Research Article

Antioxidant Potential and Effect of Extraction Solvent on Total Phenol Content, Flavonoids Content and Tannin Content of *Ficus palmata Forssk*

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ABSTRACT

Ficus palmata Forssk is evergreen, scandent shrubs, is widely distributed in most tropical countries and has been used frequently in folk medicine for varied purpose to treat different kinds of pathologies particularly diabetes, inflammation owing to the presence of numerous phytochemical constituent's residing the plant. In recent years much attention has been devoted to natural antioxidant and their association with health benefits. Plants are the potential source of natural antioxidants. The aim of this study was to assess the antioxidant activity (in vitro) of four extracts from stem bark of *Ficus palmata Forssk* and to determine phenolic content and flavonoids content of all extracts. The present study was aimed at estimation of total phenolics, flavonoids and tannins in the petroleum ether, ethyl acetate, methanol and aqueous extracts of the stem bark of Ficus palmate F. Comparative antioxidant potential was evaluated using DPPH, Nitric Oxide scavenging assay and ferric reducing power assay. The contents were determined by spectrophotometric assays by measuring the absorbance at different wavelengths. Total phenolic content were estimated by the Folin–Ciocalteu colorimetric method; the total tannin content was estimated by Folin-Denis method whereas the total flavonoid content was estimated by aluminium chloride colourimetric method. The methanol extract showed highest concentration of phenolics, flavonoids and tannins with petroleum ether extract reporting the least; ranging between 40.91-78.75 µg /mg of gallic acid equivalent, 54.44-112.31 µg/mg of (±)-catechin equivalents and 33.48-67.48 µg/mg of tannic acid, Methanol and ethyl acetate extract gave an IC50 of 98.931 ± 2.486, 85.919 ± 1.976 and 93.457 ± 2.487, 74.243 ± 1.987 for DPPH and Nitric oxide scavenging activities respectively. The results clearly indicate that Ficus. palmata Forssk is a rich source of phenolics, the basis of its traditional use in different systems of medicines.

Keywords: Phenolics, flavonoids, antioxidant, stem bark extract, tannins.

INTRODUCTION

Changing environmental conditions are giving rise to a variety of free radicals, which plants have to deal with them in order to survive. Reactive oxygen species, such as singlet oxygen, superoxide ion, hydroxyl ion and hydrogen peroxide, are highly reactive, toxic molecules, which are generated normally in cells during metabolism. They cause severe oxidative damage to proteins, lipids, enzymes and DNA by covalent binding and lipid peroxidation, with subsequent tissue injury. Natural antioxidant agents have attracted much interest because of their ability to scavenge free radicals [1]. Plants synthesize compounds with biological activity, namely antioxidant, as secondary products, which are mainly phenolic compounds serving in plant defence mechanisms to counteract reactive oxygen species (ROS) in order to avoid oxidative damage. Phenolics are secondary plant metabolites ranging from simple to highly polymerized compounds. In the case of phenolic compounds, the ability of the phenolics to act as antioxidants depends on the redox potential of their phenolic hydroxyl groups that allow them to act as reducing agents, hydrogen-donating antioxidants and oxygen quenchers [2]. Phenolic compounds are common plant secondary metabolites which have not only physiological functions in plant but also positive effect for human health because they can act as antioxidant. Antioxidants play important roles in preventing pathogenic processes related to cancer, cardiovascular disease and can enhance immune function. Antioxidant defences protect the body from the detrimental effects of free radicals generated as by -products of normal metabolism [3]. Many epidemiological studies have shown that the consumption of phenolics-rich foods is associated with the prevention of chronic diseases [4]. Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots and barks [5].

The aim of the present study was to evaluate the total phenolic content of the ethyl acetate; methanol and aqueous extracts of *Ficus palmata Forssk* stem bark.

