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Original Article

THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF THE LEAVES EXTRACT OF *CLERODENDRUM COLEBROOKIANUM* WALP, (FAM: VERBENACEAE)

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ABSTRACT

Objective: To investigate the in-vitro antioxidant and antimicrobial potential of *Clerodendrum colebrookianum* leaves extract.

Methods: The leaves of *C. colebrookianum* were collected from various parts of Aizawl, Mizoram, India. Subsequently, the leaves were extracted with solvents (chloroform, acetone, ethanol and methanol) in a Soxhlet extraction apparatus for 24hr. Further, the extracts were extensively examined for its in-vitro antioxidant (DPPH) and antimicrobial activities. The preliminary phytochemical screening was carried out using standard protocols.

Results: The existence of alkaloids, flavonoids, diterpenes, saponins, glycosides, steroids and terpeinoids were revealed in the phytochemical screening. The aqueous and acetone extract had the highest total phenolic content (2.348 mg/ml), when compared to methanol, ethanol and chloroform extracts, which was 0.549 mg/ml, 0.408 mg/ml and 0.407 mg/ml, respectively. The antioxidant activity was more significant for aqueous extract, when compared to other extracts. The antimicrobial activity was more significant for acetone extract showed significant zone of inhibition of 14±0.3, 13±0.3 and 15±0.2 for *E. coli*, S. *marcescens* and S. *aureus,* respectively.

Conclusion: The high level of antioxidant and antimicrobial potential of *C. colebrookianum* leaf extracts encourage its potential use for biomedical applications.

Keywords: Plants, Gallic acid, Drugs, Extracts.

INTRODUCTION

Since time immemorial, in search for the remedy for the diseases, people have always looked for potential drugs in medicinal plants and over the generations the plant-derived products have been a vital part of our traditional health care system. The uses of plant-derived products provide a large source of natural drug for treatments of several metabolic disorders and infectious diseases. It is estimated by the World Health Organization (WHO) that 80% of the developing countries population depends on plant derived medicines which have its own advantages like i.e. low or no adverse effects, poses minimum environmental hazards, easily available and affordable [1-3]. However, infectious diseases remain as world's major problem, accounting for 33% deaths each year and threatening to affect many more millions of people. The undesirable side effects along with the unprecedented upsurge in antibiotic resistance among pathogens upon synthetic drugs exposure have necessitated for looking at alternative therapeutics, particularly the plant based one [4-6].

Parasitic infections are known to lead for the release of free radicals which have severe consequences on functioning of cellular metabolism. Overproduction of ROS (reactive oxygen speies) either by normal oxygen metabolic process or by infections, apart from playing a beneficial role, may leads to oxidative stress thereby causing damage to vital components of the cell [7]. Generally, free radicals have benn implicated in several disorders (diabetes mellitus, heart diseases, cancer, acquired immunodeficiency syndrome, arthritis, liver disorder, ageing, etc.), and the treatment by antioxidants has gained an utmost importance in the treatment [8]. The presence of both antioxidant and antibacterial compounds in a single medicinal plant extracts, will have the dual therapeutic potential, thus helping in fighting infectious diseases and its further consequences. Although, plants used in the traditional medicine have been identified and their applications well-documented for many, however, the biological efficacy of many plants is yet to be scientifically verified [9-11].

Clerodendrum colebrookianum Walp., (Fam: Verbenaceae) locally known is "NEFAFU" is a very common and endemic medicinal plant mostly found in the North-Eastern states of India. *C. colebrookianum*, have been used for the treatment of various ailments and disorders such as cough, dysentery headache stomach disorder colics pain, hypertension, helminthic infections, diabetes and some skin diseases in the traditional system of medicine [12-16].

Khasi and *Jaintia* tribes in Meghalaya has been using *Clerodendrum* leaves preparation commonly called as "*Sla Jarem"* for high blood pressure, malaria and liver problems and in the case of rheumatic pains, application of the warmed leaf-paste on the affected area is a common traditional practice. Experimental evidences have showed hypolipidemic and hypoglycemic effect with the extract of *C. colebrookianum* [16, 17]. It has been reported that *C. colebrookianum* extract has been demonstrated to have a role in enhancing experimentally induced insulin resistance and hypertension [18]. These findings provide a first pharmacological evidence for defensive role against metabolic disorder [18]. Here we attempt to evaluate the antioxidant and antimicrobial potential of *C. colebrookianum* leaves extract and to elucidate the possible class of principal components responsible for its biological activity. This study for the first time explores the biological potential of *C. colebrookianum* extract for its antioxidant and antimicrobial activities for its further therapeutic applications.

