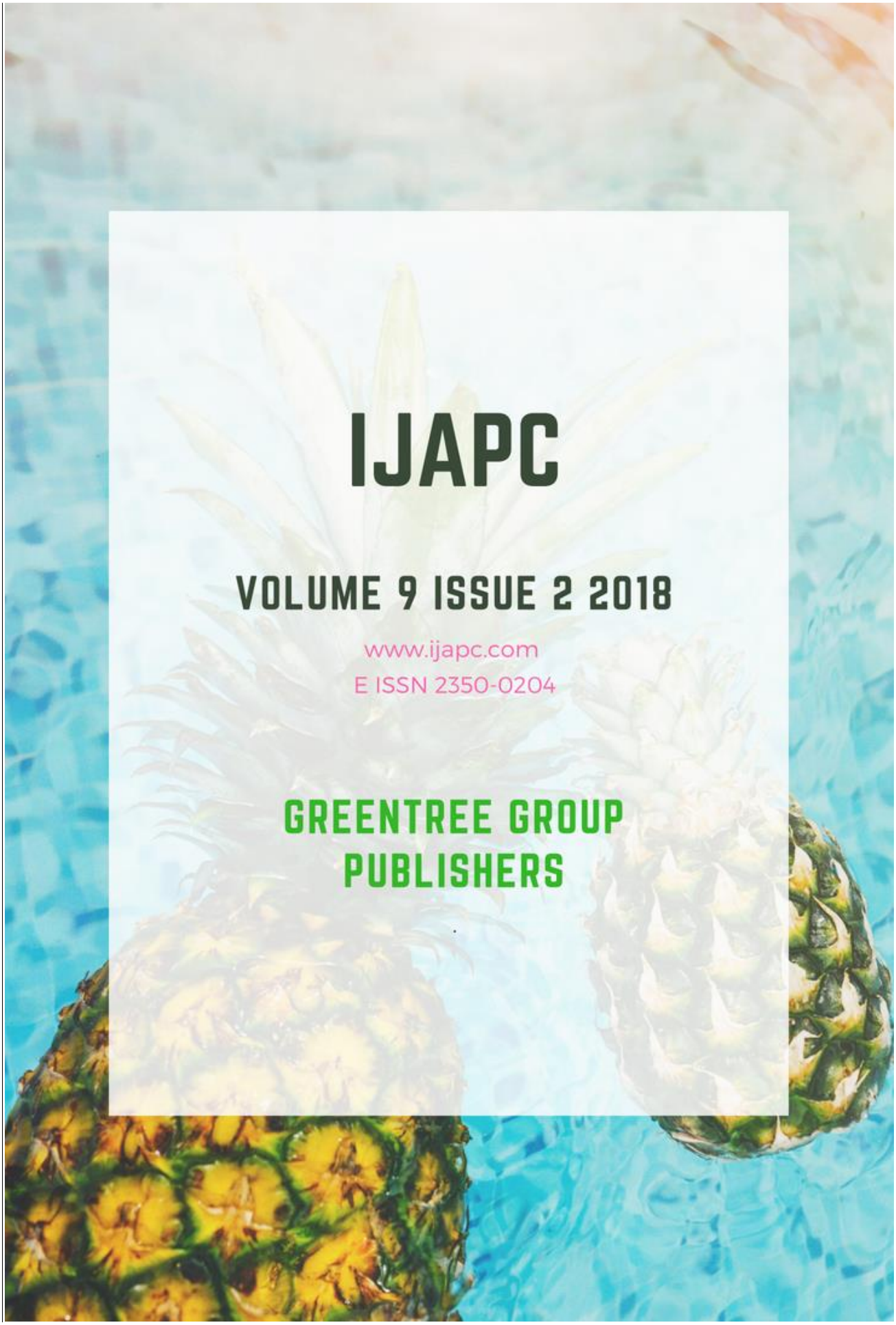

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Pharmacological Evaluation, Phytochemical Screening and Analytical Profiling of Various Extracts of Clerodendron serratum: An Medicinal Herb