Ficus palmate Forssk is an herbaceous perennial plant belonging to the family Moraceae is commonly known as Anjiri, Bedu, Khemri (Hindi) or Pepri (Gujrati) or Manjimedi (Telegu) or Phagwara (Punjab). It is a bush or a moderate sized tree, sometimes deeply 3- or 5- lobed; fruits 1/2-1 inch in diameter, auxiliary, pedunculate, sub-globose or periform, usually tormentose which become yellow or purple when ripe. The tree is found in Central and North West India and the outer Himalayas from Nepal westwards to Afghanistan, Egypt and Abyssinia. It occurs in Kumaon upto 6,000ft and is common in open places, especially along the banks of rivers and streams. The fruit is demulcent, emollient, laxative and poultice. It is used as a part of the diet in the treatment of

constipation and diseases of the lungs and bladder, decoction of leaves are also used; sometimes seeds are also used. The sap is used in the treatment of warts [6]. Their fruit were determined using free radical scavenging activities and ferric reducing activities [7, 8]. Tissue parameters such as non-protein sulfhydryl groups (NP-SH), malonaldehyde (MDA) and total protein (TP) were also measured for signifying the hepatoprotective potential of Ficus palmata. Ficus palmata total extract showed complete recovery of kidney cells with no histopathological changes [8]. The ethanolic bark extracts of Ficus palmata showed significant antimicrobial activity against Staphylococcus aureus [9].

The present evaluation of various quantitative standards will be helpful for standardizing the drug for its various pharmacological potentials, to ascertain its identity, to establish the quality and purity of this plant material in closely related species and to check the adulteration.

MATERIAL AND METHOD

Procurement of plant material

The stem bark collected from the local area of Chamoli Garhwal and was authenticated by Botanical Survey of India, Dehradun. A voucher specimen was deposited at the Department of Uttaranchal institute of Pharmaceutical sciences Uttaranchal University, Dehradun for future reference.

Chemicals and reagents

All solvents used were of analytical grade and purchased from Rankem New Delhi. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and L-ascorbic acid, Gallic acid were purchased from Sigma Chemical Co. (USA). Potassium ferricyanide, trichloroacetic acid (TCA) and ferric chloride, Aluminium chloride, potassium acetate were purchased from CDH, New Delhi. Sulphanilic acid and napthylelene diamine dichloride were purchased from Rankem, New Delhi. Folin Ciocalteu's reagents were procured from E-merck (India) Ltd, Mumbai.

Preparation of extract

Stem bark were separated from wood with the help of an axe and used for extraction. The collected materials were washed thoroughly in water, chopped, air-dried for a week at 35-40°C and pulverized in electric grinder. The powdered plant material was subjected to successive extraction with petroleum ether, ethyl acetate, methanol and water using soxhlet extractor for 24 hours with each solvent. The extracts were concentrated under reduced pressure in a rotary evaporator and dried. The percentage extractive values were recorded. The dried extracts were placed in desiccators for further studies.

In vitro antioxidant studies

All the extracts were tested for their free radical scavenging property using different in vitro models. All experiments were performed thrice and their results averaged. L-Ascorbic acid was used as standard control in each experiment. Results were expressed in IC_{50} values.

DPPH radical scavenging activity [10]

DPPH radical scavenging activity was performed according to the method of Blois, 1958 with some modifications. The 0.1 mM solution of DPPH in methanol (22.2 mg in 1000 ml) was freshly prepared. Different concentrations of extract and standards (25-400 µg/ml) were added at an equal volume to methanol solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Radical scavenging activity was calculated by the following formula (formula 1)

$$\% \text{ inhibition} = A_{control} - A_{test} / A_{control} \times 100$$

Where, $A_{control}$ = Absorbance of control reaction and A_{test} = Absorbance of samples of extracts.

Nitric oxide radical scavenging activity [11, 12]

Nitric oxide radical scavenging activity was performed according to the method of Garrat, 1964 with some modifications. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations (25-400 µg/ml) and the mixture was incubated at 25°C for 150 min From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 ml naphthyl-ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm. The nitric oxide radicals scavenging activity was calculated by the formula 1.

Determination of Total Phenolic Content

The total phenolic content of the extracts was evaluated by spectrophotometric method (Barreira et al., 2008), measuring the absorbance at 765 nm. 1 ml of sample (concentration 1 mg/ml) was mixed with 1 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and made up to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve (20–100 l g/ml, y =0.0057x + 0.1221, R^2 =0.9929) and the results were expressed as l g of gallic acid equivalents/ mg of extract (GAEs).

