



SECONDARY METABOLITES AND ANTIOXIDANT PROPERTIES OF ROOT AND LEAF EXTRACTS OF *CLERODENDRUM INFORTUNATUM* L.

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Abstract: The objective of the present study is to screen the secondary metabolites and antioxidant activity of *Clerodendrum infortunatum* L. of family Verbanaceae. Five different solvents were used to extract the bioactive compounds from the roots and leaves. Phytochemical screening reveals the presence of alkaloid, Phenolic compounds, tannins, saponins, glycosides, flavonoids, steroid, and terpenoids. Antioxidant property was evaluated using 1,1-diphenyl,2-picryl hydrazine (DPPH) radical scavenging activity. The results indicated that both root and leaf extracts exhibited good antioxidant activity.

Key Words: *Clerodendrum infortunatum*, Secondary metabolites, Antioxidant activity.

INTRODUCTION

Naturally occurring substances are of plants, animals and mineral origin. They are organic substances and could be obtained in both primary and secondary metabolic processes and also provide a source of medicine since olden days. Free radicals are the atom or group of atoms associated with an unpaired number of electrons and can be produced when oxygen interacts with certain other molecules. Many a times, free radical level exceeds up to tissue injury level and in that situation, plant-based antioxidant supply plays an important role to reduce the risk of chronic diseases. So, free radical associated disorders, can be prevented if antioxidants are provided in sufficient quantity (Asadujjaman, 2013). Natural products especially plants are the sources of different classes of phytochemicals. Recently, there has been an urge in research on the potential role of antioxidants in the treatment of atherosclerosis, heart failure, liver dysfunction, neurodegenerative disorders, cancer and diabetes mellitus, (Ajitha *et al* 2001). There is a surplus information about plants that have been found to possess strong antioxidant activity. (Badami *et al.* 2003). Keeping this in view the present study was carried out to analyze the preliminary phytochemical screening and antioxidant activity of root and leaves of *Clerodendrum infortunatum*. *C. infortunatum* is a perennial shrub belonging to the family Verbanaceae. The leaf and root are used as antipyretic, acaricide, laxative, vermifuge, anticonvulsant, antidiabetic, and for gravel, malaria, scabies, skin diseases, sores, spasm, scorpion sting, snake bite, and tumors.

MATERIALS AND METHODS

Plant material

Root and leaf of *C. infortunatum* were collected from Melghat, Maharashtra. The plant was identified with the help of floras of Cook, 1967 and Dhore, 1986 and voucher specimen was deposited to Botany Department, Govt. Vidarbha institute of science and humanities, Amravati. The collected materials were washed under running tap water to remove the surface pollutants and air dried under shade. After drying, the plant materials were ground well using mechanical blender into fine powder and stored in airtight containers with proper labeling.

Preparation of extracts

Crude plant extracts were prepared by Soxhlet extraction method (Tiwari *et al*, 2011). About 50 gm of powdered plant material was uniformly packed into a thimble and extracted with 500 ml of different solvents separately. Solvents used were Ethanol, Petroleum ether, Acetone, Ethyl acetate and Water. The process of extraction continued for 24 hours or till the solvent in siphon tube of an extractor became

colorless. After that the extracts were taken in the beakers and kept on hot plate and heated at 30-40°C till all the solvents got evaporated. Dried extracts were kept in refrigerator until used.



Clerodendrum infortunatum L

Preliminary Phytochemical screening

Chemical tests were carried out for above five extracts using standard procedures to identify the phytochemicals (Harborns, 1973)

Qualitative Phytochemical test

The solvent free extract obtain as above was then subjected to qualitative preliminary phytochemical screening for identification of various plants constituents following the standard methods.

Test for Alkaloids

Solvent free extracts, 50 mg was stirred with few ml of dilute HCL and filtered. The filtrate was tested with various alkaloidal reagents as follows:

Mayer's test

Few ml of filtrate and a drop or two of Mayers reagent were added by the side of the test tube. A white or creamy ppt indicates the presence of alkaloids.

Wagner's test

To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish- brown ppt confirms the presence of alkaloids.

Hager's test

To a few ml of filtrate, 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) were added. A prominent yellow ppt indicates the presence of alkaloids.

Test for phenolic compound

Lead acetate test

The extract (50mg) was dissolved in distilled water and to this; 3ml of 10% lead acetate solution was added. A bulky white ppt indicates the presence of phenolic compounds.

