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Original Research Paper

PHYTOCHEMICAL AND ANTIOXIDANT SCREENING OF VARIOUS EXTRACTS OF THE ROOT BARK AND LEAF OF *CLERODENDRUM INFORTUNATUM* LINN

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ABSTRACT

Clerodendrum infortunatum is a traditional Indian medicinal plant that has been documented in the traditional systems of medicines like Ayurveda, Yunani etc for the treatment of many diseases. The medicinal properties of plants are attributed to the various phytoconstituents synthesized during the plant secondary metabolism. In the present study, attempts were made to investigate the phytoconstituents present in petroleum ether, chloroform, acetone, ethanol and methanol extracts of the root bark and leaf. All the extracts were found to contain bioactive compounds. Since these compounds are of pharmacological importance, all the extracts were then subjected to *in vitro* antioxidant screening by the evaluation of total antioxidant activity, free radical scavenging activity and reducing power. All the extracts of the root bark and leaf exhibited significant antioxidant activity and radical scavenging activity. The reducing power increased with increasing concentration. Among various extracts, acetone extract exhibited the highest activity. Phytochemical screening showed phenols and tannins as the major components in the acetone extract of both root bark and leaf and thus these compounds may be responsible for the greater activity of the acetone extract.

Keywords: *Clerodendrum infortunatum*, Phytochemicals, Antioxidants, Reducing power, DPPH radical, Phenolics, Tannins.

INTRODUCTION

Plant biodiversity forms the most common source of medicines. Today, there has been a shift towards the traditional herbal medicines and a large number of drugs are derived from the natural sources either directly or indirectly. The medicinal properties offered by the plants are due to compounds synthesized in their secondary metabolism (Sukanya *et al.*, 2009) which includes flavonoids, alkaloids, tannins, steroids, glycosides, phenols, fixed oils, saponins etc. and are stored in the specific parts of plants such as leaf, bark, flower, seed, fruit and root (Sharma *et al.*, 2012). These secondary metabolites and their derivatives exhibited significant biological and pharmacological properties. They act as antioxidants, free radical scavengers, antiproliferative agents and defend the plant against microorganisms (Kennedy and Wightman, 2011). Free radicals have been implicated in the etiology of diseases such as cancer, coronary heart diseases, neurodegenerative diseases, inflammation, ageing processes etc. In the living system, oxygen consumption leads to the generation of free radicals and reactive oxygen species. Antioxidants react with free radicals and protect the body from the damaging oxidation

reactions. They reduce the oxidative damage to cellular components such as lipids, proteins, enzymes and DNA and retard the process of chronic illness and lipid peroxidation (Mandal *et al.*, 2009). A variety of natural and synthetic antioxidants are in use today. Synthetic antioxidants were reported to be responsible for liver damage and carcinogenesis (Wichi, 1986; Grice, 1988). Natural antioxidants were presumed to be safe. Medicinal plants were reported to be an important source of natural antioxidants (Rice-Evans, 2004). Therefore there is an upsurge of interest in exploring the active constituents in medicinal plants and the properties exhibited by these compounds.

Clerodendrum infortunatum is a traditional Indian medicinal plant, belongs to the family Verbanaceae. It is widely distributed throughout the plains of India. Clerodendrum is a wide and diverse genus, which has been documented in various health care systems for the treatment of diseases. The major phytoconstituents reported from the genus includes phenolics, steroids, di and tri-terpenes, flavonoids, volatile oils etc (Gouthamachandra *et al.*, 2010). *Clerodendrum infortunatum* (Bhant) has been reported to be useful in the treatment of vitiated conditions of Kapha, helminthiasis, tumors, skin diseases, bronchitis, inflammation, malarial fevers and intermittent fevers (Varier, 1994). So the plant may contain bioactive compounds, which may be a source of raw material for the drug industry. Hence in the present study, an attempt was made to investigate the phytoconstituents present in the petroleum ether, chloroform, acetone, ethanol and methanol extracts of the root bark and leaf. The antioxidant efficacy of the various extracts were also determined in terms of total antioxidant activity, reducing power and 1,1- Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity.

