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Phytochemical Investigation and Antioxidant Properties of leaves of *Daemia extensa* Containing Phenolic and Flavanoid Compounds

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ABSTRACT

To evaluate the in-vitro free radical scavenging activity of leaves Daemia extensa Petroleum ether, ethanol, aqueous extracts of Daemia extensa were prepared, with successive extraction in soxhlet apparatus. Each extract was selected to study the free radical scavenging activity by superoxide scavenging assay method. It was found that aqueous extract contained carbohydrates, glycosides amino acids flavonoids, tannins, alkaloids, steroids; ethanolic extract contained glycosides amino acids flavonoids, tannins, alkaloids, steroids. Ethanolic extract of Daemia extensa has showed 70.8±0.69 % inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity (67.8±0.58 % compared to ethanolic extract), while Petroleum ether extract showed poor inhibition of superoxide scavenging activity. All extracts showed dose and time dependent inhibition of superoxide scavenging activity. Daemia extensa had the highest total phenolic content (42.60 mg TAE (tannic acid equivalent)/ 100 g fresh weight). Total phenolic content had positive correlation with antioxidant capacity. This shows that the plants, especially Daemia extensa, may be potent source of natural antioxidants.

Key Words: Antioxidant activity, DPPH, *Daemia extensa*, Phenolic Ccontent, Superoxide scavenging, Flavanoid content

Abbreviation- DPPH; *diphenyl Picrylhydrazyl hydrate,* ***TAE;*** *tannic acid equivalent,* ***EDTA;*** *ethylene diamine tetra acetic acid.*

INTRODUCTION

Daemia extensa is a perennial twining herb, foul-smelling when bruised; Stems bears milky juice and covered with longer stiff erect hairs 1mm; Leaves are thin, broadly ovate and heart-shaped 2-12 cm long, covered with soft hairs; Greenish yellow or dull white, sweet-scented flowers born in axillary, double white corona at the base of a stamina column, long-peduncled, umbellate or corymbose clusters tinged with purple; Fruits paired with follicles 5.8 cm long and 1 cm in diameter, reflexed, beak long, covered with soft spinous outgrowth and release many seeds with long white hairs when they split open. Seeds are densely velvety on both sides. The entire plant constitutes the drug and is used as a medicine.

Literature reveals that, the carbonyl groups are responsible for free radical scavenging activity [1]. Free radicals are atoms or groups of atoms with an odd number of electrons and can be formed when oxygen interacts with certain molecules. To prevent free radical damage, the body has a defense system of antioxidants [2, 3]. Antioxidants are able to give free radicals, which becomes a companion to their unpaired electron, thus eliminating the threat of gene

alteration leading to cancer. Medicinal plants have attracted attention of not only professionals from various systems of medicines, but also the scientific community belonging to different disciplines [4, 5]. In recent years, these have been a great interest in herbal remedies for the treatment of number of ailments. Plants are promising source of drugs. In continuation of search in potential free radical scavenging agents [6], the present investigation was aimed to determine free radical scavenging activity of *Daemia extensa* Leaves. Free radical scavenging properties help in strengthening the immune system of the body which helps to overcome cancer.

A number of phytochemical studies have demonstrated the presence of several classes of chemical compounds. It is not our intention in this review to cover all the many compounds reported for *Daemia extensa*, but to summarize the major components that have been implicated in the pharmacological activities of the crude drug. Most commonly found phytochemicals from the leaves of *Daemia extensa* are flavonoids alkaloids, terpenoids, tannins, steroids and carbohydrates [7]. Although, a large number of compounds have been isolated from various parts of *Daemia extensa*. Phytochemical studies have shown the presence of cardenolides, alkaloids, triterpenes (lupeol), saponins, steroidal compounds [8]. The seeds of *Daemia extensa* contain uzarigenin, coroglaucigenin, calactin, calotropin, other cardenolides and a bitter resin, Pergularin and have a cardiotoxic action [9, 10]. It has been suggested that the plant seed action on the uterus is similar to that of pituitrin and is not inhibited by progesterone [11, 12].

Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity.The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers.In addition, they have a metal chelation potential. The phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of natural phenolic compounds from plants materials is also raising interest among scientists, food manufacturers, and consumers due to functional food with specific health effects. Several studies had been conducted to evaluate the correlation between phenolic compounds and antioxidant activity. The antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, which are known to be potent as antioxidants.

