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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT AND ANTIFUNGAL ACTIVITY OF BARK EXTRACTS OF SOYMIDA FEBRIFUGA (ROXB) A.JUSS

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ABSTRACT

The present study deals with *in vitro* antioxidant and antifungal activities of bark extracts of *Soymida febrifuga Roxb*. The antioxidant activity was done by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay, and nitric oxide scavenging assay. This study ascertains that the *S. febrifuga* Roxb bark extract could serve as an important bio-resource of antioxidants for using in food and pharmaceutical industry. Besides, 5 antifungal strains namely *Geotrichum candidum, Microsporum canis, Trychophyton rubrum, Chrysosorium tropicum* and *Rhizopus stolonifer* were used for antifungal activity. Out of five tested *G.candidum, M.canis* and *T.rubrum* were found to be positive and of these three *G.candidum* showed higher inhibition zone compared to *M. canis* and *T. rubrum*. The result reveals the higher proportion of antioxidant and antifungal properties.

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INTRODUCTION

Oxidative stress represents the existence of products calledfree radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions butbecome toxic when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems (Chanda and Dave, 2009. ROS are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders (Halliwell, 1994 and Rackova *et al*., 2007) such as cancer (Kinnula, 2004). cardiovascular disease (Singh, 2006), neural disorders (Sas *et al*., 2007), Alzheimer's disease (Smith, 2000), mildcognitive impairment (Guidi *et al*., 2006), Parkinson disease (Bolton, 2000), alcohol induced liver disease (Arteel, 2003), ulcerative colitis (Ramakrishna *et al*., 1997), ageing (Hyun, 2006), atherosclerosis (Upston, 2003). Oxygen derived free radicals such as superoxide anions; hydroxyl radicals and hydrogenperoxide are cytotoxic and give rise to tissue injuries (Jainu *et al*., 2005). Excessive amount of ROS is harmful because they initiate bimolecular oxidation which leads to cell death and creates oxidative stress. In addition, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system (Wiseman *et al*., 1996). In the body, free radicals are derived from two sources: endogenous sources, e.g. nutrient metabolism, ageing process etc. and exogenous sources e.g. tobacco smoke, ionizing radiation, air pollution, organic solvents, pesticides, etc (Buyukokuroglu, 2001). Evaluation of antioxidant activitya great number of *in vitro* methods have been developedto measure the efficiency of natural antioxidants either aspure compounds or as plant extracts. *In vitro* methods can be divided into two major groups: 1) Hydrogen atom transfer reactions like Oxygen Radical Absorbance Capacity (ORAC), Total radical trapping antioxidant potential (TRAP) and carotene bleaching; 2) Electron transfer reactions like trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power (FRAP), α, α-diphenyl- β-picryl-hydrazyl radical scavenging assay (DPPH), Superoxide anion radical scavenging assay, Hydroxyl radical scavenging assay, Nitric oxide radical scavenging assay and Total phenol assay (Huang *et al*., 2005a). These methods are popular due to their high speed and sensitivity. However, it is essential to use more than one method to evaluate antioxidant capacity of plant materials because of the complex nature of phytochemicals (Salazar *et al*., 2008). Hence this study is

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primarily focussed on2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay, and nitric oxide scavenging assay along with antifungal activities.

MATERIALS AND METHODS

Collection and Authentication

The plant was collected from Balrampur district, Chhattisgarh, India. The taxonomic identification of the plant was carried out by Dr. S. John Britto, Director and Head, The Rapinat Herbarium and Centre for Molecular Systematics St. Joseph's College (*Autonomous*) Tiruchirappalli, India. The voucher specimen was deposited at the centre (RHT 67112).

Extraction Procedure

The collected barks of plant were dried at room temperature, powdered, and was then stored in air tight container till use. It was weighed in a selected quantity and was subjected to soxhlet apparatus using solvents such as Acetone, Aqueous, Chloroform, Ethanol, Methanol and Petroleum ether respectively. The solvent was then evaporated to get dry Powder. The dried powder was preserved in an airtight bottle. The crude extracts thus obtained were used for further investigation of antioxidant and antifungal studies.

