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PHYTOCHEMICAL ANALYSIS AND FREE-RADICAL SCAVENGING ACTIVITY OF *FLACOURTIA INDICA* (BURM.F.) MERR.

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ABSTRACT

Flacourtia indica dichloromethane (DCM), butanol (BuOH), methanol (MeOH) extract of stem bark and methanolic extract of leaves were screened for *in vitro* antioxidant activity using different models. Subsequent quantification showed the presence of 15.62 and 11.53 % w/w phenolics; 1.15 and 1.80 % w/w of flavonol in methanol extract of stem bark and leaf of *Flacourtia indica* (*F. indica*) respectively. The methanolic extract showed an effective DPPH radical scavenging activity with low IC_{50} values of 17.5 and 21 µg/ml respectively and hydroxyl radical scavenging by *p*-NDA method with IC_{50} value of 350.23 µg/ml. Butanolic extract showed nitric oxide radical inhibition activity with IC_{50} value of 28.5 µg/ml. The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by *F. indica* extracts. The antioxidant property of the extracts may be due to presence of phenolic content.

Keywords: *Flacourtia indica*, DPPH, Nitric oxide radical inhibition assay, Scavenging of hydroxyl radical by *p*-NDA

INTRODUCTION

Flacourtia indica (Burm.f) Merr. Syn. *F. ramontchi* L Herit (Flacourtiaceae) is a small spinous tree or shrub found distributed throughout the Himalayas, northern districts of Uttar Pradesh, Assam, Bengal and Orissa[1-3]. The roots and fruits of *F. indica* are used in traditional medicine as a diuretic, hepatoprotective and antidiabetic[1]. The peel and pulp of *F. indica* fruits had shown radical scavenging activity[4]. The various active constituents like a phenolic glucoside ester[5], butyrolactone lignan disaccharide[6], phenolic compounds, flavonoids, tannins, sucrose and protein[4] have been isolated from this plant. This paper reports the pharmacognostical investigation and the evaluation of free-radical scavenging properties of stem bark and leaf of *F. indica* in three *in-vitro* models. The pharmacognostical investigation study includes determination of physico-chemical constants, preliminary phytochemical screening of extracts of *F. indica* stem bark. The alcoholic extract from aerial parts of *F. indica* has analgesic and anti-inflammatory effects in rats[7]. Since the stem bark and the leaves were reported to contain phenolic compounds which may act as free radical terminators so the authors thought worthwhile to carry out antioxidant activity. To the best of our knowledge, no antioxidant activity and pharmacognostical investigation has been done on the parts of this plant.

EXPERIMENTAL

F. indica stem bark and leaves were collected from forests of Shann Power House, Joginder Nagar, (Distt Mandi) Himachal Pradesh, India in October 2006. The identity of the plant material was verified by Dr. H.B Singh, Head, Raw Materials Herbarium and Museum, NISCAIR, New Delhi. A voucher specimen (NISCAIR/RHMD/Consult/06/757/74) is deposited in the herbarium of National Institute of Science Communication and Information Resources, New Delhi, India.

Physico-chemical analysis

Physico-chemical analysis i.e. percentage of ash values, loss on drying and extractive values were performed according to the official methods prescribed[8] and the WHO guidelines on quality control methods for medicinal plant materials[9]. Fluorescence analysis was carried out according to the method of Kokoski et al[10].

Preliminary phytochemical screening

Preliminary phytochemical screening was carried out by using standard procedures described by Harborne[11]. Five-hundred milligrams (500mg) of the dried methanolic extract of *F. indica* stem bark was reconstituted in 10 ml of methanol and used for preliminary phytochemical testing for the presence of different chemical groups of compounds.

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Chemicals

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) were obtained from Sigma Aldrich Co., St. Louis, USA. Folin Ciocalteu's reagents and rutin were purchased from SD Fine chemicals, India. Naphthyl ethylene diamine dihydrochloride (NEDD) was obtained from Roch-Light Ltd., Suffolk, UK. *p*-nitroso dimethyl aniline (*p*-NDA) was obtained from Across Organics, New Jersey, USA. All chemicals used were of analytical grade.

Preparation of Extracts

The dried stem barks weighing about (30gm) of *F. indica* were powdered and extracted separately with DCM, BuOH and MeOH, for 24 h by maceration. Each extract was filtered, pooled and the solvent was removed under reduced pressure (yield obtained was 4.68, 5.90, 6.40 % w/w respectively). Dried powdered leaves about (30gm) were extracted only with MeOH (yield obtained was 8.2 % w/w).