Determination of Total Flavonoid content

Flavonoid content in the extracts was determined by spectrophotometric method (Barreira et al., 2008). The extract (concentration 1 mg/ml) was mixed with 1.25 ml of distilled water and 75 µl of a 5% NaNO2 solution. After 5 min, 150 µL of 10% AlCl3 solution was added. After 6 min, 500 µl of 1MNaOH and 275 µl of distilled water were added to prepare the mixture. The solution was mixed well and the absorbance was read at 510 nm. (±)-catechin was used for constructing the standard curve (20–180 lg/mL, y= 0.016x + 0.0185, R^2 = 0.9984) and the results

were expressed as µg of (±)-catechin equivalents (CE) per mg of extract.

Determination of Total Tannin Content

Tannin content of the extracts was measured by Folin–Denis method (Oyaizu, 1986). The various extracts (50 ml) were made up to 7.5 ml by adding double distilled water. 0.5 ml Folin–Denis reagent and 1 ml of Na2CO3 were added to it. Again the volume was made up to 10 ml with double distilled water. Absorption was recorded at 700 nm. Tannic acid was used to calculate the standard curve (20–100 µg/ ml, y = 0.0054x + 0.1641, R^2 = 0.9978) and the results were expressed as µg of tannic acid equivalents (TAE) per mg of extract.

RESULT

Extractive value of different plant extract *Ficus sarmentosa* stem bark showed highest extractive value with methanol followed by water. Least extractive value was reported with petroleum ether shown in Table 1.

Table 1: Extractive values of *Ficus palmata Forssk stem* bark

Extract	Extractive Value (% w/w)
Petroleum ether Extract	1.60
Ethyl Acetate Extract	4.43
Methanol Extract	10.2
Aqueous Extract	6.89

Antioxidant activity

In present study we have evaluated the antioxidant activity of different extracts of Ficus palmate Forks stem bark. Various concentrations of extracts ranging from (25-400 µg/ml) were tested for their antioxidant activity in different in vitro models.

Among all the extracts tested for in vitro antioxidant activity using DPPH and nitric oxide radical scavenging activity ; methanol and ethyl acetate extract has shown maximum antioxidant activity with IC_{50} values of 85.919 ± 1.976 and 98.931 ± 2.486 in DPPH assay and 74.243 ± 1.987 and 93.457 ± 2.487 in Nitric oxide radical scavenging assay (Table 2).

Table 2: IC_{50} values of DPPH and Nitric oxide free radical scavenging activity of standard and various extracts

Extract	DPPH	NO
Ascorbic Acid(AA)	57.676 ± 1.92	41.222 ± 2.546
Petroleum ether(PE)	144.972 ± 3.267	175.730 ± 3.567
Ethyal acetate (EA)	98.931 ± 2.486	93.457 ± 2.487
Methanol (Me)	85.919 ± 1.976	74.243 ± 1.987
Chloroform (Ch)	287.413 ± 2.089	306.206 ± 3.987

The antioxidant activity increased with increasing concentration in all the models. The percentage inhibition of standard and extracts in various models DPPH and nitric oxide scavenging assay as shown in Figure 1 & 2. Reducing power of extracts was concentration dependent. Higher absorbance indicates more reducing power.