Test for Tannins

About (0.5g) of the plant extract was added in 10 ml of water in test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue- black coloration.

Test for proteins

To 2 ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO₄ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for amino acids

To 2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acid in the sample.

Test for reducing sugars

To 2 ml of extract 2 drops of Molisch's reagent was added and shaken well. 2ml of conc. H₂SO₄ was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

Test for glycoside

Each extract was hydrolyzed with HCL and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added to each mixture. Formation of red ppt indicates the presence of glycosides.

Test for Flavonoids

(a) 0.2 g of each extract was dissolved in diluted NaOH and few drops of HCL were added. A yellow solution that turn colorless indicates the presence of flavonoids

(b) To 2 ml of test solution, 0.5ml alcohol was mixed. Then a bit of magnesium and 1 or 2 drops of con. HCL were added and heated. The mixture was analyzed for reaction.

Test for Phenols

To 2 ml of test solution, alcohol and then few drops of neutral ferric chloride solution was added. A dark green color indicated the presence of phenolic compound.

Test for Coumarins

3 ml of 10% NaOH was added to 2 ml of aqueous extract, formation of yellow color indicates the presence of coumarins.

Test for Resins

To the 0.2 g of each extract, 10 ml of glacial acetic acid was added then heated and cooled. A drop of conc. H₂SO₄ was added. Purplish red color shows the presence of resins.

Test for Steroids/ Terpenoids

1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of con. H₂SO₄ was added by the side of the test tube. The upper layer turns red and H₂SO₄ layer showed yellow with green fluorescence indicated the presence of steroids.

Antioxidant activity with DPPH assay

The antioxidant activity of the plant extract, was estimated utilizing 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Blois,1958). Five concentrations (20, 40, 60, 80 and 100ug/ml) of each sample were prepared. 0.1 Mm solution of DPPH in methanol was prepared and 180 µl of this solution was added to 20µl of different plant extracts in 96 well plates and incubated for 30 min at room temperature in the dark. Ascorbic acid was used as a positive control. The DPPH radical-scavenging activity was determined by measuring the absorbance at 490nm and calculated using the equation (Badami and Gupta 2005).

$$I\% = (Ac - As) / Ac \times 100 \dots\dots (1)$$

Where, Ac – absorbance of the control

As – absorbance of the sample

Result and Discussion

The phytochemical characters of the root and leaves of *Clerodendrum infortunatum* were investigated and presented in table–1. The qualitative phytochemical analysis of root and leaves contains alkaloid, Phenolic compounds, tannins, saponins, glycosides, flavonoids, steroid, and terpenoids.

Table 1: Phytochemical analysis of various extracts of *C. infortunatum* (Root and Leaf)

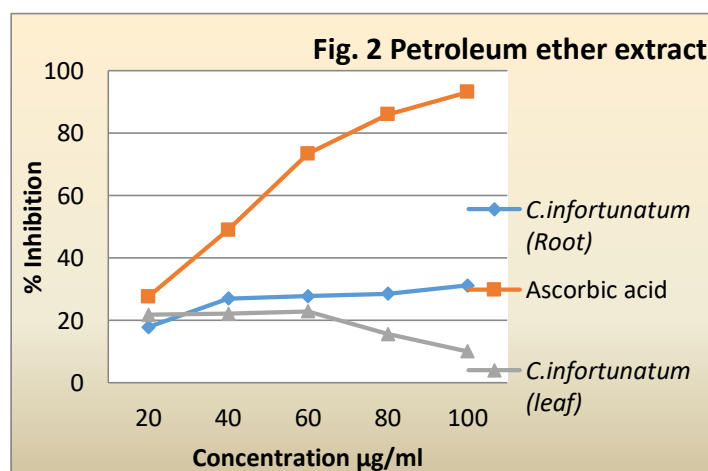
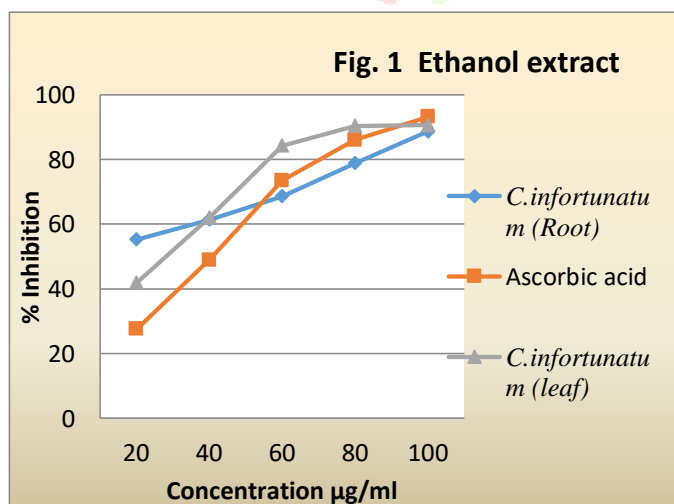
Phytochemical Components	Petroleum ether		Ethyl Acetate		Acetone		Ethanol		Aqueous	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
Alkaloids	-	-	-	+	-	+	-	+	+	+
Mayer's	+	-	+	+	+	+	+	+	+	+
Hager's	+	-	+	+	+	+	+	+	+	+
Wagner's	+	-	-	+	+	+	+	+	+	+
Dragendroff										
Saponins Foam Test	-	-	-	-	-	-	+	-	+	-
Phenolic compounds Lead acetate	+	-	-	-	-	-	+	+	+	+
Tannin Ferric	+	+	-	-	-	-	-	-	+	+
Protein	-	-	-	-	+	+	+	+	-	-
Amino acids	-	-	-	-	-	-	-	-	-	-
Reducing sugar	+	+	-	-	-	-	-	-	-	-
Glycosides	+	+	-	-	-	-	-	-	+	+

