ml of ferric chloride reagent and 4 ml of concentrated sulphuric acid was added. The reaction mixtures were incubated at RT for 30 minutes, and OD was read at 540 nm. A standard graph was plotted from which the unknown value of steroid in the test sample can be determined (Harbone, 1973).

Antimicrobial Analysis

Antimicrobial Assay by Agar Well Diffusion Method

The agar well diffusion method was used to evaluate the antimicrobial activity of the test sample. Mueller-Hinton agar (15-20 mL) was poured on glass petri plates of the same size and allowed to solidify. The standardized inoculum of the test organism was uniformly spread on the surface of the plates using a sterile cotton swab. Four wells with a diameter of 8 mm (20 mm apart from one another) were punched aseptically with a sterile cork borer in each plate. The test sample (50 and 100 µL) was added into the wells T1 and T2 from 10mg/ml stock. Gentamycin (40 µL from 4 mg/ml stock) and the solvent used for sample dilution were added as a positive and negative control, respectively. The plates were incubated for 24 h at 36°C ± 1°C under aerobic conditions. After incubation, the plates were observed, and the zone of bacterial growth inhibition around the wells was measured in mm (Valgas *et al.*, 2007).

Culture and Media Used

Muller Hinton Agar medium (HIMEDIA- M173) is used for the determination of the susceptibility of microorganisms to antimicrobial agents. Suspend 38 grams in 1000 ml distilled water. Heat until it boils to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure(121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Inoculums Details

Inoculums were procured from The Microbial Type Culture Collection (MTCC) Chandigarh (Table 1)

Antioxidant Analysis

Ferric reducing antioxidant power assay

The FRAP assay was performed according to the method (Nishaa *et al.,* 2012) with minor modifications. In the beginning, the FRAP reagent was prepared as a mixture. It was prepared with 2.5 mL of 10 mM 2,4,6-tris(2pyridyl)-s-triazine (TPTZ) in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3$ in 25 mL of 0.1 M acetate buffer pH 3.6. The freshly prepared FRAP reagent was incubated at 37°C for 10 minutes. The varying concentration of the sample was mixed with 2 mL of the FRAP reagent and added with 1 mL of distilled water. After 30 minutes of incubation, a blank was prepared using the same method described above, but distilled water was used instead of the sample. Absorbance was measured at 593 nm against blank.

Table 1: Inoculum details of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Name of microorganism	*MTCC No.*	*Incubation conditions*
Staphylococcus aureus	87	37°C for 24 hours
Pseudomonas aeruginosa	741	37°C for 24 hours

DPPH Radical Scavenging Assay

The radical scavenging activity of the leaf extract was tested against DPPH (Brand-William *et al.*, 1995). Ascorbic acid was used as the standard (prepared in distilled water (1 mg/ml; w/v)). A total of 1ml of leaf extract at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800, 1000 µg/mL) was mixed with 3.9 ml, 60µM solution of DPPH in methanol. The absorbance was measured at 515 nm after incubating for 15 minutes at room temperature. The control used here consists of only the DPPH solution. The blank solution used here consists of only 95% methanol.

For the calculation of radical scavenging activity, the following formula was used:

$$\% \text{ inhibition} = \frac{ABS^{control} - ABS^{test}}{ABS^{control}} \times 100$$ (Sakat *et al.*, 2010)

Results

Percentage Yield

The percentage yield of the four solvents used is summarised in Table 2

Phytochemical Analysis

The preliminary phytochemical analysis with four solvents revealed the presence of various phytochemicals, and the results are summarised in Table 3

Qualitative Analysis

The result shows that phenol, saponin, and alkaloid are present in all extracts. Terpenoid is only present in petroleum ether extract. The Chloroform and methanolic extracts are higher in phenolic content. The chloroform extract is higher in phenol, tannin, and alkaloid. The methanolic extract is higher in phenol and steroid content (Table 3)

