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The phytochemistry and antioxidant activity of methanolic extract of *Clerodendrum infortunatum* leaves

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Abstract

The antioxidant activity of methanolic extract *Clerodendrum infortunatum* Linn. (MECI), which is widely utilized in Indian traditional medicine for a variety of ailments, was investigated. Many antioxidant components found in plants have been recognized as active oxygen scavengers or free radical scavengers. The reagent DPPH is commonly used to assess antioxidants' ability to scavenge free radicals. MECI was found to contain a high level of polyphenolics and to have strong free radical scavenging activity. The presence of the highest amount of polyphenolics and flavonoids in it could explain the increased activity. Qualitative phytochemical analysis of extract indicated presence of alkaloids, carbohydrates, glycosides, proteins and amino acids, phytosterols, phenols, tannins, flavonoids and resins and GC-MS analysis investigated presence of total 20 active phytoconstituents.

Keywords: *Clerodendrum infortunatum*, qualitative phytochemical analysis, GC-MS analysis, DPPH radical scavenging activity

Introduction

Clerodendrum infortunatum Gaertn. is known in Ayurveda by the Sanskrit names "Bhargi", "Bhrigubhava", "Padma", "Fanji" and "Brahman yastika", as "peruvelam" in Kerala, and in Hindi as "Bhant" or "Bharangi". *Clerodendrum* is a very large and diverse genus and are widely distributed in Asia, Australia, Africa and America. Leaves of the plant are used as bitter tonic, antiperiodic, vermifuge, pain killer, laxative and cholagogue. The leaves and roots are externally used for tumors and in certain other skin diseases as paste (Suman and Gupta. 2012) [25].

Clerodendrum, a representative member of Leguminosae, elaborates a diverse array of chemical compounds including flavonoids and diterpenoids, which are reported to possess antifeeding activity, antimicrobial activity and antioxidant activity (Saha *et al.*, 2018) [21]. The ability of some species in this genus such as *C. serratum*, *C. infortunatum*, *C. inerme*, *C. viscosum*, and *C. phlomidis*, possesses a good antioxidant activity (Hafiz *et al.*, 2016) [10].

The present study was aimed to analyze the phytochemistry of *Clerodendrum infortunatum* as well as to evaluate its antioxidant capacity taking into consideration the plant's traditional medicinal qualities and its abundance of bioactive phytoconstituents.

Materials and Methods

In present study, the locally procured leaves of *Clerodendrum infortunatum* were duly authenticated vide voucher specimen No. 10577 from University Department of Botany, Rashtrasanta Tukdoji Maharaj Nagpur University, Nagpur. The leaves were shed dried, powdered and defatted with Petroleum ether and then finally extracted with 70% methanol in Soxhlet's apparatus.

Qualitative phytochemical analysis of extract

The methanolic extract of *Clerodendrum infortunatum* leaves was subjected to the preliminary qualitative phytochemical analysis to assess the presence of active principles (Rosenthaler and Ghosh, 1930 and Raaman, 2006) [20, 18].

GC-MS analysis of extract

The GC-MS analysis of hydro-methanolic extract of seeds of *C. infortunatum* was carried out using Agilent GC 7890 with triple axis's 5975 MS detector.

The capillary column was Agilent HP-5MS (30 m × 250 μm × 0.25 μm) composed of 5% phenyl methyl silox. The initial oven temperature was 40 °C for 0 min which was raised at rate of 25 °C/min upto 160 °C for 15 min and than at rate of 2 °C/min upto 280 °C for hold time of 5 min. The injector volume was 4 μl. The gas used as carrier with constant flow rate of 1ml/min with split ratio of 25:1. The MS operating conditions were; source temperature 230 °C (max 250 °C), quad temperature 150 °C (max 200 °C), solvent delay time of 4 min. Compounds were identified in terms of RT values and mass spectra with those obtained from the NIST search library. The obtained compounds were searched for detailed information.