MATERIALS AND METHODS

Collection of Plant

The root bark and leaf of *Clerodendrum infortunatum* were collected from the Idukki District of Kerala during the month of April, 2013. The root bark and leaf were washed properly, air dried in shade, chopped and powdered in a kitchen blender. The powdered plant material was stored in air tight containers until further analysis.

Preparation of Plant Extracts

About 40 g of powdered leaf and root bark were extracted separately and sequentially with petroleum ether, chloroform, acetone, ethanol and methanol using soxhlet apparatus for 72 hrs. The extracts were filtered and concentrated to dryness under reduced pressure in a rotary evaporator. Percentage yield of various extracts were determined and the extracts were stored in sterile bottles at 4 ºC for further studies.

Phytochemical Screening

Preliminary phytochemical screening of the various extracts of the root bark and leaf was carried out for the detection of alkaloids, flavonoids, steroids, diterpenes, glycosides, saponins, tannins and phenols using standard protocols (Harborne, 1973; Trease and Evans, 1989; Tiwari *et al*., 2011).

In Vitro Antioxidant Screening

Reducing Power

The reducing power of the various extracts was determined by the method of Oyaizu (1986). 2.5 ml of various extracts of the root bark and leaf (100-1000 µg) was mixed with 2.5 ml phosphate buffer (0.2M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was kept in a water bath at 50 ºC for 20 minutes. After cooling, 2.5 ml of trichloroacetic acid (10%) was added and centrifuged at 3000 rpm for 10 minutes, whenever necessary. The upper layer of solution (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml of freshly prepared ferric chloride solution (0.1%). The absorbance was read at 700 nm in a UV-Vis spectrophotometer (Hitachi U-500). Ascorbic acid at various concentrations (10-100 µg) was used as standard. Increased absorbance of the reaction mixture indicated increase in reducing power.

Free Radical Scavenging Activity

The free radical scavenging potential of different extracts was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) by the modified method

of Blois, (1958). 2 ml reaction mixture containing 1 ml methanolic solution of DPPH (0.1mM) and 1 ml of plant extracts at various concentrations (1000-1.95 µg) was incubated in dark at 37 ºC for 30 minutes. 1ml of DMSO serves as the control. Ascorbic acid (1000-1.95 µg) was used as the standard. After incubation the absorbance was read at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and can be calculated using the formula

$$\% \text{ inhibition of DPPH radical} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

IC_{50} value was calculated using the graph by plotting inhibition percentage against extract concentration.

Total Antioxidant Activity

The total antioxidant activity of the different extracts of *Clerodendrum infortunatum* was determined by the phosphomolybdenum method described by Prieto*et al*., (1999). 0.1ml of various extracts (100µg) was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95 ºC for 90 minutes. After cooling the samples to room temperature, the absorbance was measured at 695 nm against a blank in a UV-Vis spectrophotometer (Hitachi U-500). 0.1 ml of ascorbic acid (5-30 µg) serves as the standard. Methanol in the place of extract is used as the blank. The antioxidant activity was expressed as mg ascorbic acid equivalents per gram of sample on a dry weight basis.

RESULTS

The present study revealed the presence of medicinally active constituents in the root bark and leaf of *Clerodendrum infortunatum*. The phytochemical characteristics of the various extracts were summarised in tables 1 and 2. The results revealed the presence of alkaloids, phenolics, flavonoids, tannins, steroids and saponins in different extracts of both the root bark and leaf.