MATERIALS AND METHODS

Materials

Quercetin and Folin–Ciocalteu reagent were procured from Qualigens, Mumbai, India. 2,2-diphenyl-1-picrylhydrazyl (DPPH),

thiobarbituric acid (TBA), gallic acid, and ascorbic acid, were from SRL Chemicals, India. All the other solvents and reagents are of analytical grade.

Plant material

C. colebrookianum leaves were collected in January month (2012) from various parts of Aizawl, Mizoram, India. Dr. S. Sundara Rajan authenticated the plant material and the voucher specimen (JU-RUV-81) were deposited at the Research centre of Vrkshayurveda, Jain University, Bangalore. The whole plant samples were dried under controlled temperature (shade dry). The dried leaves were stored in a closed vessel. By using a grinder (mixer) the dried plant material was pulverized into fine powder

Preparation of extract

Twenty-five milligram of the powdered material was extracted in Soxhlet extraction apparatus for 24h with 200 ml of each of chloroform, acetone, ethanol and methanol solvents. Under reduced pressure using a Rotavapor (BuchiFlawil, Switzerland) the extracts were evaporated to dryness and the sticky greenish substances which were obtained was suspended in dimethyl sulphoxide (DMSO) and stored at-20^{0}C until further use for the phytochemical screening, antibacterial and antioxidant properties.

Phytochemical analysis

The preliminary qualitative phytochemical analyses of carbohydrates, alkaloids, saponins, phenolics and tannins, flavonoids, fixed oils and fats, glycosides, phytosterols and triterpenoids in the extracts were carried out using the standard methods as describe[19-22].

Quantitative analysis

Determination of total phenolic content

The *C. colebrookianum* leaf extracts (ethanol, petroleum ether, chloroform and aqueous) total phenolics were determined using Folin-Ciocalteau reagent method, employing Gallic acid as standard [23]. Briefly, 200 ml of extracts (2 mg/ml) were made up to 3 ml with distilled water, and then mixed thoroughly with 0.5 ml of FC reagent for 3 min. Further, 2 ml of sodium carbonate i.e. 20% (w/v) were added to the mixture and allowed to stand for 60 min in the dark. Finally, the absorbance was measured as OD at 650 nm and results was expressed as mg of gallic acid equivalent (GAE)/g of dry weight.

Antioxidant activity

Free radical scavenging activity [DPPH]

C. colebrookianum leaf leaves extracts radical scavenging properties were evaluated by the method of Blois, [24]. 0.1 solution of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH*) in methanol was prepared and to this 1 ml of solution, 3 ml of the extracts at different concentration (1-15µg/ml) was added. This reaction mixture was incubated in the dark for 30 min and measured at 517 nm, which represented the discoloration. As a positive control, ascorbic acid was used. The DPPH* radical scavenging capacity was calculated and expressed as percent inhibition using the following equation:

I %= (Absorbance of control–Absorbance of test/Absorbance of control) x100

The IC_{50} values were calculated from the regression equation prepared from the different concentrations of the extracts.

Antibacterial activity

Microorganisms

Escherichia coli (MTCC DH5α), Serratia marcescens (MTCC 7103) and Staphylococcus aureus (MTCC 4301) were the bacterial strains used for this study, which was maintained on nutrient agar slope at 4 ºC. Stock culture was prepared by inoculating each culture from slants to flask in sterile nutrient broth and incubated at 37 ºC for 24 h. Further, the stock culture was serially diluted by ten-fold with sterile peptone water. From this one tenth of ml of each dilution was spread on a plates of nutrient agar and incubated at 37 °C for 24 h and the number of colony forming units (CFU) was counted from plates of each dilution and thereby the total CFU was calculated in the stock culture. For antimicrobial screening, the stock cultures of 1×10^5 CFU per ml were used.