DPPH radical scavenging activity

The extract was dissolved at a concentration of 0.005-0.075 mg/ml in methanol. Methanolic solution of DPPH at a concentration of 0.02 mg/ml was prepared and 1.5 ml of it was added with 0.75 ml of diluted methanolic extract of different concentrations. Methanol 0.75 ml was serve as a blank. Quercetin and Rutin at a concentration of 0.001 - 0.020

mg/ml diluted in methanol were served as positive controls. The prepared samples were incubated for 15 minutes at room temperature and absorbance was measured at 517 nm with UV spectrophotometer. The DPPH radical scavenging activity was evaluated using following equation-% inhibition= [(Abs. of blank – Abs of sample)/(Abs. of blank)] X 100

The mean of three IC₅₀ (concentration of sample which is required to scavenge 50% of the DPPH free radicals) values of extract or standard was determined (Meda *et al.*, 2005 and Kumaran and Karunakaran, 2007)^[11].

Results

The fraction extracted had 17.07% extractability, brownish green colour and semisolid consistency. The preliminary phytochemical analysis detected the presence of active compounds alkaloids, carbohydrates, glycosides, proteins and amino acids, phytosterols, phenols, tannins, flavonoids and resins. The GC-MS analysis of extract revealed presence of total 20 active phytoconstituents. Its chromatogram and details are depicted in Figure 1 and Table 1, respectively.