Reducing Power

The reducing power has significant correlation with the antioxidant activity. The concentration of Fe^{2+} formed by the reduction of fe^{3+}/ferricyanide complex was monitored by measuring the formation of Prussian blue at 700 nm. Reducing power of all the extracts of root bark and leaf increased linearly with increasing concentration (figure1 and 2). The results were comparable with the standard antioxidant, ascorbic acid. Among the various extracts, the acetone extract of both the root bark and leaf showed highest reducing ability.

Free Radical Scavenging Activity

The degree of discolouration of DPPH by its reduction indicated the radical scavenging activity of the antioxidant. The DPPH scavenging activity of all the extracts increased steadily with increase in concentration and then it remains almost stable. Table 3 gives the IC_{50} values of various extracts and the standard antioxidant, ascorbic acid. The lowest IC_{50} values of the acetone extract points to its greater scavenging activity.

Total Antioxidant Activity

Total antioxidant activity of the root bark and leaf was given in figure 3 and 4. All the extracts exhibited significant antioxidant activity. Highest antioxidant activity was noted in the acetone and methanol extract of the root bark and leaf. The antioxidant activity of petroleum ether, chloroform and ethanol extracts of root bark and leaf were almost comparable.

DISCUSSION

Plant derived drugs are of great importance today. The phytochemical analysis of the plant extracts revealed the presence of alkaloids, steroids, terpenoids, phenolics, flavonoids, tannins and saponins and these compounds are known to exhibit the physiological activities and medicinal properties of plants. Flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart diseases (Kahkonen *et al*., 1999). Steroids are reported to possess antimicrobial activity. Tannins have astringent properties, it hastens the healing of wounds and inflamed mucous membranes. Characteristics of saponins include the precipitation and coagulation of red blood cells (Doss, 2009). These results proved

Clerodendrum infortunatum to be a valuable reservoir of potential drugs. Reactive oxygen species produced in the tissues are closely linked to malignant diseases like cancer, inflammation, cardiovascular diseases etc. Antioxidants delay the process of oxidative stress and protect the cells from lipid peroxidation. Medicinal plants are an important source of natural antioxidants. Reducing power of a compound may serve as an indicator of its antioxidant activity (Meir *et al.*, 1995). Compounds with reducing power indicated that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes. The reduction of potassium ferricyanide by the various extracts of the root bark and leaf of *Clerodendrum infortunatum* showed their hydrogen donating ability. The increase in the intensity of colour indicated increasing reducing potential. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. It is widely used as a substrate to evaluate the antioxidant activity. The ability of the extracts to decolourize the DPPH radical points to their radical scavenging activity. The results revealed that various extracts of the root bark and leaf of *Clerodendrum infortunatum* possess hydrogen donating ability to act as an antioxidant. IC_{50} value is the effective concentration at which the antioxidant activity is 50%. Smaller IC_{50} value indicated the greater antioxidant capacity. The acetone extracts of root bark and leaf exhibited higher scavenging activity compared to the other extracts (IC_{50} value is 14.84 and 15.63 µg/ml respectively).

The total antioxidant activity was evaluated on the basis of the reduction of Mo (VI) to Mo (V) by the extract and the subsequent formation of green phosphate/Mo (V) complex. All the extracts of the root bark and leaf exhibited significant antioxidant activity.

Antioxidant screening assays revealed that all the extracts of the root bark and leaf exhibited significant antioxidant activity. Among the various extracts, acetone extract of the root bark and leaf exhibited highest reducing capacity at all concentrations. These results were consistent with total antioxidant activity and DPPH radical scavenging activity. From the phytochemical analysis phenolics and tannins were the major components of acetone extract of both root bark and leaf. So these may contribute to the free radical scavenging and antioxidant activities exhibited by the plant extract.

CONCLUSION

The present study demonstrated the antioxidant capability of various extracts of the root bark and leaf of *Clerodendrum infortunatum*. This may be due to the presence of phytoconstituents present in the plant. Tannins and phenols present in the acetone extract may contribute to the greater antioxidant activity of the acetone extract. Therefore further studies on phenolics and tannins from *Clerodendrum infortunatum* will be beneficial to the pharmacological industry.