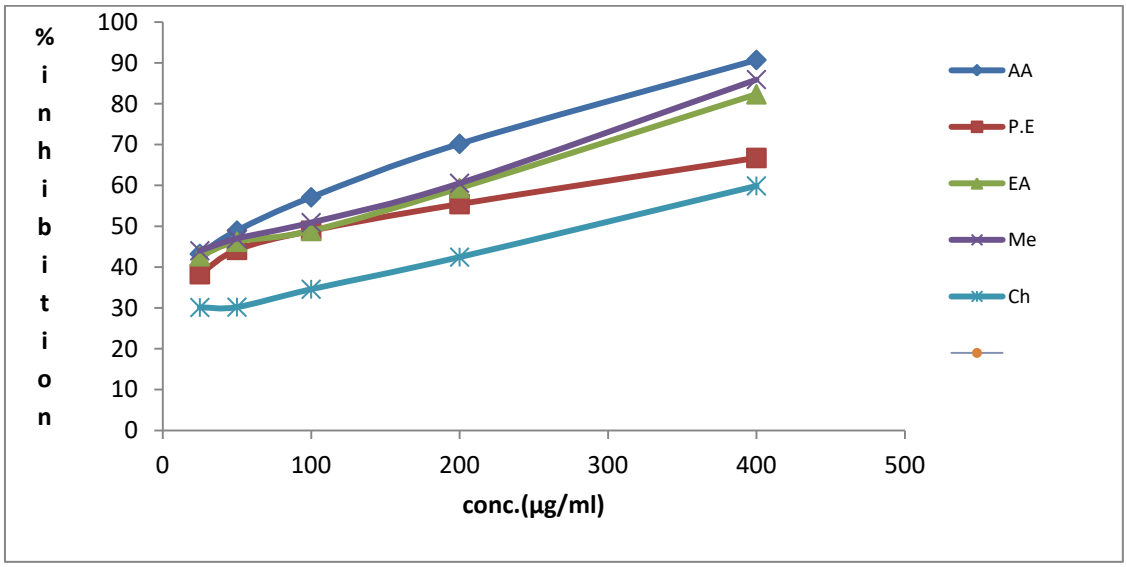

Figure 1: DPPH radical scavenging effect of Ascorbic acid and various extracts of Ficus palmata stem Bark

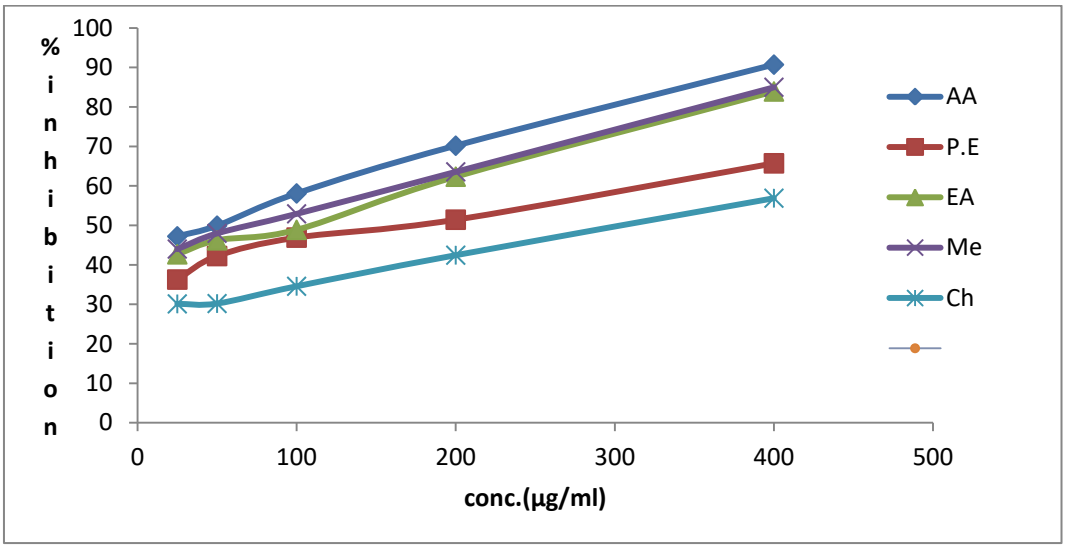

Figure 2: Nitric oxide scavenging activity of Ascorbic acid and various extracts of Ficus palmate stem Bark

Total phenolic content

The standard ascorbic acid and extracts were determined for its total phenolic content on the basis of its ascorbic acid equivalent Folin-Ciocalteu phenol assay. The amount of ascorbic acid equivalent was determined from the calculation of calibration curve of ascorbic acid (Figure 3).

The methanol extract recorded the highest content of about 101.71 µg/mg of extract. The next highest was observed in aqueous extract. The least content was reported in petroleum ether extract the result shown in Table no-4.

