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Preliminary Phytochemical Analysis and DPPH Free Radical Scavenging Activity of *Trewia nudiflora* Linn. Roots and Leaves

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Abstract: Oxidative stress is one of the major causative factors of many chronic and degenerative diseases. Plants have been used in traditional medicine in different parts of world for thousands of years and continue to provide new remedies for human kind. The present study was to investigate the preliminary phytochemical analysis of various extracts of roots and leaves of *Trewia nudiflora* (Euphorbiaceae) and antioxidant activity by 1, 1, diphenyl-2-picryl hydrazyl (DPPH) radical scavenging method. The preliminary phytochemical screening showed the presence of several phytochemicals including alkaloids, glycosides, flavonoids, steroids, phenolic compounds and tannins. The ethanol and aqueous extracts of roots and leaves of *Trewia nudiflora* showed significant antioxidant activity compared to standard drug ascorbic acid.

Key words: Phytochemical analysis, total phenol, total flavonoid, DPPH, *Trewia nudiflora*

INTRODUCTION

Oxidation is essential biological process of many living organisms for the production of energy. The ROS are formed during metabolism and they may contribute to pathogenesis of many diseases such as diabetes, cancer and cardiovascular diseases (Zovko Koncic *et al*., 2010) as well as ROS are believed to damage cell membranes and DNA (Finkel and Holbrook, 2000). The phenolic classes (simple phenolics, flavonoids, phenolic acids and anthocyanins) of phytochemical have the structural requirements of free radical scavengers as antioxidant property (Sun *et al*., 2002). Hence, now days more attention has been paid to find out natural non toxic antioxidants to protect the human body from free radicals.

The plant *Trewia nudiflora* (Euphorbiaceae) is a small sized tree grows up to 5 m in height. Leaves are simple, cordate, acuminate, both surface pubescent and long petiolate. Flowers are arising from axilla or from terminal spikes. Fruits are hard, greenish yellow pods, which is staple food of Indian Rhinoceros. Trewia nudiflora is found in various parts of India. Root contains resinous matter and fat, while root decoction used as stomachic and alterative in flatulence, gout, rheumatism (Rathore *et al*., 2007) and malignancy especially leukemia and hepato-biliary affections. A decoction of shoots and leaves of Trewia nudiflora is used as traditional medicine to relieve swelling and to treat flatulence, excessive bile and sputum. The leaves are applied on wounds to heal them with good efficiency (Nadkarni and Nadkarni, 2002; Ram *et al*., 2004). The plant contains a pyridine alkaloid, N-methyl-5-carboxamide-2-pyridone, nudiflorine. Bark yields taraxerone and betasitosterol. Seeds are reported to contain ricinidine and maytansinoid compound trewiasine (Balakrishnan *et al*., 2012). The antioxidant activity of *Trewia nudiflora* has not been reported yet. Therefore, the present study was designed for the preliminary phytochemical screening of various extracts of roots and leaves of *Trewia nudiflora* and DPPH free radical scavenging activity of ethanol and aqueous extracts of this medicinal plant.

MATERIALS AND METHODS

Plant material and extraction: The roots and leaves of *Trewia nudiflora* were collected from the local area of Kerakat, Jaunpur District, Uttar Pradesh, India month of November 2012 and authenticated at Department of Botany, Safia College, Bhopal, Madhya Pradesh. The voucher specimen (435/Bot/Saifia/13) has been preserved in our laboratory for further collection and reference. The roots and leaves were dried under shade, powdered with a mechanical grinder and passed through a 40-mesh sieve. The successive solvent cold extraction method used to obtain various extracts including petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts.

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The solvents were removed from the extracts under reduced pressure by using a rotary vacuum evaporator (Buchi model, Jyoti Lab, Gwalior, India). The percentage of yield of extracts was noted.

Chemicals: DPPH (1, 1, diphenyl-2- picryl hydrazyl) was obtained from Himedia, Mumbai, India and ascorbic acid was purchased from Merk, Mumbai, India. All other chemicals and solvents used were of the highest purity and analytical grade.