Preparation of Test and Standard Solutions

The extracts and the standard antioxidants, ascorbic acid and rutin, were dissolved in distilled dimethyl sulphoxide (DMSO) separately and used for *in-vitro* antioxidant study. The stock solutions were serially diluted with DMSO to get required dilutions.

Estimation of total phenolics

The total phenol content of all the extracts was determined by using the standard Folin-Ciocalteu method[12]. Each extract solution (100 ìl) was mixed with 2 ml of Folin-Ciocalteu reagent and 1.6 ml of sodium carbonate, shaken well and kept for 2 h. The absorbance was measured at 750 nm using Beckman (DU 640B) Spectrophotometer. Using gallic acid monohydrate as standard, standard curve was prepared and linearity was obtained in the range of 2.5 to 25 ìg/ml. The total phenol content of the extracts was obtained by using the standard curve. The total phenol content was expressed as gallic acid equivalent in % w/w of the extracts.

Estimation of total flavonols

The total flavonol content of the extracts was determined by aluminium chloride colorimetric method[13]. Each extract (0.5 ml) was mixed with 1.5 ml methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Beckman (DU 640B) Spectrophotometer. Using rutin as standard, standard curve was prepared and linearity was obtained in the range of 1-10 µg/ml. Using the standard curve the total flavonol content was expressed as rutin equivalent in % w/w of the extracts.

DPPH radical- scavenging assay

The antioxidant activitiy[14, 15] of the methanol extracts and standard compounds (rutin and ascorbic acid) were assessed on the basis of radical scavenging effect of the stable DPPH free radical. To 6ml of DPPH (20µg/ ml) methanolic solution, 20 µl of DMSO solution of each extract was added separately, at room temperature. The mixture was shaken vigorously and kept aside for 5 min and absorbance was measured at about 517nm with Beckman (DU 640B) spectrophotometer against corresponding test blanks. All tests were run in triplicate and mean values were taken for calculation. IC_{50} value is the concentration of sample required to inhibit 50% of DPPH radical.

Nitric oxide radical inhibition assay

Nitric oxide is a free radical and scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide[16-17]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite, which can be estimated by the use of Griess Illosvog reagent[18]. In the present investigation, Griess Illosvog reagent is modified by using naphthylethylene diamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (1.5 ml) containing sodium nitroprusside (10 mM, 1 ml), phosphate buffer saline (0.25 ml) and extract solution (0.25 ml) was incubated at 25 ºC for about 2 h. After incubation, 0.5 ml of the reaction mixture containing nitrite ions was removed and 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was added, mixed well and allowed to stand for 5 min for completing diazotization, then 1 ml of naphthylethylene diamine dihydrochloride (0.1%) was added, mixed and allowed to stand for 30 min. A pink colored chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions with Beckman (DU 640B) spectrophotometer. IC_{50} value is the concentration of sample required to inhibit 50% of nitric oxide radical.

Scavenging of hydroxyl radical by *p*-NDA method

To a solution containing ferric chloride (0.1 mM, 0.5 ml), EDTA (0.1 mM, 0.5 ml), ascorbic acid (0.1 mM, 0.5 ml), hydrogen peroxide (2mM, 0.5ml) and *p*-NDA (0.01 mM, 0.5 ml) in phosphate buffer (pH 7.4, 20 mM) various concentrations of extract or standard in distilled DMSO (0.5 ml) was added to produce a final volume of 3 ml. Absorbance was measured at 440 nm[19].

RESULTS AND DISCUSSION

Physico-chemical parameters i.e. ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 1) of the powdered *F. indica* stem bark revealed a high concentration of total ash. The total ash, water soluble ash and acid insoluble ash which are important parameter for detecting the presence of inorganic substances were found to be 9.46, 1.06 and 0.58 % w/w respectively in stem bark

Table 1. Ash values and loss on drying (LOD) of the stem bark and leaves of F. indica