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ABSTRACT

Clerodendron serratum belongs to family *verbenaceae* known as *Bharangi* commonly found in the India. The *Clerodendron serratum* showed anti-fungal, anti-oxidant, tuberculosis, anti-asthmatic, anti-bacterial and anti-inflammatory properties. The *Clerodendron serratum* contains D-mannitol, cleroflavone, apigenin, scutellarein, serratagenic acid, queretaroic acid and γ-sitosterol.The phytochemical screening was performed on petroleum ether, chloroform and methanolicextractsof the plant. Analytical techniques (Thin Layer Chromatography, Fourier Transmittance Infrared and High Performance Thin Layer Chromatography) and chemical test confirmed the presence of alkaloids, flavanoids, phenols, terpenoids, steroids and saponins in plant extracts. Most of the phytochemicals were present in chloroform and methanol extracts.The alkaloid found in chloroform and methanol extract respectively at R_f 0.42 and 0.45. Flavanoids and phenol were present in chloroform and methanol extract. Terpenoids and saponins were present in methanol extract while steroids were observed in chloroform extract only. Further antibacterial and antifungal activities were also performed which showed positive result for plant extracts.

KEYWORDS

Clerodendronserratum, Antibacterial, Antifungal, Phytochemical Screening, TLC, HPTLC

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INTRODUCTION

In a present scinerio drugs have been derived directly or indirectly from traditional medicinal plants. Microscopic and macroscopic descriptions of medicinal plants is the first step towards establishing the identity and purity and such work should be carried out before performingother tests. Anatomical characters are also helpful for the identification of drug when morphological features are indistinct[1-5].

In India there are about 7500 species of the flowering plants known to have medicinal properties. Various parts of the plant may be used for medicinal purposesuch as; root, rhizome, wood, bark, flower, fruit and seed. Bark is outermost covering of old stem and trunk. The term bark is used most often in a non-technical context and refers to all tissue outside the vascular cambium of the axis, in either a primary or secondary state of growth[6-8]. The outer layer protects the tree from hot or chilly winds and it may be impregnated with certain chemical substances. The secondary metabolites like tannins, phenolic, steroids, alkaloids, crystals of calcium carbonate, calcium oxalate and magnesium oxide etc. embedded in the barks. Therefore bark is used as medicine to treat several diseases includingstem bark as well as root barks. In Ayurvedicsystem the barks are used in the form of fine powder (*Churnas*), infusion (*Fanta*), decoction (*Kadha*) or fermented decoction (*Arishtha*) or can be made into pills (*Vati* or *Guti*)[9-12].

Considering importance of bark present investigation were planned to perform phyto-chemical and pharmacological evaluation of bark extracts of *Clerodendronserratum*. The study was aimed to explore folklore use of plant bark.

MATERIALS AND METHODS

Sample collection

The *Clerodendron serratum* was collected from the Indore (*Malwa*) region India. The authentication of plant was done in the Ayurveda department Indore. The bark of plant was scraped with the help of knife, in the month of November 2016 and dried in shade. After drying the bark was grinded into course powder and stored in plastic vessel.

Extract preparation

The course powder of bark of *Clerodendron serratum* was divided into three parts. Each part having 25 g of bark powder extracted with petroleum ether, chloroform and methanol. The first 25g bark powder was wrapped into thimble and kept in upper chamber of Soxhlet apparatus (Fig.1) and in lower portion solvent was

present, heated to evaporation and solvent reaches to thimble (upper portion) and passes through the sample powder as a result extraction was starts. same procedure was adopted for chloroform and methanol extracts.