Phytochemical analysis: The extracts were subjected to tests to identify the presence of various phytoconstituents, i.e. alkaloids (Dragendorff's test), steroids and terpenoids (Liebermann Burchard test), tannin and phenolic compounds (ferric chloride test), flavonoids (Shinoda test), amino acids (Ninhydrin test), etc., by the usual methods prescribed in standard texts (Harborne, 1998).

Determination of total phenol: Total phenolic contents in the ethanol and aqueous extracts were determined by the using the Folin-Ciocalteu method (McDonald *et al*., 2001). A dilute solution of plant extracts (0.5 mL of 1:10 g m L^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 mL, 1:10 diluted with distilled water) and aqueous sodium carbonate (4 mL, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by UV-VIS spectrometer (Pharmaspec 1700, Shimadzu) at 765 nm and the content expressed in terms of milligram gallic acid per gram of dry extract.

Determination of total flavonoid: Aluminum chloride colorimetric method was used for flavonoids determination (Chang *et al*., 2002). Plant extracts (0.5 mL of 1:10 g m L^{-1}) in methanol were separately mixed with 1.5 mL of 1M potassium acetate and 2.8 mL of distilled water. It was maintained at room temperature for 30 min and then the absorbance of the mixture was measured at 415 nm with UV-VIS spectrometer (Pharmaspec 1700, Shimadzu). The total flavonoids content was expressed in terms of milligram catechin per gram of dry extract.

Determination of DPPH radical scavenging activity: The free radical scavenging activity of ethanol and aqueous extracts of roots and leaves of *Trewia nudiflora* and ascorbic acid were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Bhaskar and Balakrishnan, 2009). DPPH solution (0.1 mM) in ethanol was prepared and 1 mL of this solution was added to 3 mL of extract solution in water at different concentrations (100-1000 μg mL^{-1}). After 35 min, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenged (\%)} = \frac{A_{cont}-A_{test}}{A_{CONT}} \times 100$$

where, A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

Statistical analysis: The amount of extracts needed to inhibit free radicals concentration by 50% (IC_{50}) was graphically estimated using linear regression lines (Bhaskar and Balakrishnan, 2009).

RESULTS AND DISCUSSION

Phytochemical studies of various extracts showed the presence of several phytochemicals including alkaloids, glycosides, flavonoids, steroids, phenolic compounds and tannins (Table 1). The percentage yield of aqueous and ethanol extracts of roots and leaves were found to be more than the other extracts (Table 2). Results obtained in the present study revealed that the levels of these phenolic compounds in the aqueous and ethanol extracts of roots and leaves of *Trewia nudiflora* were considerable (Table 3). DPPH is frequently used to determine radical scavenging activity of natural compounds and its radical form absorbs at 517 nm due to the antioxidant activity, the absorption decreases may be the formation of its non radical form such as DPPH–H (Blois, 1958). Polyphenol are the major plant compounds

Table 1: Preliminary phytochemical screening of various extracts of roots and leaves of *Trewia nudiflora* linn

Phytoconstituents/	Petroleum ether		Chloroform		Ethyl acetate		Ethanol		Aqueous	
extracts	Root	Leaves	Root	Leaves	Root	Leaves	Root	Leaves	Root	Leaves
Alkaloids	-	-	+	+	-	-	+	+	-	-
Glycosides	-	-	+	+	+	+	+	+	+	+
Flavonoids	-	-	+	+	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	+	+	-	-
Phenolic and tannins	-	-	-	-	-	-	+	+	+	+
Fixed oils	-	+	-	-	-	-	-	-	-	-