Table 1: Preliminary phytochemical analysis of the root bark of *Clerodendrum infortunatum*

Phytoconstituents	Test	PECI	CECI	AECI	EECI	MECI
Alkaloids	Meyer's test	+ + +	+ +	-	-	-
	Wagner's test	+ + +	+ +	-	-	-
	Dragendroff's test	+ + +	+ +	-	-	-
Flavonoids	Shinoda test	-	+	+	+ +	+ + +
	Alkaline reagent test	-	+	+	+ +	+ + +
	Lead acetate test	-	+	+	+ +	+ + +
Steroids	Salkowski's test	+	+	+	-	-
	Acetic acid test	+	+	+	-	-

Diterpenes	Copper acetate test	+	+	+	-	-
Glycosides	Keller killiani test	+	+	-	-	-
	Baljet's test	+	+	-	-	-
Saponins	Foam test	-	+	+	+	+ +
Tannins	Gelatin test	-	+	+ + +	+	+ +
	Potassium hydroxide test	-	+	+ + +	+	+ +
Phenols	Ferric chloride test	-	+	+ +	+ +	+ +

Faintly: (+); Moderately: (++); Highly: (+++); Absent: (-)

PECI- Petroleum ether extract of Clerodendrum infortunatum, CECI- chloroform extract, AECI- acetone extract, EECI- ethanol extract, MECI- methanol extract

Table 2: Preliminary phytochemical analysis of the leaf of *Clerodendrum infortunatum*

Phytoconstituents	Test	PECI	CECI	AECI	EECI	MECI
Alkaloids	Meyer's test	+	+	-	-	-
	Wagner's test	+	+	-	-	-
	Dragendroff's test	+	+	-	-	-
Flavonoids	Shinoda test	-	+	+	+ +	+ + +
	Alkaline reagent test	-	+	+	+ +	+ + +
	Lead acetate test	-	+	+	+ +	+ + +
Steroids	Salkowski's test	+	+ +	-	-	-
	Acetic acid test	+	+ +	-	-	-
Diterpenes	Copper acetate test	+	+	-	-	-
Glycosides	Keller killiani test	-	-	-	-	-
	Baljet's test	-	-	-	-	-
Saponins	Foam test	-	-	+	+ +	+ +
Tannins	Gelatin test	-	-	+ + +	-	+
	Potassium hydroxide test	-	-	+ + +	-	+
Phenols	Ferric chloride test	-	+	+ +	+ +	+ +

Faintly: (+); Moderately: (++); Highly: (+++); Absent: (-)

PECI- Petroleum ether extract of Clerodendrum infortunatum, CECI- chloroform extract, AECI- acetone extract, EECI- ethanol extract, MECI- methanol extract

Table 3: IC_{50} values of different extracts of *Clerodendrum infortunatum* for DPPH radical scavenging assay

Plant part	Extract	IC_{50} (µg/ml)
Standard	Ascorbic acid	13.28
Leaf	Petroleum ether extract	21.88
	Chloroform extract	23.44
	Acetone extract	15.63
	Ethanol extract	18.76
	Methanol extract	20.3
Root bark	Petroleum ether extract	15.65
	Chloroform extract	28.13
	Acetone extract	14.84
	Ethanol extract	21.88
	Methanol extract	20.32