Bacterial susceptibility testing

The bacteria strains grown on nutrient broth were swabbed on sterile plates of nutrient agar separately. Using a sterilized cork borer agar wells were cut with a dimension of 6 mm diameter and 100 µl of different concentrations (0.2, 0.4, 0.6, 0.8, and 1µg/ml) of leaf extracts (aqueous, methanol, ethanol and acetone) were added to different wells in the plate. DMSO served as negative control and 100 µg/100 ml of *Tetracycline, Ampicillin* and *Methicillin* served as positive controls. The plates were then incubated at 37ºC for 24 and the diameter of inhibition zones was measured in millimeter and the results were recorded [25-27].

Statistical analysis

SPSS version 10.0.1(Chicago, IL) using a one-way student's t-test was done for statistical analysis. When compared to relevant controls, the value of $p<0.05$ was considered statistically significant. The results are expressed as mean±standard deviation (SD).

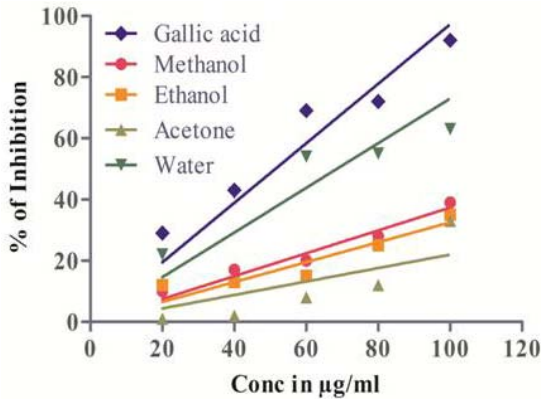

Fig. 1

Table 1: Phytochemical evaluation of *C. colebrookianum* leaves extracts

Test	Methanol	Acetone	Chloroform	Ethanol	Aqueous
Steroids	+	-	-	-	-
Terpernoids	+	-	-	+	+
Flavonoids	+	-	-	-	-
Diterpenes	-	+	-	+	+
Saponins	+	+	+	+	+
Glycosides	-	-	-	+	+
Alkaloids	-	-	-	-	-
Tannins	-	-	-	-	-
Anthraquinones	-	-	-	-	-

Table 2: Total phenolic and total flavonoid content of *C. colebrookianum* leaves extracts

Extract	Total phenolic (mg of GAE/ml)
Acetone	2.348
Water	1.25
Methanol	0.549
Ethanol	0.408
Chloroform	0.407

Table 3: IC_{50} Values of *C. colebrookianum* leaves extracts

DPPH antioxidant assay					
Extracts	**Acetone**	**Ethanol**	**Methanol**	**Water**	**Gallic acid**
IC_{50} values (µg/ml)	227.27	153.84	134.04	68.49	46.9

Table 4: Antibacterial activity of leaf extracts of *C. colebrookianum* leaves extracts

Extracts	Conc (µg/ml)	Bacterial strains [Zone of inhibition (mm)]		
		Escherichia coli	*Serratia marcescens*	*Staphylococcus aureus*
Acetone	0.2	0	0	12±0.1
	0.4	11±0.1	9±0.1	13±0.2
	0.6	12±0.2	13±0.2	14±0.2
	0.8	13±0.3	13±0.1	15±0.2
	1	14±0.3	13±0.3	15±0.2
Methanol	0.2	8±0.1	0	6±0.1
	0.4	9±0.1	8±0.1	7±0.1
	0.6	9±0.1	10±0.1	8±0.1
	0.8	10±0.2	11±0.2	10±0.1
	1	11±0.2	12±0.3	11±0.2
Chloroform	0.2	0	3±0.1	0
	0.4	7±0.1	9±0.2	0
	0.6	9±0.1	9±0.1	13±0.1
	0.8	10±0.2	15±0.3	11±0.1
	1	11±0.2	17±0.2	9±0.2
Ethanol	0.2	0	0	0
	0.4	0	0	8±0.1
	0.6	0	0	8±0.1
	0.8	0	0	9±0.1
	1	0	0	10±0.2
Aqueous	0.2	0	0	0
	0.4	0	0	0
	0.6	0	0	0
	0.8	0	0	0
	1	0	0	0
Controls	Tetracycline(2)	15±0.2	31±0.3	33±0.2
	Ampicillin (2)	1±0.1	0	1±0.1

The test was done in triplicate. Diameter of the zone of inhibitions is given here as mean±standard deviation.