+: Positive, -: Negative

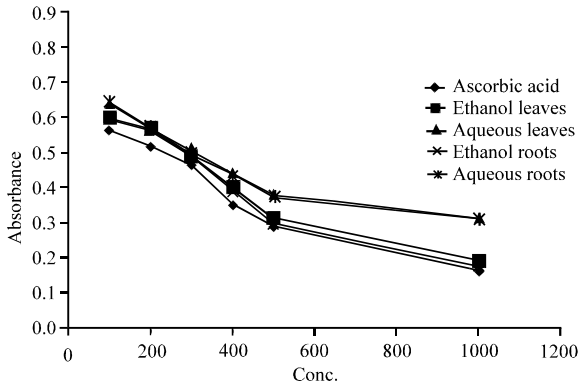

Fig. 1: DPPH free radical scavenging activity of ethanol and aqueous extracts of roots and leaves of *Trewia nudiflora* (conc. vs. absorbance)

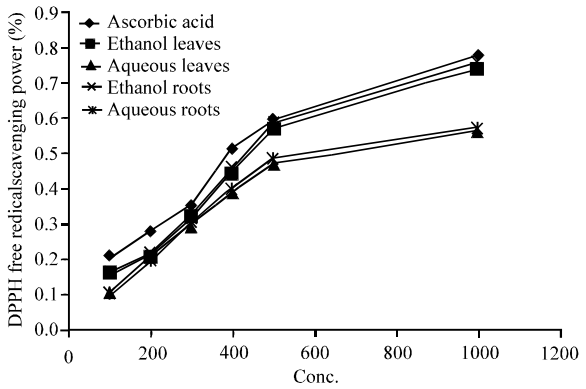

Fig. 2: DPPH free radical scavenging activity of ethanol and aqueous extracts of roots and leaves of *Trewia nudiflora* (Concentrations vs. % DPPH free radical scavenging activity)

Table 2: Percentage yield of various extracts of roots and leaves of *Trewia nudiflora* Linn

	Percentage yield of extracts (w/w)	
Extracts	Root	Leaves
Petroleum ether	0.06	1.63
Chloroform	0.1	1.66
Ethyl acetate	0.03	0.36
Ethanol	0.16	0.76
Distilled water	1.66	3.26

Table 3: Total phenolic and flavonoid content of ethanol and aqueous extracts of roots and leaves of *Trewia nudiflora* Linn

	Total phenolic content[a]		Total flavonoid content[b]	
Extract	Roots	Leaves	Roots	Leaves
Ethanol	8.53±0.1201	7.89±0.1345	4.56±0.1421	6.53±0.1201
Aqueous	10.72±0.1430	9.73±0.1390	7.03±0.1531	10.02±0.0650

Values represent Mean±SEM (n = 3), [a]Expressed as mg of gallic acid equivalents g^{-1} of dry plant extract, [b]Expressed as mg of catechin equivalents g^{-1} of dry plant extract

with high level of antioxidant activity due to their ability to absorb, neutralize and to quench free radicals as well as their redox properties presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Bhaskar and Balakrishnan, 2009). Hence, the radical scavenging activity in the presence of a hydrogen donating antioxidant can be monitored as a decrease in absorbance of DPPH solution (Fig. 1). The DPPH free radical scavenging activity of the ethanol and aqueous extracts of roots and leaves of *Trewia nudiflora* and ascorbic acid showed at different concentrations (Fig. 2). The investigated extracts demonstrated that the DPPH free radical scavenging activity and IC_{50} value of ethanol and aqueous extracts of roots were found to be 561.80 and 704.23 μg mL^{-1}, while IC_{50} value of ethanol and aqueous extracts of leaves were found to be 581.80 and 714.29 μg mL^{-1}, respectively. The IC_{50} value of ascorbic acid was found to be 537.63 μg mL^{-1}. These studies suggest that *Trewia nudiflora* has potential to develop a new antioxidant agent to use to treat various oxidative stress related diseases.

CONCLUSION

In present study, antioxidant activities of the ethanol and aqueous extracts of roots and leaves of *Trewia nudiflora* were investigated. Both the extracts of roots and leaves were found to possess antioxidant activity as determined by scavenging effect on the DPPH free radical. The antioxidant activity correlated well with the content of main plant antioxidants such as phenolic and flavonoid compounds, which suggests an important role of these extracts in overall antioxidant activity of investigated plant organs. Further study is necessary for isolation and characterization of the active antioxidants.

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