MATERIALS AND METHODS

Collection and preparation of extract

Leaves of *Daemia extensa* were collected from Indore (Madhya Pradesh). The authentication was done by Prof. S. R. Upadhyaya Indore (M.P.) INDIA.

Preparation of Extracts

The leaves of *Daemia extensa* were collected and shade dried. The dried leaves were coarse powdered and the powder was packed in to soxhlet column and extracted successively with petroleum ether (60 – 80°C), ethanol (64.5 – 65.5°C) and distilled water. The extracts were concentrated under reduced pressure (bath temp 50°C). The dried extracts were stored in airtight container in refrigerator below 10-20°C.

Preliminary Phytochemical Screening

The preliminary phytochemical screening was carried out on petroleum ether, ethanol and aqueous extracts of *Daemia extensa* leaves for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods [13, 14].

Determination of total phenolic contents

The concentration of total phenolics in extract was determined using Folin– Ciocalteu reagent according to the method described by Singleton and Rossi (1965) with slight modification using tannic acid as a standard. Briefly, 1.0 ml of extract solution (5mg/ml) was added in a 100 ml volumetric flask that contained about 60 ml distilled water. 5.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. 15.0 ml sodium carbonate (20 % w/v) was added after 8 mins and the volume was made up to 100 ml using distilled water. The mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U. K). The total phenolic content was determined as mg of tannic acid equivalent (TAE) using an equation obtained from the standard tannic acid calibration graph.

Determination of total flavonoid content

The total flavonoid content was determined using the Dowd method [15]. 5 mL of 2 % aluminium trichloride (AlCl3) in methanol was mixed with the same volume of the extract solution (0.4 mg/mL). Absorption readings at 415 nm using PerkinElmer UV spectrophotometer were taken after 10 minutes against a blank sample consisting of a 5 mL extract solution with 5 mL methanol without AlCl3. The total flavonoid content was determined using a standard curve with catechin (0 - 100 mg/L) as the standard. Total flavonoid content is expressed as mg of catechin equivalents (CE) / g of extract.

Superoxide scavenging activity

Petroleum ether, aqueous and ethanolic extracts were screened for anti-oxidant activity using superoxide free radical scavenging activity in dose and time dependent manner [15]. The assay was based on the capacity of the samples to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. The reaction mixture contains 50 mM phosphate buffer, pH 7.6, 20µg riboflavin, 12 mM EDTA, O.1 mg/3 ml NBT, added in that sequence. The reaction was started by illumination the reaction mixture with different concentrations (5-100 µ g/ml) of samples for 15, 30 and 45 min. The absorbance was measured immediately after illumination at 590 nm. Ascorbic acid was used as standard drug. Percentage inhibition and IC_{50} were calculated (Results are shown in Fig. 4).

DPPH radical scavenging assay

Radical scavenging activity of plant extracts against stable DPPH (2, 2-diphenyl-2 picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were measured at 520 nm. Radical scavenging activity of extracts was measured using modified method of Brand-Williams 1995, as described below. Extract solutions were prepared by dissolving 0.025 g of dry extract in 10 ml of methanol. Three ml of Freshly prepared solution of DPPH in methanol was mixed with 77 µl extracted solution to produce final mass ratio of extracts approximately 3:1, 1.5:1, 0.75:1 .Analysis was done using disposable microcuvettes of 1 cm path length. Similar concentrations of rutin were used as reference standard. The samples were kept in the dark for 15 min at room temperature and decrease in absorption was measured. Absorption of blank sample containing the same concentration of methanol and DPPH solution was prepared and measured daily. The experiment was carried out in triplicate. Radical scavenging activity was calculated using following formula:

% inhibition = [(AB -AA)/AB] x 100

Where: AB —absorption of blank sample (t=0 min).
AA —absorption of tested extract solution (t=15 min.).

RESULTS AND DISCUSSION

Phytochemicals investigations

It was found that petroleum ether extract contained steroids, fat and fixed oils; aqueous extract contained carbohydrates, amino acids, steroids, flavonoid, alkaloids, glycosides and tannins; ethanolic extract also showed almost similar phytochemicals as compared to aqueous extract.

Total phenolic content of the extracts

The total phenolic content of the *Daemia extensa* extracts in tannic acid equivalents are presented in Table 1. The highest value was obtained for *Daemia extensa* in ethanol and lowest by the extract with petroleum ether.