Flavonoides	+	-	-	-	+	+	+	+	-	+
Phenols	+	+	+	+	+	+	+	+	+	+
Coumarins	+	+	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	-	-	-	-	-
Steroids/ Terpenoids	+	+	-	-	-	-	-	-	+	+

Antioxidant activity

The antioxidant activity of root and leaves of *Clerodendrum infortunatum* were evaluated by measuring reducing ability free radical scavenging activity with various extracts (Ethanol, Petroleum ether, Acetone, Ethyl acetate and Water) by using DPPH assay and results are depicted in Figure 1 to 5. Ascorbic acid was used as standard control (Figure -6).

The DPPH scavenging activity of different extracts of *C. infortunatum* root and leaf was studied and ethanol extract was found to be more active in almost all concentrations than the other solvent extracts in case of root as well as leaf of *C.infortunatum*. In case of leaf extract highest antioxidant activity of 90.65% was observed at 100 µg/ml concentration. Results reveals that ethanol extract of *C.infortunatum* (root) showed good antioxidant activity which is comparable with ascorbic acid (93.13%). This can also be explained by comparing their IC₅₀ values as higher DPPH radical scavenging activity is associated with lower IC₅₀ value. The IC₅₀ values for DPPH radical scavenging assay showed significant variation from 11.44µg/ml to 277.57 µg/ml. Modi *et al.*, (2010) have revealed that *C. infortunatum* leaf extract has significant antioxidant activity and are encouraging for further assessment to elucidate the mechanism of action and to identify bioactive compounds implicated in the antioxidant effect. According to Prakash *et al.*, (2011) *C. infortunatum* exhibited good antioxidant activity with IC₅₀ 50.90 µg/ml. Rahman *et al.*, (2011) showed that *C.viscosum* extract exhibited moderate antioxidant property. DPPH(1,1 -diphenyl-2-picryl-hydrazyl) is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant (Modi *et al.*,2010). In many *Clerodendrum* species similar antioxidant activity has been reported earlier such as in *C. serratum* (Ismail and Leelavathi, 2011); methanolic extract of roots from *C. viscosum* (Pankaj *et al.*, 2007); methanolic extract of leaves from *C. inermiae* (Gurudeeban *et al.*, 2010); acetone extract of bark of *C. phlomidis* (Bhatnagar and Pattanaik, 2012) and ethanolic extract of *C. phlomidis* capable of better free radical scavenging, (Satish *et al.*, 2011) which is similar as reported in our study. Overall study highlighted that plant *C. infortunatum* is proving to be the better candidate for antioxidant activity and could be proposed as better antioxidant agent for future drugs. Presence of Phenolic comp is responsible for the antioxidant activity was reported by Bhatnagar and Patnaik, 2012; Ogunwa *et al.*, 2016 and Hutke and Tayde, 2019. Same could be true for present study. The results of the present study showed that the ethanol extract of the leaves and root of *C.infortunatum* possess promising antioxidant activity.



