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Study of Secondary Metabolites and Antibacterial Activity of *Clerodendrum chinense* found in Chhattisgarh Region, India

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Asian Journal of Organic & Medicinal Chemistry

Volume: 7 Year: 2022
Issue: 3 Month: July–September
pp: 270–272
DOI: https://doi.org/10.14233/ajomc.2022.AJOMC-P398

Received: 4 August 2022
Accepted: 20 September 2022
Published: 30 September 2022

ABSTRACT

Clerodendrum chinense a plant native to south Asia is a perennial shrub with triangular ovate shaped leaves. Present study involves the study of secondary metabolites and antibacterial activity of *Clerodendrum chinense*. Phytochemical analysis of plant extract shows the presence of active phyto-constituents like protein, carbohydrate, sterols, alkaloids, tannins, saponins. TLC analysis revealed the presence of β-sitosterol in the extract, which is an important phytosterol. Antibacterial activity shows characteristic inhibition against two different strain *i.e. E. coli* and *Pseudomonas*, which suggests the plant might show promising results in the field of antibiotics.

KEYWORDS

Antimicrobial activity, Secondary metabolites, Sterols, β-Sitosterol, Chhattisgarh.

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INTRODUCTION

Phytochemical constituents of medicinal plant have shown promising results in the advancement of pharmaceutical research. It has been recorded that about 450-500 or more plants growing or available in Indian forest possess therapeutic values [1], therefore necessary to study the biodiversity of plant in particular area and also literate the people of that area for conservation of plants to ensure its availability for future generation [2]. Chhattisgarh state has one of the oldest, richest and most diverse culture tradition related with the use of medicinal plants [3]. *Clerodendrum chinense* (glory bower/ hazar beli) belonging to family Lamiaceae is native to southeast Asia, is now widely used as popular garden plant as well as for some medicinal purposes [4]. The plant is a perennial shrub that grows up to 3 m tall with triangular-ovate shaped leaves and generally used locally in the treatment of arthritis, rheumatism, dropsy, swellings, edema, gout, for general healing purposes and as painkiller [5]. Present study intends to amplify the study of medicinal properties of *Clerodendrum chinense* by analyzing its secondary metabolites and its antibacterial activity.

EXPERIMENTAL

Sample preparation: Leaves of *Clerodendrum chinense* were collected from rural localities of Dantewada district of

India. Leaves were thoroughly washed under running tap water and then with distilled water to remove all the dirt from the surface and shade dried, since some plant constituents are photosensitive [6]. The dried leaves were further incubated at 36 °C for 48 h, which was followed by fine grounded powder by a mixer.

Extraction method: The powdered sample was then subjected to successive cycles of Soxhlet's apparatus using 3 solvents *i.e.* ethanol (99%), petroleum ether, aqueous (water); 200 mL each with 20 g of dried sample. The resulting extract was then filtered and concentrated in hot water bath.

Determination of extractive value: The extract was filtered by using Whatmann filter paper no. 1 and filtrate was dried in hot water bath and weighed [6]. Extractive values in percentage were calculated by using the following formula:

$$\text{Extractive value (\%)} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times 100$$

Phytochemical screening of extract of *C. chinense*

Biuret test for proteins: An extract (3 mL) was taken and equal volume of 5% solution of NaOH and 1% copper sulphate were added. The resulted pink or purple colour confirmed the presence of proteins.

Molisch's test for carbohydrate: To an extract (2 mL), 2-3 drops of alcoholic α-naphthol solution was added, shaken well and then 1 mL conc. H_2SO_4 was added carefully along the sides of the test tube. The formation of the violet ring formed at the junction confirmed the presence of carbohydrate.

Alkaloids (dry extract precipitation test): A solution containing 4 mL methanol, 400 mL of glacial acetic acid, along with a few drops of ammonia was added to the small quantity of dry plant extract. The appearance of the precipitation indicated the presence of alkaloids [7].