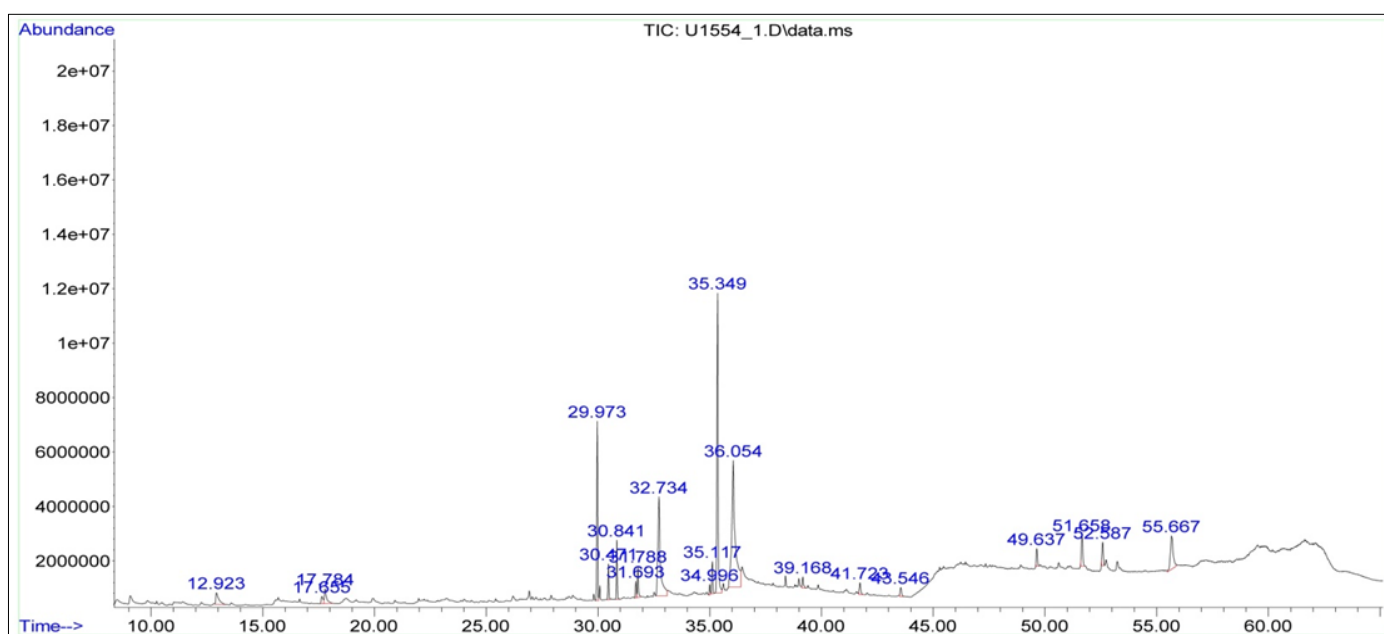


Fig 1: Chromatogram of GC-MS

Table 1: Details of active principles observed in GC-MS analysis

Sr. No.	Compound Identified	Formula	Peak Height	RT (Min.)	Percent Area
1.	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₈ O ₄	429269	12.923	2.213%
	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	263345	17.655	0.862%
	Ethanone, 1-(2-hydroxy-5-methylphenyl)				
2.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	541581	17.784	1.955%
	Phytol				
3.	3-Eicosyne	C ₂₀ H ₃₈	6600301	29.973	9.210%
4.	9-Eicosyne	C ₂₀ H ₃₈	1319616	30.471	1.843%
5.	Tetradecanal	C ₁₄ H ₂₈ O	2185714	30.841	3.085%
	Octadecanal	C ₁₈ H ₃₆ O			
6.	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	618959	31.693	0.974%
7.	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	1145149	31.788	1.775%
8.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	3646289	32.734	15.010%
9.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C ₁₉ H ₃₂ O ₂	384252	34.996	0.580%
10.	Phytol	C ₂₀ H ₄₀ O	1181456	35.11	2.055%
11.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	C ₁₈ H ₃₀ O ₂	1102432	35.349	20.08%
12.	Ethanol, 2-(9-octadecenyl)-, (Z)-	C ₂₀ H ₄₀ O ₂	4655560	36.054	26.27%
	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	C ₁₆ H ₂₈ O ₃			
13.	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₉ H ₁₇ NO ₂	418424	39.168	0.807%

14.	3,6,6-Trimethylundecane-2,5,10-trione	C ₁₄ H ₂₄ O ₃	441738	41.723	0.992%
	Digitoxin	C ₄₁ H ₆₄ O ₁₃			
15.	1-Cyclohexanol, 2-[1-(phenylsulfonyl)methylidene]	C ₁₃ H ₁₆ O ₃ S	333561	43.546	0.825%
	Tetradecanedioic acid, 3,6-epoxy-, dimethyl ester	C ₁₆ H ₂₈ O ₅			
16.	Stigmasterol	C ₂₉ H ₄₈ O	641274	49.637	1.237%
17.	Phenol, 2-(1-methylpropyl)-	C ₁₀ H ₁₄ O	1154183	51.658	2.685%
18.	Phenol, 4-(1-methylpropyl)-	C ₁₀ H ₁₄ O			
19.	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	C ₁₂ H ₂₃ NO ₂	867302	52.587	1.897%
	Cyclobutanecarboxylic acid, 2-dimethylaminoethyl ester	C ₉ H ₁₇ NO ₂			
20.	17-Pentatriacontene	C ₃₅ H ₇₀	1229375	55.667	5.637%
	Octadecanal, 2-bromo	C ₁₈ H ₃₅ BrO			

The results of IC₅₀ values of DPPH radical scavenging activity of plant extract and standards i.e. Quercetin and Rutin are summarized in Table 2. Quercetin showed the highest DPPH radical scavenging activity with IC₅₀ mean value of 0.012 + 0.005mg/ml. The IC₅₀ value observed for Rutin was 0.014 + 0.008mg/ml, which was followed by *C. infortunatum* and it was 0.05 + 0.006mg/ml.

Table 2: Results of DPPH radical scavenging activities

Sr. No.	Sample	RSA:IC ₅₀ (mg/ml + SD)
1.	Quercetin	0.012 + 0.005
2.	Rutin	0.014 + 0.008
3.	Extract	0.05 + 0.006

Discussion

The physical characteristics of extract of *Clerodendrum infortunatum* and its extractability percentage is in agreement with Gouthamachandra *et al.*, (2009)^[8] and Das *et al.*, (2011)^[24] who observed extractability percentage of 16.5% and 13.5%, respectively. Overall, the methanolic extract indicated presence of alkaloids, carbohydrates, glycosides, proteins and amino acids, phytosterols, phenols, tannins, flavonoids and resins. In some earlier studies Rajurkar (2011)^[19]; Ghosh *et al.*, (2015)^[7]; Limsay *et al.*, (2018)^[12, 13], Prashith *et al.*, (2019)^[17] and Dongare *et al.*, (2020)^[4] also reported presence of similar pharmacologically active phytochemicals like alkaloids, carbohydrates, glycosides, proteins and amino acids, phytosterols, phenols, tannins, flavonoids and resins in the extract of *C. infortunatum*.