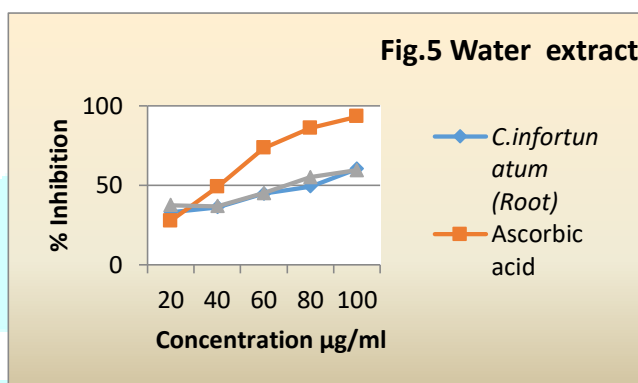
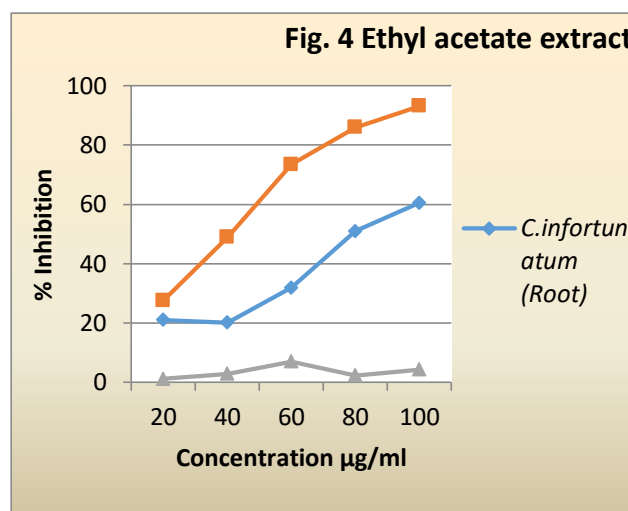
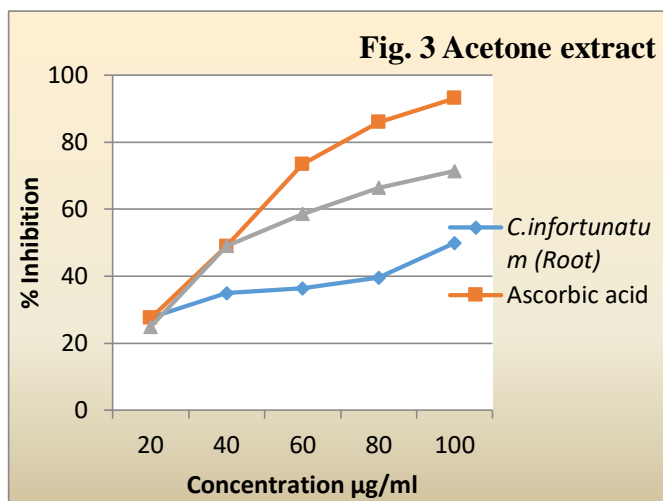


Fig. 1 - 5 DPPH radical scavenging activity (% inhibition) of *C.infortunatum* (Root and Leaf)

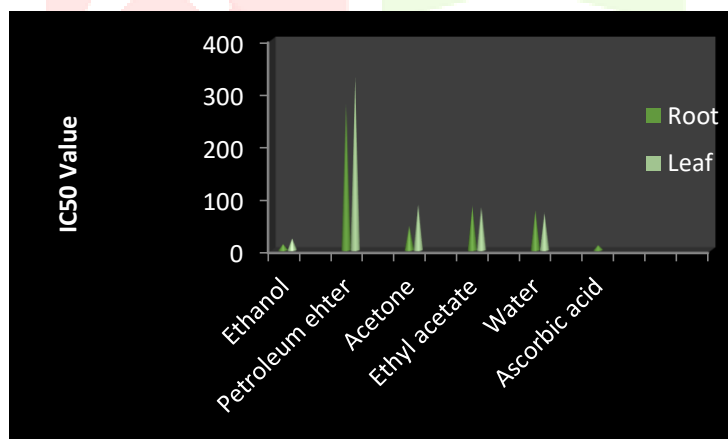


Fig . 6 DPPH radical scavenging activity (IC50) of Root and stem of *C.infortunatum*

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