Table No. 1

S. No.	Extracts	TAE[a]
1	C_2H_5OH	15.3
2	Aqueous	13.5
3	Petroleum ether	5.4

[a] *Total phenolic content is expressed as tannic acid equivalents (TAE; mg catechin/g of extract)*

Studies on total phenolic content had been published in several papers. Total phenolic content of *Daemia extensa* in three different climates (India, Nicaragua and Niger) ranged from 2940 - 4250 mg GAE/ dry weight [16] and water plants extracts studied by Noriham et al. (2004) ranged from 257 - 3234 mg TAE/100 g dry weight. In addition, Jerez et al. (2007) evaluated the total phenolic from the bark of two kinds of pine, *Pinus pinaster* and *Pinus radiata*. Different levels reported in these studies may be attributed to the different plants, procedures and standards used to express as total phenolic contents used by individual groups of investigator. The usage of Folin-Ciocalteu reagent also was measured based on the colour measurement which was non-specific on phenol. Perhaps there were other components that can react with the reagent such as ascorbic acid [17]. Besides, various phenolic compounds have different response to this assay [18]. However, the measurement of color changes after two hours storage could be used to determine the existence of phenol in samples. This may due to the antioxidant properties of plant extract that react as reductant agent which known as redox action.

Total Flavanoid content of the extracts

Daemia extensa extracted with ethanol expressed the highest total flavonoid content in catechin equivalents, as compare to petroleum ether and aqueous extract shown in Table 2.

Table No. 2

S. No.	Extracts	CE[a]
1	C_2H_5OH	12.3
2	Aqueous	9.5
3	Petroleum ether	3.4

[a] *Total flavonoid content is expressed as catechin equivalents (CE; mg catechin/g of extract).*

Free radicals scavenging activity

Ethanolic extract of *Daemia extensa* had showed 57.6±0.62 % inhibition in superoxide scavenging model. Aqueous extract also showed almost similar activity (55.3±0.48% compared to ethanolic extract), while Petroleum ether extract showed poor inhibition of superoxide scavenging activity. All extracts showed dose and time dependent inhibition of superoxide scavenging activity. The results are reported in Table 3and shown in Fig. 1, 2, 3.

DPPH radical scavenging activity

The aqueous extract of *Daemia extensa* exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC_{50}) at a concentration of 11.4 µg/ml. The IC_{50} value of the extract was found to be close to that of the standard; rutin (IC_{50} 10 µg/ml). Compared to rutin the extract exhibited a similar curve of antioxidant activity. This result demonstrated that *Daemia extensa* extract has inhibitory activity against the DPPH radical, Fig. 5.