Antioxidant Activity

Total antioxidant capacity assay

The total antioxidant capacity assay was determined as described (Prieto, 1999). Different concentrations of the ethanolic and methanolic bark extracts of *Soymida febrifuga* (10-50µg/ml) were taken and added 1.0 ml ofthe reagent solution (0.6 M Sulphuric acid, 28 mM Sodium phosphate and 4 mM Ammoniummolybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid.The antioxidant activity is calculatedas follows:

Antioxidant activity (%) = (A0 – A1 / A0) X 100

Where; A0 is the absorbance of control and A1 is the absorbance of test.

DPPH Radical Scavenging activity

Radical scavenging activity was measured by using DPPH scavenging method of (Blois, 1958). A solution of DPPH in methanol (24µg/ml) was prepared and 2ml of this solution was added to ethanol and methanol bark extract at different concentrations (10- 50µg/ml). Absorbance at 517 nm was determined after 30 min at room temperature and the scavenging activity were calculated as a percentage of the radical reduction. Each experiment was performed in triplicate. Ascorbic acid was used as standard. The percentage of inhibition was calculated using the formula:

Inhibition (%) = (A0 – A1 / A0) × 100

Where; A0 is the absorbance of control and A1 is the absorbance of test.

Nitric oxide scavenging assay

Nitric oxide scavenging activity was measured spectrophotometrically (Govindarajan, 2003). The Ethanolic and Methanolic bark extracts of *S. febrifuga* were added to different test-tubes in varying concentrations (10-50 µg/ml). Sodium nitroprusside (5mM) in phosphate buffer was added to each test tube to make up volume to 1.5ml. Solutions were incubated at 25ºC for 30 minutes. Thereafter, 1.5ml of Griess reagent (1% Sulphanilamide, 0.1% Naphthylethylenediamine dichloride and 3% Phosphoric acid) was added to each test tube. The absorbance was measured immediately at 546 nm. The percentage of scavenging activity was measured with reference to ascorbic acid and the inhibition was calculated using the formula:

Inhibition (%) = (A0 – A1 / A0) × 100

Where; A0 is the absorbance of control and A1 is the absorbance of test.

Antifungal studies

For the antifungal studies, five fungal species were used in Potato Dextrose agar medium. The fungal species were *Geotrichum candidum, Microsporum canis, Trychophyton rubrum, Chrysosorium tropicum* and *Rhizopus stolonifer*. Well diffusion method was adopted for the present study with the size of well as 6mm. The concentration of the extracts was 200µg/well while that of the control was 100µl of distilled water per well. The experiments were carried out in six replicates for five pathogenic fungi and the mean and standard deviations were calculated using standard formulae.

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \overline{x})^2}$$

Where;

σ = standard deviation
Xi = each value of dataset
X (with a bar over it) = the arithmetic mean of the data
N = the total number of data points
∑ (xi - mean)^2 = the sum of (xi - mean)^2 for all datapoints

RESULTS AND DISCUSSION

The total antioxidant capacity of ethanolic and methanolic bark extracts of *S. febrifuga* Roxbwas determined by phosphor molybdenum using Ascorbic acid as standard. In phosphor molybdenum assay, the concentrations range from 10-50µg/mL, ascorbic acid showed higher dose dependent reducing activity 89.17±1.59 at 50µg/ml followed by ethanol 75.47±1.60 at50µg/mland methanol extracts 73.73±2.05 at50µg/ml (Table 1, Chart 1). This result showed that ascorbic acid exhibited excellent total antioxidant activity.

Table 1. Total antioxidant capacity assay

S. No.	Conc.µg/ml	Ethanol	Methanol	Ascorbic acid
1.	10	51.04±2.49	37.87±1.75	45.65±2.05
2.	20	63.12±1.84	53.44±2.18	56.52±1.96
3.	30	67.25±1.18	61.19±0.34	65.94±2.04
4.	40	70.85±1.30	68.42±1.31	78.91±1.65
5.	50	75.47±1.60	73.73±2.05	89.17±1.59

Data given are Mean of three replicates ± Standard Deviation.