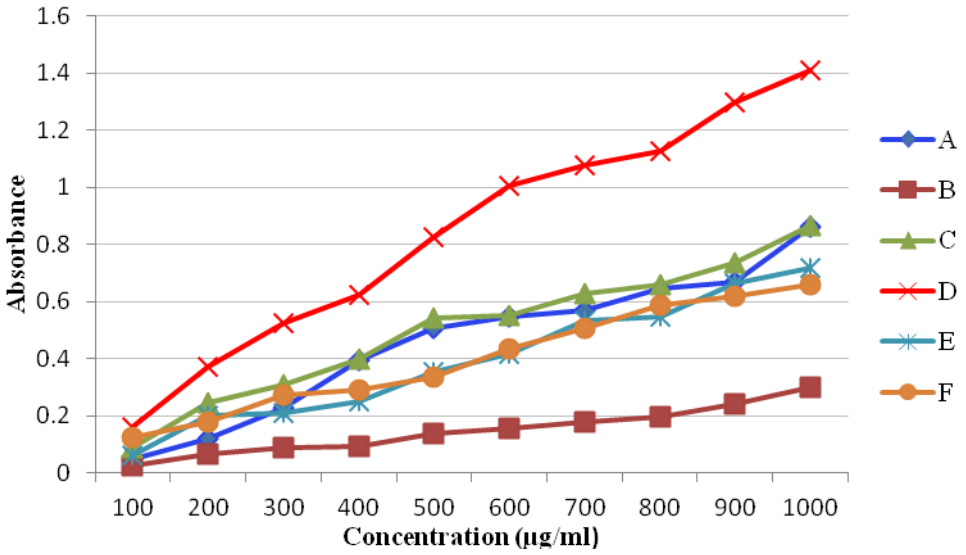

Figure 1: Reducing power of various extracts of the root bark of *Clerodendrum infortunatum* and ascorbic acid

Ascorbic acid is diluted 1:10. Values are the mean, where n=4.

A- Ascorbic acid, B- Petroleum ether extract, C- Chloroform extract, D- Acetone extract, E- Ethanol extract, F- Methanol extract.

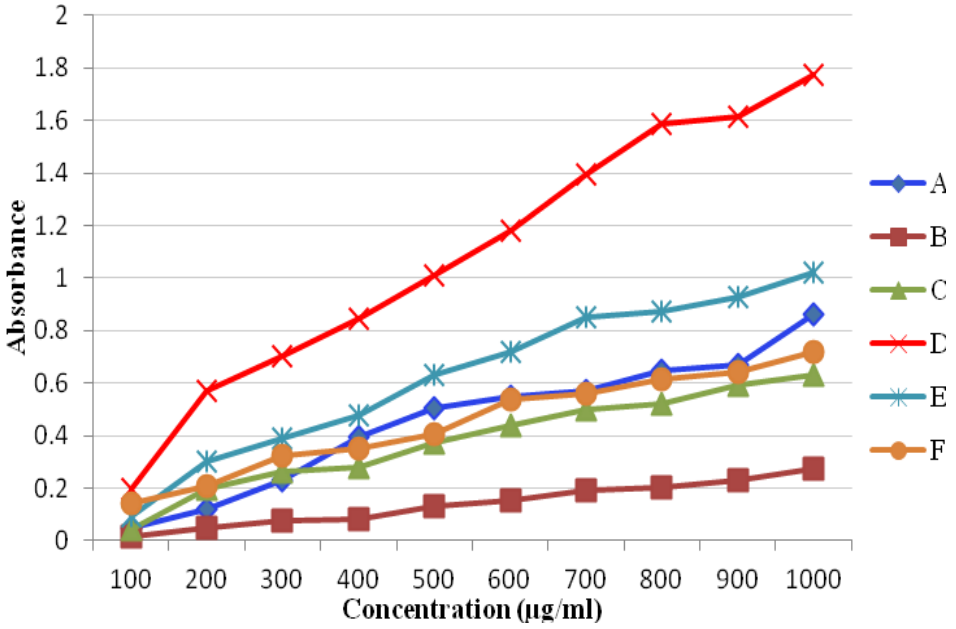

Figure 2: Reducing power of various extracts of the leaf of *Clerodendrum infortunatum* and ascorbic acid

Ascorbic acid is diluted 1:10. Values are the mean, where n=4.