Ash values (% w/w)				Loss on drying (% w/w)
Plant Part	Total ash	Water-soluble ash	Acid-insoluble ash	LOD
Stem bark	9.46	1.06	0.38	9.41
Leaves	6.73	1.2	0.55	7.05

and 6.73, 1.2 and 0.55 % w/w respectively in leaves. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water, ethanol, butanol, methanol and dichloromethane soluble extractives, which are indicator of total solvent soluble component, are 5.0, 6.12, 5.90, 3.72, 4.68 % w/w respectively (Table 2). Loss on drying of the powdered *F. indica* root revealed the presence of 9.41% of moisture in a drug. The results of fluorescence

Table 2. Extractive values of the stem bark of F. indica

Parameters	Values % (w/w)
Water soluble	5.0
Ethanol soluble	6.12
Butanol soluble	5.90
Methanol soluble	3.72
Dichloromethane soluble	4.68

analysis of the powdered stem bark and leaves are presented in Table 3 & 4 respectively, which helps in detecting the presence of various constituents like phenolic compounds, flavonoids, steroids and other natural compounds based on different fluorescence with different chemical reagents. These studies help in authentication of the plant since there is no work reported on their pharmacognostical investigation previously.

Table 3: Results of Fluorescence analysis of powdered stem bark of F. indica

Drug + Reagent	**UV light**		**Visible light**
	Short (UV 254nm)	**Long (UV 366nm)**	
Powder as such	Dark brown	Light brown	Light brown
Powder + 1N NaOH (Aq)	Brown	Brown	Light brown
Powder + 1N NaOH (Alc)	-	-	Whitish brown
Pwder + 1N HCl	-	-	Very light brown
Powder + NH_3	Light brown	Light green	Light brown
Powder + 5% Iodine	Dark yellowish brown	Dark yellowish brown	Dark yellowish brown
Powder + 5% $FeCl_3$	Dark brown	Dark brown	yellowish brown
Powder + acetic acid	Dark green	Light orange	Light green
Powder + 1N H_2SO_4	Dull green	Orangish brown	Dark brown
Powder + 1N HNO_3	Dark brown	Dark brown	Yellowish brown

Table 4: Results of Fluorescence analysis of powdered leaves of F. indica

Drug + Reagent	**UV light**		**Visible light**
	Short (UV 254nm)	**Long (UV 366nm)**	
Powder as such	Dark green	Whitish green	Light green
Powder + 1N NaOH (Aq)	Yellowish brown	Light purple	Yellowish brown
Powder + 1N NaOH (Alc)	-	Light pink	Pale yellow
Pwder + 1N HCl	-	-	Very light green
Powder + NH_3	Light yellowish green	Light fluorescent green	Yellowish green
Powder + 5% Iodine	Dark yellowish brown	Dark yellowish brown	Dark yellowish brown
Powder + 5% $FeCl_3$	Dark brown	Dark brown	Dark yellowish green
Powder + acetic acid	Dark green	Light purple	Light green
Powder + 1N H_2SO_4	Dark brown	Orangish brown	Dark green
Powder + 1N HNO_3	Dark green	Dark green	Light yellowish green

and leaf of *F. indica* showed the presence of phytosterols, lipids, phenolic compounds, carbohydrates, flavonoids and tannins. Subsequent quantification showed the presence of 15.62 and 11.53 % w/w phenolics (calculated as gallic acid), 1.15 and 1.80 % w/w of flavonol in methanol extract of stem bark and leaf of *F. indica* respectively.

Plant phenolics including flavonoids are known to possess strong antioxidant properties[20]. The results of antioxidant activity of standards ascorbic acid, rutin and different extracts are shown in Table 5a and Table 5b. The methanolic extract of stem bark and leaf of *F. indica* showed a concentration-dependent DPPH radical scavenging activity by bleaching it with low IC_{50} values of 17.5 and 21 µg/ml respectively. Butanolic extract of *F. indica* stem bark showed good nitric oxide radical inhibition activity with IC_{50} of 28.5 µg/ml in a dose-dependent manner. The methanolic extract of stem bark

Table 5a. In vitro antioxidant activity of different extracts of F. indica stem bark and leaves

	IC_{50} values ± SEM (µg/ml)*			
Plant extract	Yield of extract (%)	DPPH	Nitric oxide	*p*-NDA
MeOH[SB]	6.4± 0.4	17.5± 1.0	150± 2.8	350.23± 3.4
MeOH[L]	8.2± 0.5	21± 1.2	> 700	>1000
DCM[SB]	4.68± 0.6	19± 1.3	>700	>1000
BuOH[SB]	5.9± 0.8	16± 1.0	28.5± 1.0	>1000
Standards				
Ascorbic acid	-	2.69± 0.02	-	>1000
Rutin	-	5.83± 1.2	68.44± 1.4	205.83± 0.4

*SEM: ± standard error mean, average of three determinations, SB-stem bark, L-Leaves.