RESULTS

Phytochemical screening of the leaves of *C. colebrookianum* confirmed the existence of alkaloids, flavonoids, diterpenes, saponins, glycosides, steroids and terpenoids (Table.1). The total phenolic contents of *C. colebrookianum* extracts were determined (table 2) and the results obtained indicates that, the aqueous exact (1.25 mg/ml), and the acetone extract (2.348 mg/ml) had the highest total phenolic content, when compared to the methanol, ethanol and chloroform 0.549 mg/ml, 0.408 mg/ml and 0.407 mg/ml extracts, respectively. These results shown that the extracts of aqueous and acetone possessed significant secondary metabolites releasing activity from leaves of *C. colebrookianum*.

The DPPH inhibition activities were expressed in percentage of the different extracts of *C. colebrookianum* as shown in fig. 1 and the IC_{50} values of different extract are summarized in table 3. From the results it is observed that, the aqueous extract showed highest scavenging activity of 60% at 100µg/ml concentration, whereas acetone, ethanol and methanol extracts showed 28%, 30% and 38% scavenging activity at the identical concentration, respectively. As the IC_{50} values of aqueous extract were found to be 227.27 µg/ml (table 3), exhibiting higher DPPH scavenging activity than the other extracts. Therefore, the observed noticeable effect on scavenging free radicals of aqueous extract can be related to the high phenolic constituents present (table 2).

In the antimicrobial activity among all the extracts tested, acetone extract significantly inhibited the bacterial activity with a zone of inhibition of 14±0.3, 13±0.3 and 15±0.2 for *E. coli*, S. *marcescens* and S. *aureus,* respectively. However, It was interesting to observe that the aqueous extract did not possess anti-bacterial activity (table 4).

DISCUSSION

Parasitic infections are known to lead for the release of free radicals which have severe consequences on cellular metabolism. Overproduction of ROS either by normal oxygen metabolic process or by infections, apart from playing a beneficial role, may leads to oxidative stress thereby causing damage to vital components of cell. Presence of both antioxidant and antibacterial compounds in single medicinal plant extract, have tremendous potential in fighting infectious diseases and its further consequences. In these lines, the leaves extracts of *C. colebrookianum* was evaluated for its antioxidant and antibacterial activities.

C. colebrookianum leaves extracts phytochemical screening reveals the presence of alkaloids, flavonoids, diterpenes, saponins, glycosides, steroids and terpenoids. A high phenolic content was observed in the acetone and aqueous extracts and this higher amount of total phenolic content in the extracts suggests the high antioxidant activity it process. For evaluating the free radical-scavenging activities of antioxidants, DPPH is a stable free radical, which has been generally accepted as a tool extensively [28]. The results showed that the aqueous extract exhibited higher DPPH scavenging activity (fig. 1 and table 3), which also possessed higher phenolic content and this may be due to the fact that phenolic compounds are often extracted in higher amounts by using polar solvents [29]. It is also reported that a wide variation in the polyphenolic contents of the extract are existing due to differences in the polarity of the extracting solvents [30,31]. These all results indicate that the aqueous extracts have a significant effect on scavenging free radicals and can be related to the high phenolic constituents present. As a secondary metabolism in plants the phenolic antioxidants are produced and due to their redox properties the antioxidant activity. Phenolic antioxidants chelating the transitional metals in known to inhibit lipoxygenase [32]. The phenolic constituents are reported to be as good antioxidants as they are effective hydrogen donors [33]. The higher antibacterial potency of acetone and aqueous extracts can be attributed to the high phenolics which might be enhanced in the presence of solvents. It has been well documented that the alkaloids, phenols, flavonoids, and tannins are well known for their antimicrobial activity [34].

CONCLUSION

The *C. colebrookianum* leaves extract showed promising antibacterial and antioxidant activity. This study also supports the view that the extracts obtained using high polarity solvents (aqueous) was considerably more effective in radical scavenging activity. Further, it was observed that there was a strong correlation of higher antibacterial activity with that of high total phenolic content in the acetone extracts of *C. colebrookianum* leaves. The results encourage the use of *C. colebrookianum* leaves extracts for nutraceuticals applications, functional food and medicinal application. Future work on the chemical composition and better understand the mechanism of action of the principal component present in the extract will be interesting for developing it as a drug for therapeutic application.

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CONFLICTS OF INTERESTS

Declared None

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