Table 2: Percentage yield of solvents used

Solvent used (500ml)	*Weight of sample (g)*	*Weight of extract (g)*	*% Yield*
Petroleum Ether (PE)	50	1.63	3.26
Chloroform (Chl)		0.89	1.78
Methanol (MeOH)		7.18	14.36
Water (H_2O)		4.50	9.00

Table 3: Qualitative analysis of phytoconstituents in different solvents of the leaf extract of *Canthiumera neilgherrensis* (Wight) K.M. Wong

Sl.No	Name of test	Extract			
		PE	Chl	MeOH	H_2O
1	Phenol	+	++	+++	+
2	Tannin	++	++	-	+
3	Flavonoid	++	+++	+	+
4	Saponin	+	++	++	++
5	Terpenoids	++	+++	++	+
6	Alkaloid	+	+++	+	+
7	Glycoside	+	++	+	-
8	Steroid	+	++	+++	++
9	Quinones	++	++	-	-
10	Fatty acid	++	+	-	-

PE- Petroleum ether, Chl- Chloroform, MeOH- Methanol, H_2O- Distilled Water

Quantitative Analysis

The result shows that the methanol extract is rich in phenols and steroids. Out of the five quantified phytochemicals, terpenoids showed the highest quantity (227.14 mg/ml) in the chloroform extract. The phytochemicals such as flavonoids, alkaloids, and terpenoids were highly concentrated in chloroform extract. These results show that the suitable extract is chloroform, and methanol is next to it. Both the petroleum ether and the distilled water extract have the lowest concentration of phytoconstituents (Table 4)

Antibacterial Activity

The result of antibacterial activity by the agar well diffusion method is summarized in Table 5. The zone of inhibition ranges from 8 mm to 11 mm. The highest inhibition was found in water extract against *Pseudomonas aeruginosa*, which is a gram-negative bacterium. A satisfactory inhibition was found in petroleum ether and chloroform extracts against *Pseudomonas aeruginosa.* Only the chloroform extract shows a satisfactory inhibition zone (8 mm) against the gram-positive bacteria *Staphylococcus aureus.*

Table 4: Quantitative analysis of phytoconstituents in different solvents of the leaf extract of *Canthiumera neilgherrensis* (Wight) K.M. Wong

Phytochemical	Petroleum ether	Chloroform	Methanol	Distilled water
Phenol(mg GAE/g extract)	8.39	17.96	47.18	7.44
Flavonoid (Tannic acid mg/ml)	59.15	136.76	20.24	20.46
Alkaloid(mg/ml)	24.56	77.33	29.00	31.22
Terpenoid(mg/ml)	111.23	227.14	180.77	49.41
Steroid (mg/ml)	24.15	55.40	65.13	38.60

PE- Petroleum ether, Chl- Chloroform, MeOH- Methanol, H_2O- Distilled Water

Table 5: Antibacterial activity of different extracts using agar well diffusion method

Sl. No:	Name of microorganism	Sample code	Zone of inhibition (mm)			
			Standard Gentamycin (160 mcg)	Negative control	T1 (50µl from 10 mg/ml)	T_2 (100µl from 10 mg/ml)
1	*Pseudomonas aeruginosa*	PE	31mm	-	-ve	+ve (8mm)
2		Chl	31mm	-	-ve	+ve (8mm)
3		MeOH	31mm	-	-ve	-ve
4		H2O	31mm	-	-ve	+ve (11mm)
1	*Staphylococcus aureus*	PE	20mm	-	-ve	-ve
2		Chl	20mm	-	-ve	+ve (8mm)
3		MeOH	20mm	-	-ve	-ve
4		H2O	20mm	-	-ve	-ve

PE- Petroleum ether, Chl- Chloroform, MeOH- Methanol, H_2O- Distilled Water

There is no inhibitory zone in the case of methanolic extract against *Pseudomonas aeruginosa.* The most effective extract is water extract against *Pseudomonas aeruginosa* (Table 5).