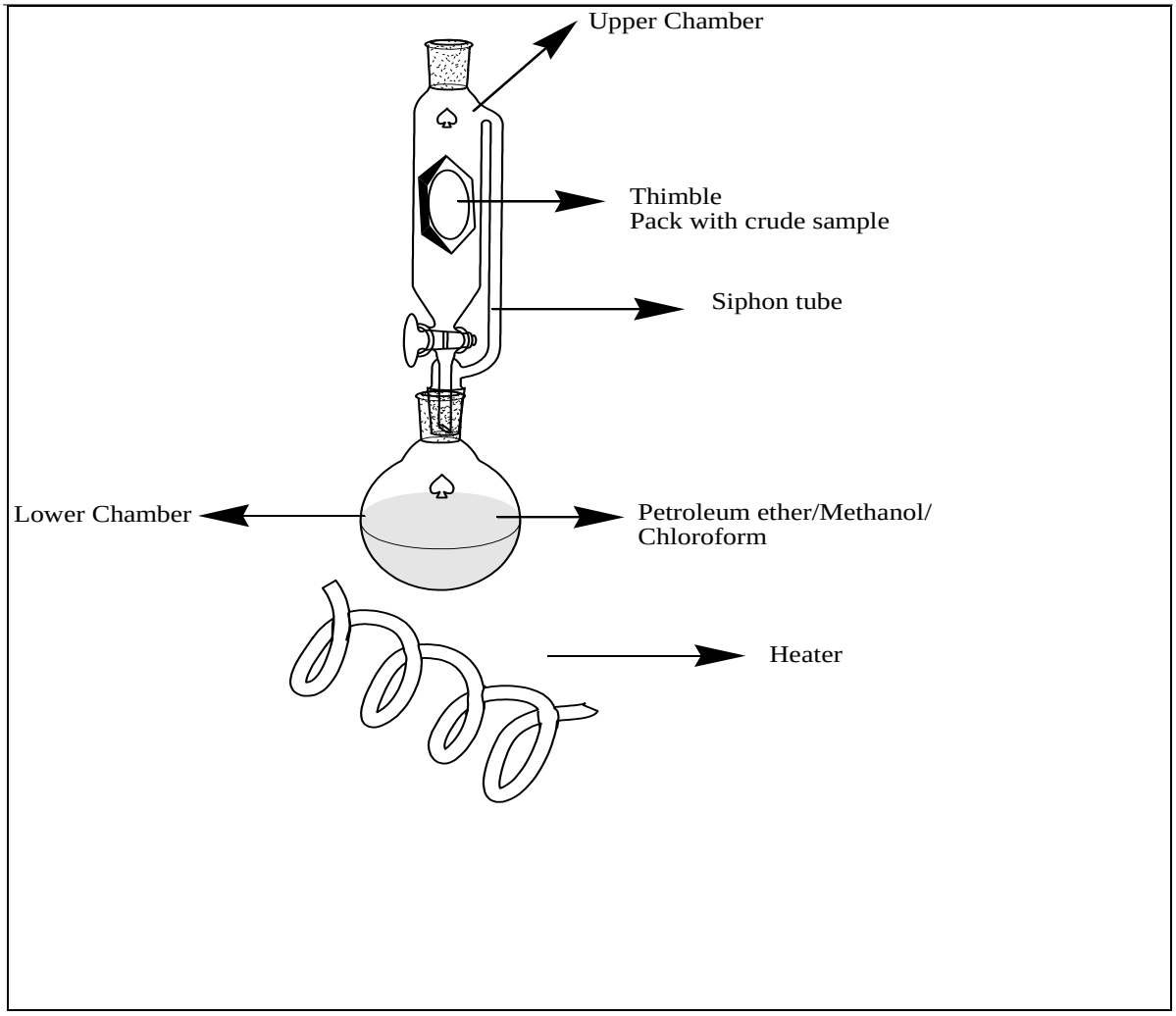

Fig. 1 The Soxhlet apparatus used in extraction

The petroleum ether, chloroform and methanolic extracts were collected, filtered with Whatmann No. 1 filter paper, evaporated till drying, stored in air tight container and analyzed further[13].