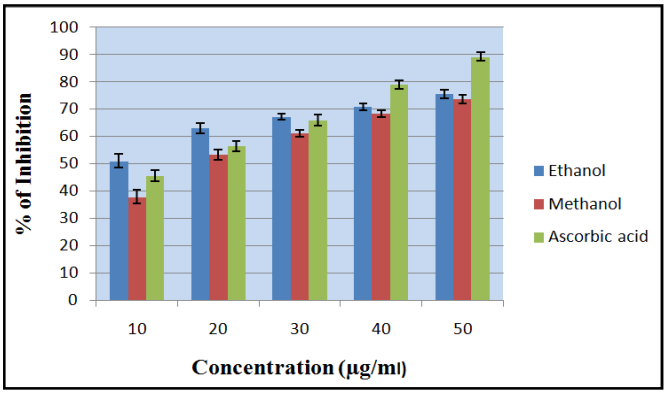


Chart 1. Total antioxidant of *S.febrifuga* bark

Table 2. DPPH Radical Scavenging activity

S.No.	Conc.µg/ml	Ethanol	Methanol	Ascorbic acid
1.	10	32.35±1	48.27±1.1	45.65±2.05
2.	20	45.63±1.1	53.7±0.95	56.52±1.96
3.	30	62.4±1.35	61.49±0.58	65.94±2.04
4.	40	78.3±0.87	78.13±1.45	78.91±1.65
5.	50	88.22±0.75	87.92±1.11	89.17±1.59

Data given are Mean of three replicates ± Standard Deviation.

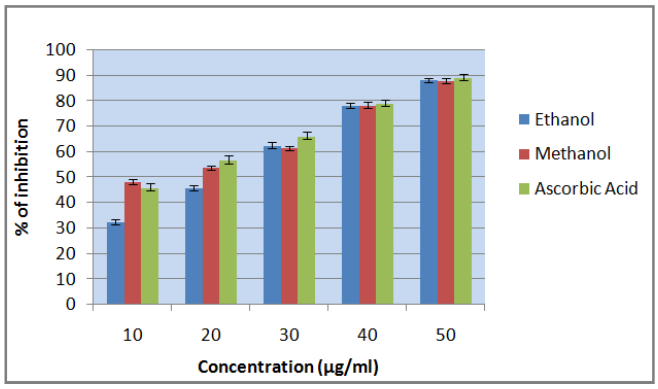


Chart 2. DPPH Radical Scavenging activity of *S.febrifuga* bark

DPPH Radical Scavenging activity of *S.febrifuga* Roxb. bark was determined by using ascorbic acid as standard. The DPPH scavenging activity was high in ascorbic acid 89.17±1.59 at 50µg/ml conc. followed by ethanol 88.22±0.75at 50µg/ml conc. and methanol 87.92±1.11 at 50µg/ml conc (Table 2, Chart 2).

Table 3. Nitric oxide scavenging assay

S.No.	Conc.µg/ml	Ethanol	Methanol	Ascorbic acid
1.	10	42.6±0.65	38.48±1.35	40.3±1.91
2.	20	55.27±1.23	48.49±0.88	51.6±2.4
3.	30	60.83±1.58	57.33±0.78	59.7±2.5
4.	40	69.44±1.26	70.03±0.9	67.1±1.89
5.	50	77.7±0.88	78.97±1.1	78.8±2.5

Data given are Mean of three replicates ± Standard Deviation.

Nitric Oxide (NO) scavenging assay is based on the scavenging ability of ehtanolic and methanolic bark extract of *S.febrifuga* Rxob, as well as ascorbic acid, which is used as standard. The scavenging of NO was found to increase in dose dependent manner. Maximum inhibition of NO was observed in the methanolic extracts 78.97±1.1 at 50µg/ml concentration followed by ascorbic78.8±2.5 at 50µg/ml concentration and ethanolic77.7±0.88 at 50µg/ml concentration (Table 3, Chart 3).