Dutta *et al.*, (2016)^[5] observed presence of bioactive phytochemicals like phenolics, flavonoid, alkaloids and glycosides along with some essential vitamins in the roots of extract of *Clerodendrum infortunatum* and while comparing phytochemical profile of *Clerodendrum infortunatum* with other medicinal plants, it was observed that *Clerodendrum infortunatum* is having highest quantity of phenols, lipids and tannins. The presence of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl was demonstrated by Ghosh *et al.*, (2015)^[7] and Dey *et al.*, (2015)^[2] in the extract of *Clerodendrum infortunatum*. Dey *et al.*, (2015)^[2] reported presence of 2-Methoxy-4-vinylphenol. Nandi and Lyndem (2016)^[26] reported presence of linolenic acid in the leaves and stigmasterol in the aerial parts of *Clerodendrum infortunatum*. The presence of Hexadecanoic acid, methyl ester was indicated by Ghosh *et al.*, (2015)^[7]. Ghosh *et al.*, (2015)^[7] and Dey *et al.*, (2015)^[2] showed presence of n-Hexadecanoic acid similarly the presence of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester; Phytol; 9,12,15-Octadecatrienoic acid, (Z,Z,Z) and Ethanol, 2-(9-octadecenyloxy)-, (Z)- were reported by Dey *et al.*, (2015)^[2] and Mohammed *et al.*, (2016)^[6] in various GC-MS studies of extract of *Clerodendrum infortunatum*.

Molehin *et al.*, (2017)^[16] reported presence of 3-Eicosyne; 9-

Eicosyne; Hexadecanoic acid, methyl ester; n-Hexadecanoic acid; 9,12-Octadecadienoic acid (Z,Z)-, methyl ester in leaf extract of another *Clerodendrum* species *Clerodendrum volubile*. They further reported that, Hexadecanoic acid has proven to exhibit antimicrobial, anti-inflammatory, anti-oxidant and other pharmacological activities. The GC-MS analysis of medicinal plants *Eichhornia crassipes* (Mart) Solms. exhibited presence of 3-Eicosyne; 9-Eicosyne; n-Hexadecanoic acid; Phytol; 9,12,15-Octadecatrienoic acid, (Z,Z,Z); Ethanol, 2-(9-octadecenyloxy)-, (Z)-; stigmasterol and 17-Pentatriacontene which were reported to have various pharmacological activities like antimicrobial, anti-inflammatory, anti-cancer, hypocholesterolemic and anti-oxidant properties (Kumar *et al.*, 2018). The findings of GC-MS analysis shows the presence of various phytoconstituents in the extract of *Clerodendrum infortunatum* and are in agreement with above reports. Limsay *et al.*, (2018)^[12, 13] and Prashith *et al.*, (2019)^[17] studied the DPPH radical scavenging activity of leaves of extract of *Clerodendrum infortunatum* and found it 0.047mg/ml and 3.69 µg/ml (0.0036mg/ml), respectively. The results obtained in present study are in agreement with the reported findings. Sannigrahi *et al.*, (2009)^[22]; Gurudeeban *et al.*, (2010)^[9]; Modi *et al.*, (2010)^[15]; Suman and Gupta (2012)^[25]; Limsay (2018)^[12, 13] and Siddik *et al.*, (2021)^[23] found that extract of *Clerodendrum infortunatum* Linn. Contains a high amount of polyphenolics and possesses significant free radical scavenging activity in the assay like total amount of polyphenolics compounds, DPPH radical scavenging, nitric oxide scavenging, superoxide anion radical scavenging, hydroxyl radical scavenging and reductive power assay. The higher activity was may be due to presence of richest amount of polyphenolics and flavonoids in it.

Conclusions

The 70% methanolic extract of *Clerodendrum infortunatum* leaves possesses pharmacologically active phytoconstituents including phenols, flavonoids etc. when subjected to qualitative and quantitative estimation. It also demonstrated the antioxidant potential in DPPH radical scavenging activity and therefore the plant needs to be systematically investigated for medicinal properties to validate its folklore medicinal claims.

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