Salkowski test for sterols: A solution of 3 mL $CHCl_3$ and conc. H_2SO_4 was added with crude sample, which results in the formation of lower layer of red colour and confirmed the presence of sterols.

Analysis of cardiac glycosides: An extract (0.5 mL) dissolved in 2 mL glacial acetic acid was added to 1 mL of conc. H_2SO_4 followed by the addition of 1-2 drops of 1% $FeCl_3$. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Analysis of anthraquinones: Aqueous ammonia was added to 1 mL of extract (shaken well) and observed for change in colour of aqueous layer. The formation of red, pink and violet for ethanol (99%), petroleum ether and aqueous (water), respectively [8] confirmed the presence of anthraquinones.

Analysis of flavonoids: To a aqueous extract, 2.5 mL of dil. NH_3 was added, followed by a few drops of conc. H_2SO_4. The appearance of yellow colour indicated the presence of flavonoids, which usually disappear on standing.

Analysis of tannins: Few drops of 1% $FeCl_3$ were added to 5 mL of extract filtrate. The appearance of brownish green or blue black colouration confirmed the presence of tannins.

Analysis of saponins (frothing test): About 0.5 mL of extract was diluted in 5 mL of distilled water and the suspension was shaken vigorously for about 15 min. Formation of 1-2 cm thick layer of foam indicated the presence of saponins.

TLC analysis: TLC analysis of extract of medicinal plants from Chhattisgarh region, showed the characteristic spots and R_f value of 0.98, which were comparable to standard β-sitosterol [6]. In present analysis, the silica gel coated plates were used on which the plant extract was applied. The movement of samples was characterized by a retardation factor (R_f):

$$R_f = \frac{\text{Distance moved by the analyte}}{\text{Distance moved by the mobile phase front}}$$

Antibacterial activity: Nutrient agar media (NAM) was prepared by mixing peptone, beef extract, NaCl and agar in distilled water and sterilized in autoclave at 15 lb pressure for 15 min. The sterilized media were poured into petri dishes and wells were created in the media using a borer. About 50 µL of methanolic extract were pour into each wells and tested against two pathogenic bacterial strains (24 h old) *i.e. E. coli* and *Pseudomonas*.

RESULTS AND DISCUSSION

Experimental data revealed that the extractive value of ethanol solvent was the highest followed by aqueous and petroleum ether (Table-1). Preliminary phytochemical analysis of *Clerodendrum chinense* showed the presence of phytoactive constituents like proteins, alkaloid, sterols, tannins, saponins, *etc*. It was observed that flavonoid was absent in ethanol and petroleum ether extract whereas, glycoside was absent only in ethanolic extract (Table-2). The antibacterial activity was observed only in the methanolic extract (*E. coli*: 4.93 mm and. *Pseudomonas*: 4.20 mm). TLC analysis of the extract showed characteristic spot and R_f value of 0.974 which was approx. near and comparable with the standard value of β-sitosterol (0.98).

TABLE-1
EXTRACTIVE VALUES OF AIR DRIED PLANT EXTRACT FROM VARIOUS SOLVENTS

Solvents	Colour and consistency	Extractive value (g)
Aqueous	Dark yellowish brown, viscous	3.12
Ethanol	Dark green, sticky	3.56
Petroleum ether	Dark green, sticky	1.94

TABLE-2
PHYTOCHEMICAL SCREENING OF EXTRACT OF *Clerodendrum chinense* IN DIFFERENT SOLVENTS

Test	Aqueous	Ethanol	Petroleum ether
Protein	+	+	+
Carbohydrate	+	+	–
Alkaloids	+	+	+
Sterols	+	+	+
Glycosides	+	–	+
Anthraquinones	+	+	–
Flavonoids	+	–	–
Tannins	+	+	+
Saponins	+	+	+

Conclusion

Present study reveals the presence of the active phytochemical constituents in *Clerodendrum chinense* and also shows that the plant have some antibacterial properties, which might

show promising results in the field of antibiotics subject to further investigations.

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