Table 5b. Total phenolic and total flavonol content of different extracts of F. indica stem bark and leaves

Plant Extract	Total Phenol content ± SEM (% w/w)	Total Flavonol content ± SEM (% w/w)
MeOH[SB]	15.62± 1.5	1.15± 0.07
MeOH[L]	11.55 ± 1.2	1.80± 0.3
DCM[SB]	8.84± 0.9	0.819± 0.13
BuOH[SB]	7.15± 1.1	1.50 ± 0.4

*SEM: ± standard error mean, average of three determinations, SB-stem bark, L-Leaves.

showed hydroxyl radical scavenging by *p*-NDA method with IC_{50} value of 350.23 µg/ml and all other extract showed IC_{50} > 1000 µg/ml. This free- radical scavenging activity can be attributed to the high amounts of flavonoids and phenolics present in different extracts of the plant. It also resulted in showing the lower IC_{50} values in *in-vitro* antioxidant studies. The lower IC_{50} values indicated the high antioxidant potency of the extracts. On the basis of the deep phytochemical findings it was found that the different extracts of *Flacourtia indica* stem bark were rich in polyphenolic compounds like butyrolactone lignan, coumaroyl glucopyranose, flavonoids, tannins which may act as free radical scavengers. In view of this the authors have performed free-radical scavenging activity of different extracts of *F. indica* stem bark. The fruits of *F. indica* were analyzed for DPPH radical scavenging activity[21]. There is no such previous report of antioxidant activity of stem bark in the literature. This work may serve as a model for detailed free radical scavenging property of this plant extract.

CONCLUSION

In conclusion, the extracts from the stem bark and leaves of *F. indica* possess significant antioxidant activity. As there is no pharmacognostic work on record of this traditionally much valued drug, the present work was taken up with a view to lay down standards, which could be useful to detect the authenticity of this medicinally useful plant.

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REFERENCES

1. Duthie JS. Flora of the upper gangetic plain, Bishen singh Mahendra pal singh New Connaught place, Dehra doon. Vol. I; 1994, p 215.
2. Kirtikar KR, Basu BD. Indian Medicinal plants, Vol II, L.M. Basu Publication, Allahabad, 1935, p 1565.
3. The Wealth of India, Raw materials, Vol. 4, Council of Scientific and Industrial Research; New Delhi, 1956, p 43.
4. Ndhlala AR, et al., Food Chemistry. 2007; 103: 82.
5. Bhaumik PK, et al., Phytochemistry. 1987; 26:3090.
6. Satyanarayana V, et al., Phytochemistry. 1991; 30: 1026.
7. Gurav S, et al., Pharmacologyonline. 2007, 2: 20.
8. Anonymous: Indian Pharmacopoeia 4th edn, Vol.2, Government of India, Ministry of Health and Welfare, Controller of Publications, New Delhi, 1996, A53.
9. WHO/PHARM/92.559/rev.1. Quality Control Methods for Medicinal Plant Materials Organisation Mondiale De La Sante, Geneva. (1992); 9: 22.
10. Kokoski J, et al., J Am Pharmacol Assoc. 1958; 47: 715.
11. Harborne JB and Williams CA. Phytochemistry. 2000; 55: 481.
12. Sadasivam S, Manickam A. Biochemical Method for Agricultural Sciences, Wiley Eastern Ltd, New Delhi, 1992, p 187.
13. Woisky R and Salatino A. J Agric Res. 1998; 37: 99.
14. Schmeda-Hirschmann G, et al., Econ Bot.1999; 53:177.
15. Germano MP, et al., J Agric Food Chem. 2002; 50: 1168.
16. Badami S, et al., J Ethnopharmacol. 2003; 84:227.
17. Marcocci L, et al., Methods Enzymol. 1994; 234: 462.
18. Garrat DC. The Quantitative Analysis of Drugs, Chapman and Hall Ltd, Japan, 1964, p 456.
19. Elizabeth K and Rao MNA. Int J Pharm.1990; 58: 237.
20. Chu YH, et al., J Sci Food Agric. 2000; 80: 561.
21. Ndhlala AR, et al., Food chemistry. 2007;103:82.