Thin layer chromatography

The thin layer chromatography profiling of dried extracts of petroleum ether, chloroform andmethanol was performed using silicagel plate. The three TLC plates were taken and 50 µl crude extracts applied on 1 centimeter above the TLC plate with the help of micro-pipette. After sample application the plates were dried and kept in the chamber equipped with solvent system; Ethyl Acetate: Chloroform: Water: methanol 5:3:1:1. The solvent mixture was allowed to travel ¾th of plate height, after

that plate was removed and dried. The dried plate kept in UV chamber and then in iodine chamber to detect spots. The spots were scraped and analyzed further for chemical evaluation[14].

The scraped bands of TLC plate were dissolved in10 mL respective solvent, filtered and then subjected to following tests:

Chemical Test

Discussed in Table 1.

Table 1 Phytochemical screening chemical test and TLC results (R_f Value) of extracts of *Clerodendron serratum*

S. No.	Constituents	Test performed	Extracts of *Clerodendron serratum*					
			Petroleum Ether Extract		Chloroform		Methanol extract	
			Results	R_f Value	Results	R_f Value	Results	R_f Value
1	Alkaloids	Mayer's Test Wagner's Test	-ve -ve	-	+ve +ve	0.42	+ve +ve	0.45
2	Flavanoids	Ammonia Reduction Test	-ve	-	+ve	0.30	+ve	0.32
3	Phenol	Ferric Chloride Test	-ve	-	+ve	0.70	+ve	0.71
4	Terpenoids	Salkowski's Test	+ve	0.54	-ve	-	+ve	0.53
5	Steroids	Salkowski's Test	-ve	-	+ve	0.60	-ve	-
6	Saponins	Froth Test	+ve	0.80	-ve	-	+ve	0.80

IR Spectroscopy

The dried petroleum ether, chloroform and methanol extract of plant *Clerodendron serratum* kept in oven for drying then triturated in mortal pestle along with dried KBr**.** The blank reading of KBr analyzed in FTIR (Fourier transform infrared) to avoid the interference of it in sample**.** The triturated extract kept in sample cell of FTIR and instrument allowed to run spectra which generated peaks of functional group present in sample[10].

High performance thin layer chromatography (HPTLC)

The silica gel GF HPTLC plate were used for analysis and activated in oven prior to spotting**.** The sample of dried petroleum ether, chloroform and methanol extract of plant was applied with an automatic applicator**.** Thesolvents used asmobilephase were of composed of Ethyl Acetate: Chloroform: Water: methanol 5:3:1:1**.** The solvent mixture was filtered and kept in chamber for saturation**.** The silica gel GF HPTLC plate kept in HPTLC chamber and mobile phase was allowed to run for few hours**.** After development plate was removed from chamber dried to avoid contamination**.** The plate was kept in UV chamber for confirmation of spot**.** The TLC scanner used to detect spot at 200 and 800 nm[15].

Pharmacological evaluation

The pharmacological evaluation of extracts of *Clerodendron serratum* was performed as follows:

a. Anti-bacterial activity*:* Cup-plate method

The culture medium was made with nutrient agar in Ultra-Violet laminar air flow and sterilization was done at 60°C in an autoclave**.** The medium was poured on glass plate and mixed with bacterial suspension and after drying cups were prepared over it**.** The samples were then poured over bacterial cup and plate and activity was measured by visual inspection.[16-18]

b. Antifungal activity by inhibitory zone estimation:

Disk diffusion method was used to determine antifungal activity. Sample was prepared and disk was dipped in it with solid culture medium**.** All plates of different extract were kept for incubation at 37 °C in UV chamber for 48 h. after incubation inhibition was measured by the round scale[16-18].