Antioxidant Analysis

Ferric Reducing Antioxidant Power Assay

The results of the antioxidant analysis are summarised in Tables 6 and 7. From the results, it is clear that chloroform extract is potentially reducing Fe^{3+} to Fe^{2+}. The reducing power is lower in petroleum ether extract. In all extracts higher the concentration, the higher is the reducing potential.

DPPH Radical Scavenging Assay

The DPPH radicle scavenging assay results are depicted in Tables 8 and 9. All solvents had The highest antioxidant activity at 1000 µg/ml concentration. The methanolic extract markedly reduced the concentration of DPPH with a lower capability (the value of % inhibition is half of the standard) than that of the standard (Ascorbic acid). The percentage inhibition is approximately similar in both petroleum ether and water extracts.

Table 6: Absorbance of Ferrous Sulphate as standard

Standard	*Concentration (ng/ml)*	*OD at 593 nm*			*Avg. OD at 593 nm*
FeSO4 (Standard 3.6µM/ ml)	5	0.1215	0.1217	0.1212	0.1215
	10	0.2340	0.2340	0.2340	0.2340
	20	0.4112	0.4110	0.4115	0.4112
	40	0.8232	0.8235	0.8233	0.8233
	60	1.3611	1.3610	1.3612	1.3611
	80	1.8324	1.8325	1.8323	1.8324
	100	2.094	2.0941	2.0940	2.0940

Table 7: Reducing power of different extracts of *Canthiumera neilgherrensis* in different concentration (FRAP assay)

Extracts	*Conc. in ng/mL*	*Avg. OD at 593 nm*	*Fe^{2+}(mg/mL)*
Petroleum Ether	20	0.018	0.49
	40	0.025	0.81
	60	0.063	2.56
	80	0.071	2.93
	100	0.086	3.62
Chloroform	20	0.036	1.32
	40	0.085	3.58
	60	0.154	6.76
	80	0.250	11.18
	100	0.282	12.65
Methanol	20	0.016	0.40
	40	0.053	2.10
	60	0.057	2.29
	80	0.060	2.42
	100	0.090	3.81
Distilled water	20	0.014	0.30
	40	0.023	0.72
	60	0.050	1.96
	80	0.074	3.07
	100	0.102	4.36

Table 8: Absorbance of different extract of *Canthiumera neilgherrensis* at 515 nm by UV-Visible Spectrophotometer (DPPH assay).

	OD at 515 nm				
Conc. (µg/ml)	Ascorbic acid	PE	Chl	MeOH	H_2O
Control	1.28	0.8462	0.8462	0.8462	0.8462
1.56	1.20	0.8452	0.8253	0.8224	0.8451
3.12	1.19	0.8175	0.8055	0.8006	0.8038
6.25	1.12	0.7993	0.7755	0.7805	0.7885
12.5	0.96	0.7747	0.7545	0.7671	0.7732
25	0.65	0.7513	0.7390	0.7415	0.7641
50	0.09	0.7237	0.7084	0.7127	0.7233
100	0.09	0.6752	0.6764	0.6517	0.7003
200	0.08	0.6565	0.6314	0.6116	0.6855
400	0.08	0.6447	0.6206	0.5733	0.6662
800	0.09	0.6204	0.5646	0.5308	0.6416
1000	0.09	0.5822	0.5445	0.4947	0.5842

PE- Petroleum ether, Chl- Chloroform, MeOH- Methanol, H_2O- Distilled Water

Table 9: Percentage inhibition of different extracts with ascorbic acid as standard

	Percentage inhibition				
Conc. (µg/ml)	Ascorbic acid	PE	Chl	MeOH	H_2O
1.56	6.0	0.12	2.47	2.81	1.31
3.12	6.8	3.40	4.81	5.38	5.01
6.25	12.2	5.54	8.35	7.77	6.82
12.5	25.0	8.45	10.83	9.34	8.62
25	48.7	11.22	12.66	12.37	9.71
50	93.0	14.48	16.28	15.78	14.52
100	93.2	20.21	20.07	22.98	17.24
200	93.5	22.42	25.38	27.72	18.99
400	93.2	23.82	26.66	32.25	21.27
800	93.2	26.68	33.28	37.28	24.17
1000	93.2	31.19	35.65	41.54	30.97