A- Ascorbic acid, B- Petroleum ether extract, C- Chloroform extract, D- Acetone extract, E- Ethanol extract, F- Methanol extract

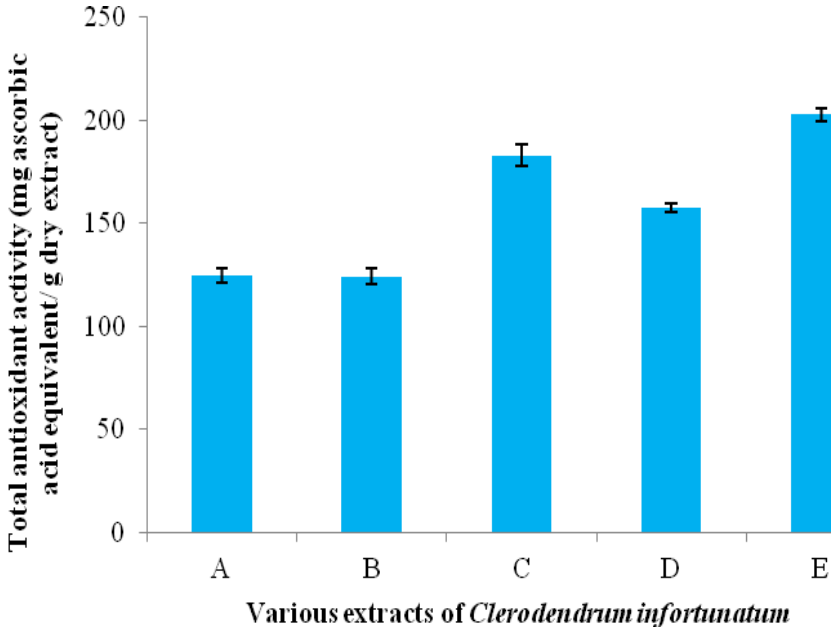

Figure 3: Total antioxidant activity of various extracts of the root bark of *Clerodendrum infortunatum*

Values are the mean ± SD (n=4).

A- Petroleum ether extract, B- Chloroform extract, C- Acetone extract, D- Ethanol extract, E- Methanol extract.

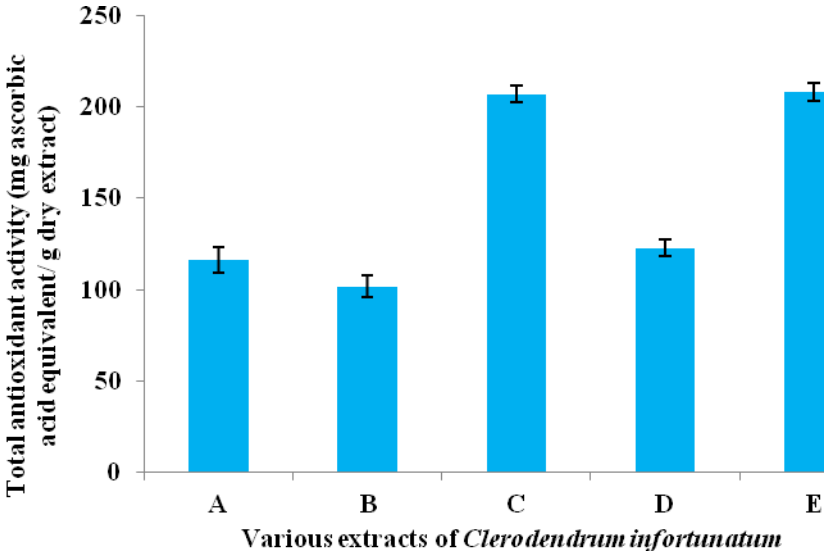

Figure 4: Total antioxidant activity of various extracts of the leaf of *Clerodendrum infortunatum*

Values are the mean ± SD (n=4).

A- Petroleum ether extract, B- Chloroform extract, C- Acetone extract, D- Ethanol extract, E- Methanol extract.

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