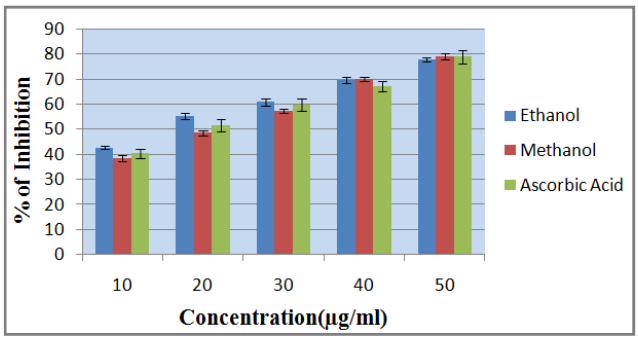
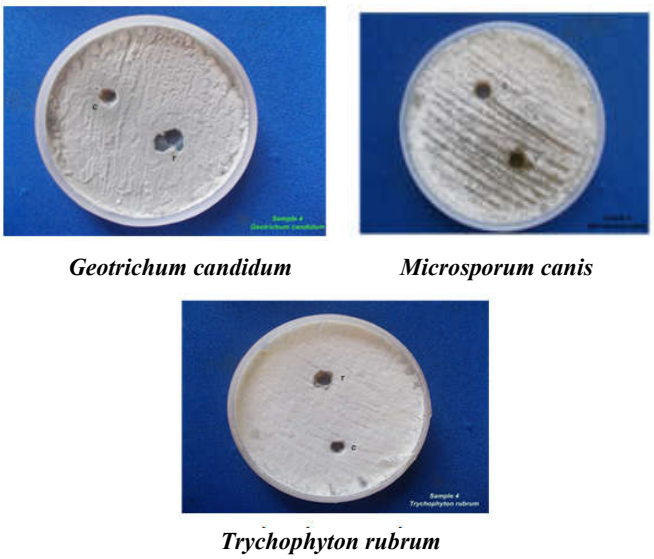


Chart 3. Nitric oxide scavenging assay of *S.febrifuga* bark

Antifungal studies



Geotrichum candidum ***Microsporum canis***

Trychophyton rubrum

Plate 1. Antifungal activity of methanolicextraxt of *S. febrifuga* (Roxb.)A. Juss. Bark

Table: 4 Antifungal activity of methanol extract of *S. febrifuga* (Roxb.)A. Juss. Bark

S.No.	Test Microorganisms	Zone of Inhibition (mm)
1.	*Geotrichum candidum*	13.67±0.82
2.	*Microsporum canis*	10.33±0.82
3.	*Trychophyton rubrum*	10.33±0.52

Data given are Mean of six replicates ± Standard Deviation.

The antifungal activities of methanolic extracts of *S.febrifuga* (Roxb.) A. Juss bark was carried out against five pathogenic fungi, namely *Geotrichum candidum*, *Microsporum canis, Trychophyton rubrum, Chrysosorium tropicum*and *Rhizopus stolonifer*. The extract exhibited maximum inhibition against *G. candidum* 13.67±0.82(mm) whereas *M. canis* and

T.rubrum showed 10.33±0.82(mm) and 10.33±0.52 (mm) inhibition zones respectively. The fungi C.tropicum and R.stolonifer were found to be negative (Plate1, Table 4 and Chart 4).

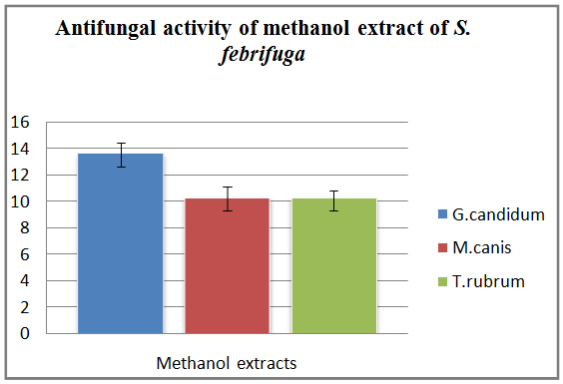


Chart 4. Antifungal activity of methanol extract of *S. febrifuga* bark

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Conclusion

The sample was subjected toscreening for their possible antioxidant activity by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical, total antioxidant assay, and nitric oxide scavenging assay. Results showed that the *S.febrifuga* Roxb. Possessed a strong degree of antioxidant activity. The *S. febrifuga* bark remarkably showed the inhibition zone against tested fungal strains. Hence the plant can be used as alternative drug to cure disease caused by pathogenic fungi. Hence it also requires further studies to isolate, Characterize elucidate and to purify the bioactive compounds for the better therapeutic values.

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