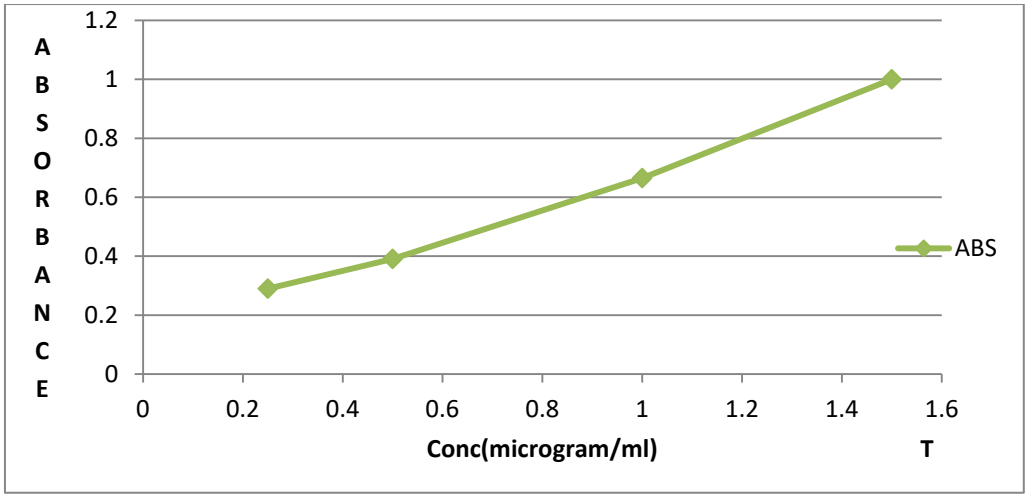

Figure 3: The calibration curve of standard gallic acid.

Table 4: Total phenolic content of various extracts of *F Ficus. palmata Forssk*

Extract	Total Phenolic Content* (µg/mg of extract -GAE)
Petroleum Ether	40.91 ± 0.20
Ethyl Acetate	52.42 ± 1.40
Methanol	101.71 ± 1.14
Aqueous	78.75 ± 1.12

Total flavonoid content

The methanol extract reported the highest content for flavonoid too (140.79 µg/mg of extract), then stood the ethyl acetate and aqueous extracts. Least value was obtained for petroleum ether extract the result shown in Table -5.

Table 5: Total flavonoid content of various extracts of *Ficus sarmentosa*

Extract	Total Flavonoid Content* (µg/mg of extract - CE)
Petroleum Ether	54.44 ± 2.12
Ethyl Acetate	125.16 ± 2.80
Methanol	140.79 ± 3.34
Aqueous	112.31 ± 1.30

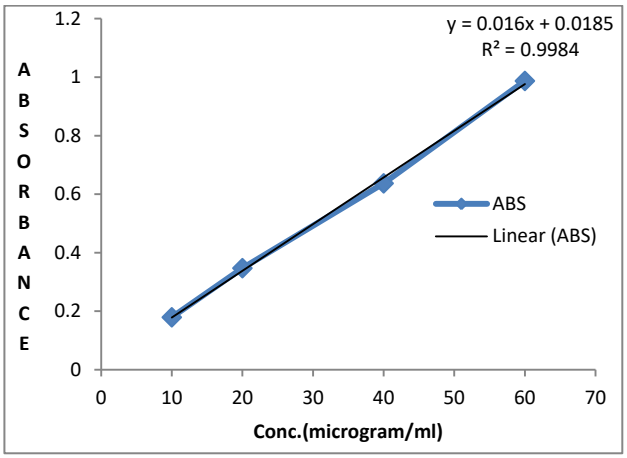

Figure 4: The calibration curve of standard Catechin.

Estimation of tannins

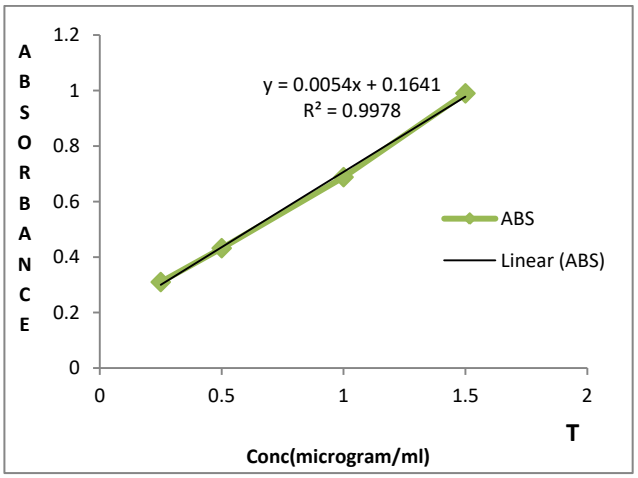

Figure 4: The calibration curve of standard Tannic acid.