PE- Petroleum ether, Chl- Chloroform, MeOH- Methanol, H2O- Distilled Water

Discussions

Phytochemical Analysis

The leaf extract of *Canthiumera neilgherrensis* (Wight) K.M. Wong revealed the presence of phenol, flavonoids, alkaloids, tannin, terpenoids, steroids, saponins and glycosides and which are the compounds responsible for various therapeutic properties. These compounds are responsible for various properties such as antioxidant, antibacterial, antifungal, antiviral, anti-cancerous, anti-inflammatory, analgesic, etc. The presence of phenol and flavonoid suggests that the leaf extract of *Canthiumera neilgherrensis* (Wight) K.M. Wong has antioxidant activity.

Phenolic compounds act as chemopreventive, anti-inflammatory, and anticoagulant agents and are used in the pharmaceutical industry (Gueboudji, 2022). Flavonoids have numerous biological activities such as anti-inflammatory, antiviral, antimicrobial, anti-cancerous, cardioprotective, hepatoprotective, etc. (Kumar *& Pandey,* 2013). Alkaloids are important for their anesthetic, cardioprotective and anti-inflammatory activities (Kurek, 2019). Mainly terpenoids have physiological activities such as anti-inflammatory, antimicrobial, antimalarial, etc. (Yang *et al.*, 2020). Steroids are known to have anti-tumor, anti-inflammatory, and neuroprotection activities etc (Dembitsky *et al.*, 2018).

Antibacterial Analysis

Out of the four extracts tested, only water extracts of *Canthiumera neilgherrensis* (Wight) K.M. Wong exhibited good antibacterial activity and gave a zone of inhibition against *Pseudomonas aeruginosa.* Arif *et al.* (2022) reported that water was one of the effective solvents used in the extraction, and they showed good activity against microbes. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most suitable microbes in plant-based extracts (Mostafa *et al.,* 2018). It is widely known that gram-negative bacteria are more resistant to microbes than gram-positive. In the present study, *Pseudomonas aeruginosa*, gram-negative bacteria, was also found susceptible to the extracts of *Canthiumera neilgherrensis* (Wight) K.M. Wong. The presence of alkaloids, saponins, tannins, flavonoids, and steroids also substantiate the antimicrobial capacity

Antioxidant Analysis

Antioxidants are substances that counteract free radicals by interfering with the oxidative process mediated by free radicals (Cui *et al.*, 2004). Some antioxidants naturally occur in many foods and are produced by various biological systems (Halliwell, 1997). Ascorbic acid should be used as the standard for showing antioxidant efficiency (Molyneux, 2004). This method is more efficient than the direct determination of DPPH obtained from the calibration curve (Leitao *et al.*, 2002). In the FRAP assay, chloroform leaf extract is more effective than others in reducing Fe^{3+} to Fe^{2+}. In the DPPH radical scavenging assay, methanolic extract exhibits greater inhibition potential than the other three. The amount of inhibition in both the FRAP and DPPH assays is concentration-dependent, i.e., the higher the concentration, the greater the inhibition. The presence of phenol along with flavonoids also substantiates the antioxidant capacity.

Conclusions

Leaf extract of *Canthiumera neilgherrensis* (Wight) K.M. Wong has potential antioxidant and antibacterial activity along with significant levels of total flavonoid content. Both the DPPH and FRAP assays proved that the antioxidant activity of the extract was appreciable. It can be used as a potential source of natural antioxidants. Multiplication, propagation, and conservation of *Canthiumera neilgherrensis* (Wight) K.M. Wong is essential as it is confined to an area (endemic to the Southern Western Ghats). Moreover, *Canthiumera neilgherrensis* (Wight) K.M. Wong is a vulnerable species, and its protection is essential.

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Authors Contribution

The first author conceived and designed the analysis, performed the analysis, interpreted the data, and wrote the paper. The second author edited and critically revised the paper and approved the version to be published.

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