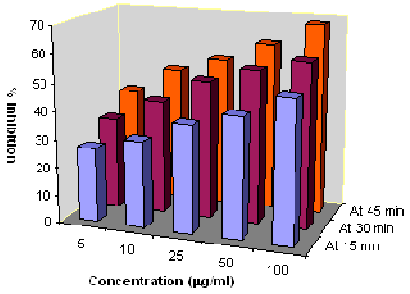

Fig.1: Effect of petroleum ether extract on superoxide free radicals

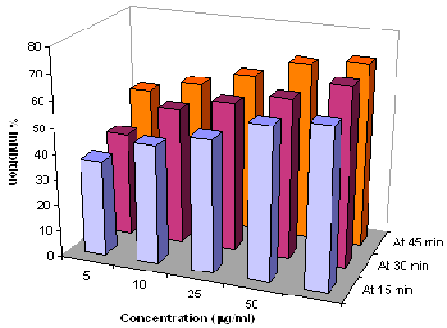

Fig. 2: Effect of ethanolic extract on superoxide free radicals

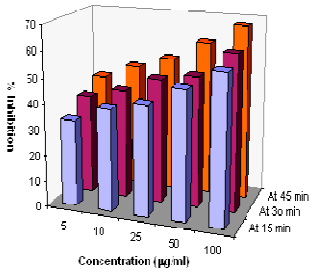

Fig. 3: Effect of aqueous extract on superoxide free radicals

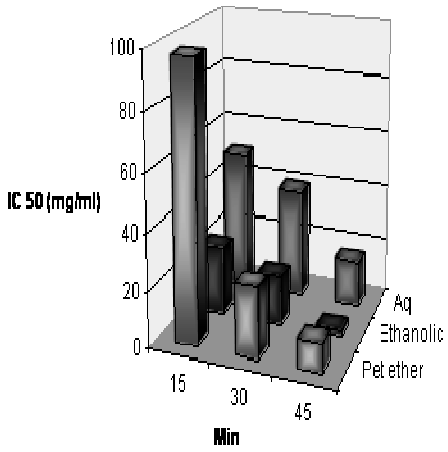

Fig. 4: IC_{50} of tested extracts

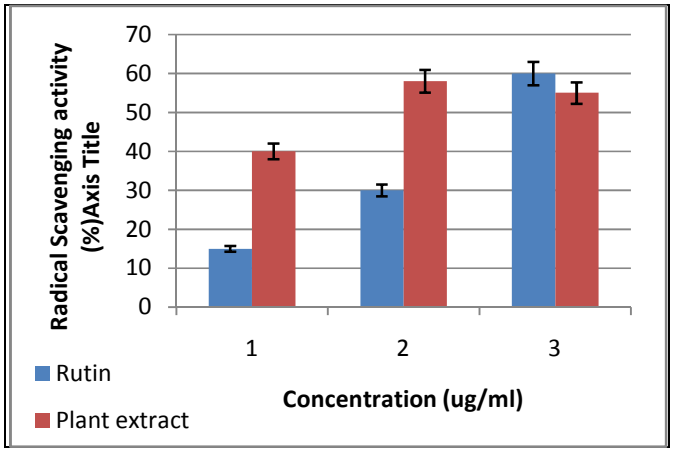

Fig. S5: DPPH radical scavenging activity of *Jatropha gossypifolia* extract added to a ethanolic solution of DPPH radical scavenging activity was measured at 520 nm as compared to rutin

Table No. 1. Percentage inhibition of superoxide free radical scavenging activity of petroleum, ethanolic and aqueous extracts

S. No.	Concentrations (µg/ml)	% Inhibition								
		Minutes								
		15			30			45		
		Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous	Petroleum ether	Ethanolic	Aqueous
1.	5	26.8±0.28	37.0±0.32	33.6±0.22	32.9±0.34	40.4±0.38	38.7±0.39	39.4±0.44	51.4±0.49	43.1±0.33
2.	10	31.5±0.31	44.8±0.49	38.4±0.27	39.7±0.39	54.9±0.45	42.4±0.47	47.6±0.45	62.8±0.58	48.2±0.51
3.	25	38.6±0.32	47.8±0.53	42.7±0.39	48.3±0.54	57.5±0.55	48.3±0.49	53.6±0.57	61.8±0.66	52.8±0.57
4.	50	45.0±0.52	57.6±0.62	55.3±0.48	54.9±0.53	61.6±0.59	50.9±0.52	60.4±0.63	68.4±0.65	60.0±0.63
5.	100	50.6±0.47	61.2±0.51	59.7±0.53	59.7±0.59	68.6±0.61	60.5±0.64	68.5±0.67	70.8±0.69	67.8±0.58

Data are mean±S.D of three measurements. Statistica l analysis was performed by the Student's t-test and by ANOVA

Statistical analysis

All analyses were run in triplicates. Data were analyzed by an analysis of variance (ANOVA). Statistical analysis was performed by the Student's *t*-test and by ANOVA.

The traditional medicine all over the world is nowadays revalued by an extensive activity of research on different plant species and their therapeutic principles. Experimental evidence suggests that free radicals (FR) and reactive oxygen species (ROS) can be involved in a high number of diseases [19]. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

In the present study, aqueous and ethanolic extract were selected as they contain alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds. This may have active constituents for producing the free radical scavenging effect.

Free radicals are produced under certain environmental condition and during normal cellular function in the body. These molecules are missing an electron, giving them an electric charge. To neutralize this charge, free radicals try to withdraw an electron from, or donate an electron to, a neighboring molecule. Other antioxidants works against the molecules that form free radicals, destroying them before they can begin the domino effect that leads to oxidative damage. For example, certain enzymes in the body, such as superoxide dimutase, work with other chemical to transfer free radical into harmless molecules. Vitamin C; an antioxidant that may prevent cataracts and cancers of the stomach; throat, mouth, and pancreas. It may also prevent the oxidation of LDL cholesterol, lowering the risk of heart disease. Literature reveals that, the carbonyl groups present in the flavonoids and phenolic compounds were responsible for free radical scavenging activity. This investigation revealed that the *Daemia extensa* Contains pharmacologically active substance such as alkaloids, glycosides, saponins, tannins, flavonoids and phenolic compounds, which are responsible for the Superoxide scavenging activity.

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