The highest value was reported for the methanol extract (106.21µg/mg) followed by ethyl acetate, water and petroleum ether extracts result shown in Table-6.

Table 6: Total tannin content of various extracts of *Ficus. palmata Forssk*

Extract	Total Tannin Content* (µg/mg of extract - TAE)
Petroleum Ether	33.48 ± 3.31
Ethyl Acetate	70.30 ± 3.17
Methanol	106.21 ± 4.45
Aqueous	67.48 ± 13.07

*All trials were carried out in triplicate. The values are means of three replicates with standard deviations (mean ± S.D.; n = 3), $p < 0.05$.

DISCUSSION

Phenolics are important plant secondary metabolites with antioxidant activity owing to their redox potential, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[13].In previous studies the aqueous and ethanolic extracts of *Ficus. palmata Forssk* have shown significant antioxidant activity. The % free radical scavenging activity gradually increases with increasing concentrations of *Ficus. palmata Forssk* extracts in DPPH radical scavenging assay. Dose dependent antioxidant activity pattern was also observed in phosphomolybdate assay. Antioxidant activity was directly correlated with the amount of total phenolic contents in the extracts [14]. Approaches can be made to identify the individual polyphenolic compounds that are responsible for the antioxidant properties.

DPPH is very convenient for the screening of number of samples of different polarity. The measurement of the scavenging of DPPH radical allows determining the intrinsic ability of substance to donate hydrogen atom or electrons to this reactive species in a homogenous system [15]. Methanolic DPPH solution get reduced because of the presence of antioxidant substances having hydrogen-donating groups such as phenols and flavonoid compounds due to the formation of non-radical DPPH-H form [16].

Nitric oxide is a diffusible free radical that plays an important role as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antitumor activities [17]. Although, NO is involved in host defence, over production of these radical contributes to the pathogenesis of various diseases [18]. The petroleum ether extract and ethyl acetate extract significantly inhibited NO in a dose dependent manner with the IC_{50} being 72.39±1.53 and 94.81±2.56 µg/ml as compared with the standards ascorbic acid having the IC_{50} values of 42.8±2.61 µg/ml. The results indicated that both petroleum ether and ethyl acetate extract contain the compounds which are able to inhibit NO and act as an antioxidant.

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action [19]. In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe3+ to Fe2+ by donating an electron [20]. Being good electron donors, a phenolic compound shows the reducing power and has ability to convert ferric ion to ferrous ion by donating an electron [21]. Increasing absorbance at 700 nm indicates an increase in reductive ability [22].

In our study, the different extracts of *F.Palmata* showed high total antioxidant and DPPH activity, NO and ferrous iron reducing capacities. Considerable correlations were observed between DPPH and NO scavenging, reducing power and total phenols.

Structurally, phenols comprise an aromatic ring having one or more hydroxyl groups. The antioxidant activity of this type of molecule is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons or chelate metal cations [23].

CONCLUSION

Amongst all the extracts, methanol and ethyl acetate extracts of *Ficus palmata* showed potent antioxidant potential as determined by different procedures. Presence of phenolic compounds in the extracts confirmed their utility as potent antioxidant agent.