RESULTS AND DISCUSSION

The plant sources were widely used for medicinal purpose since ancient times due to active chemical constituents present in it. The results of phytochemical screening are compiled in table-1 which confirms presence of secondary metabolite which contributed towards antimicrobial activity. The TLC was also performed using mixture of ethyl acetate: chloroform: water: methanol as mobile phase. The alkaloid was present in the chloroform and methanolic extract of plant respectively at R_f value 0.42 and 0.45. The alkaloid was confirmed in scrap part of TLC by Mayer's Test and Wagner's Test. The flavonoid was confirmed by ammonia reduction test at R_f value 0.30 and 0.2 in chloroform and methanol extract, respectively. The terpenoids was confirmed by Salkowski's Test at R_f value 0.54 and 0.53 in petroleum ether and methanol extract of plant respectively. The steroid was present in chloroform extract of plant at R_f value 0.60. The saponin was confirmed by Froth Test at R_f value 0.80 and 0.80 in petroleum ether and methanol extract respectively. The investigation confirmed presence of important phytochemical in plant extracts which are known for therapeutic value.

The FTIR technique was used to identify functional group present in the extract. The narrow -NH group peak obtained at 3502 cm^{-1}, broad -OH peak at 3400 cm^{-1}, =C-H peak at 2200 cm^{-1}, -C-H peak at 2000 cm^{-1}, C-O peak at 1800 and -CHO peak at 1700 cm^{-1}. The results of IR study confirmed presence of characteristics peaks of Alkaloids, Flavanoids, Terpenoids, Steroids and Saponins.

The HPTLC also performed which

confirmed the presence of Alkaloids, Flavanoids, Phenol, Terpenoids, Steroids and Saponinsin the extract. The spots for Alkaloids, Flavanoids, Phenol, Terpenoids, Steroids and Saponins found in TLC respectively at R_f value 0.45, 0.32, 0.71, 0.53, 0.60 and 0.80. The TLC and HPTLC results were calculated and found similarity between both.

The antibacterial activity of extract (petroleum ether, chloroform and methanolic) of *Clerodendron serratum* performed by a cup-plate technique. The petroleum ether, chloroform and methanolic extract showed positive result against *Staphylococcus aureus* and *Salomonella typhimurium* (Table 2).

Table 2 Anti-bacterial activity of petroleum ether, chloroform and methanol extract of *Clerodendron serratum*

S.No.	Bacteria	*Clerodendron serratum*		
		Petroleum Etherextract	**chloroform Extract**	**Methanol extract**
1	*Staphylococcus aureus*	+ve	+ve	+ve
2	*Salomonellatyphimurium*	+ve	+ve	+ve

The Anti-fungal activity of extract (petroleum ether, chloroform and methanolic) of *Clerodendron serratum* performed through a zone inhibition technique. The petroleum ether extract showed negative result against *A.flavus* while showed positive result against *P. notatum*, *A.niger* and*A.fumigatus*. The chloroform extract showed positive result against *A.flavus* and *P. notatum*, while showed negative result against *A.niger* and *A.fumigatus*. The methanolic extract showed positive result against *A.flavus* and *P. notatum* **(Table 3)**.

Table 3 Anti-fungal activity of petroleum ether, chloroform and methanol extract of *Clerodendron serratum*

S.No.	Fungus	*Clerodendronserratum*		
		Petroleum ether extract	**Chloroform extract**	**Methanol extract**
1	*A.flavus*	-ve	+ve	+ve
2	*P. notatum*	+ve	+ve	+ve
3	*A.niger*	+ve	-ve	-ve
4	*A.fumigatus*	+ve	-ve	-ve

CONCLUSION

The *Clerodendron serratum* is an important medicinal used plant from ancient times. The results of phytochemical analysis suggested that *Clerodendron serratum* possess bioactive phytochemicals of medicinal importance. The phytochemical screening initiated by TLC profiling and chemical test confirmed presence of Alkaloids, Flavanoids, Phenol, Terpenoids, Steroids and Saponins. The pharmacological evaluation of extracts was also performed. The petroleum ether, chloroform and methanolic extract of plant showed potential therapeutic response against bacteria and fungus. The study concluded that the bark of plant *Clerodendron serratum* may be further recommended as potent antimicrobial agents.

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