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REFERENCES

1. Shoib A,Baba,Shahid A, Malik,Determination of total phenolic and flavonoid content,antimicrobial and antioxidant activity of root extract of Arisaema jacquemontii Blume,Journal of Taibha University for Science, 9, 2015, 449-454.
2. Negi A, Sharma N, Pant R, Farswan MS, Determination of total phenol content of the stem bark of Bauhinia variegate Linn;An approach to standardization, The Pharma Research , 7(2), 2013, 16-22.
3. Souhila Mahmoudi, Mustapha Khali, Abderahim Benkhaled, Karima Benamirouche,Imen Baiti, phenolic and flavnoid contents, antioxidant and antimicrobial activities of leaf extracts from Algerian Ficus carica L.varieties,Asian Pacific Journal of Tropical Biomedicine, 6 (3), 2016, 239-245.
4. Hertog MGL, Feskens EJM, Kromhout D, Hollman PCH, Katan MB. 1993. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. Lancet 342, 1007–1011.
5. Pratt, D.E., Hudson, B.J.F., 1990. Natural antioxidants not exploited commercially. In: Hudson, B.J.F. (Ed.), Food Antioxidants, first ed., Elsevier, Amsterdam, pp. 171 -192.
6. Negi A, Rawat A, Standardization and preliminary phytochemical screening of Ficus Palmata Forssk stem barks, J Pharm Biol Sci 5(1), 2017, 15-20.
7. Saini R, Garg V, Dangwal K. comparative study of three wild edible fruits of uttarakhand for antioxidant, antiproliferative activities and polyphenolic composition. International Journal of Pharma and Bio Sciences 3(4), Oct 2012, 158-167.
8. Alqasoumi SI, Basudan OA, Al-Rehaily AJ, Abdel-Kader MS. Phytochemical and pharmacological study of Ficus palmate growing in Saudi Arabia. Saudi Pharmaceutical Journal. In Press; http: // www. sciencedirect. Com /science /article / pii /S1319016413001217.
9. Saklani S, Chandra S. Antimicrobial activity, nutritional profile and quantitative study of different fractions of Ficus palmata. International Research Journal of Plant Science 2(11), November 2011, 332-337.
10. Blois MS, Antioxidant determinations by the use of a stable free radical. Nature, 29, 1958, 1199-2000.
11. Garrat DC, The Quantitative Analysis of Drugs. Chapman and Hall.1964 pp. 456.
12. Oyaizu, M. (1986). Studies on products on browning reaction prepared from glucose amine. Japanese Journal of Nutrition 44, 1986, 307-315.
13. Meghashri, S., Kumar, V. & Gopal, S. Antioxidant properties of a novel flavonoid from leaves of *Leucas aspera*, Food Chemistry, 122, 2010, 105–110.
14. Mishra A, Kumar S, Bhargava A, Sharma B, Pandey AK, Studies on in vitro antioxidant and antistaphylococcal activities of some important medicinal plants, Cellular and Molecular Biology, 57(1), 2011, 16-25.
15. Gautam B, Vadivel V, Stuetz W, Biesalski HK, Bioactive compounds extracted from Indian wild legume seeds: antioxidant and type II diabetes related enzyme inhibition properties, International Journal of Food Sciences and Nutrition, 2011, 1-4.
16. Mensour, L.L., Menezes, F.S., Leitao, G.G., Reis A.S, Dos Santos, T.C. and Coube, C.S. (2011). Screening of Brazilian Plant Extracts for Antioxidant Activity by Use of DPPH Free Radical Method. Phytotherapy Research 15, 127-130.
17. Hagerman, A.E., Riedel, K.M., Jones, G.A., Sovik, K.N. Ritchard, N.T. Hartzfeld P.W. Riechel, T.L., (1998): High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of Agriculture and Food Chemistry 46, 1887-92.
18. Guo, L., Fatig, R.O., Orr, G.L., Schafer, B.W., Strickland, J.A. and Sukhapinda, K. (1999). Photorhabdus luminescens W-14 insecticidal activity consists of at least two similar but distinct proteins. Indian Journal of Experimental Biology. 274, 9836-9842.
19. Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F., Fazelian, M. and Eslami, B. (2009). In vitro antioxidant and free radical scavenging activity of Diospyros lotus and Pyrus boissieriana growing in Iran. Pharmacognosy Magazine 4(18), 123-127.
20. Ebrahimzadeh, M.A., Nabavi, S.M., Nabavi, S.F., Bahramian, F. and Bekhradnia, A.R. (2010). Antioxidant and free radical scavenging activity of H. officinalis, Var. angustifolius, V. odorata, B. hyrcana and C. speciosum. Pakistan Journal of Pharmaceutical Sciences 23(1), 29-34.
21. Kar, A. and Borthakur, S.K. (2008). Medicinal plants used against dysentery, diarrhea and cholera by the tribes of erstwhile Kameng district of Arunachal Pradesh. Natural Product Radiance 7, 176181.
22. Sravani, T. and Paarakh, P.M. (2012). Antioxidant activity of Hedychium spicatum Buch.-Ham. Rhizomes. Indian Journal of Natural Products and Resources 3(3), 354-58.
23. Amarowicz, R., Pegg, R.B., Rahimi, M., Barl, B. and Weil, J.A. (2004). Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies, Food Chemistry. 84, 551–562.

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