

Evaluation of Antioxidant and Antimicrobial Activity of *Ficus Carica* Leaves: an *In Vitro* Approach

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Abstract

Ficus carica L. (fig) belongs to the mulberry tree (Moraceae) which is one of the oldest fruits in the world. It is used in our traditional system of medicine for healing various diseases. In a continuous bid to explore new biocompatible antioxidant and antimicrobial with the least associated side-effects *Ficus carica* were tested against pathogenic microorganisms; *S. epidermidis*, *K. Pneumoniae*, *B. Subtilis*, *E. aerogens*, and *B. cereus*. The methanol extracts were prepared and screened for *in-vitro* antioxidant activities an antimicrobial activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and agar well diffusion method respectively. In addition, extract of *Ficus carica* prepared by soxlet apparatus and were partially purified by preparatory Thin Layer Chromatography (TLC). In conclusion, the results indicate antimicrobial activity of the extract which could be further explored for purification of antioxidant compounds.

Keywords: *Ficus carica*, Antioxidant, Free Radicals, Antimicrobial, DPPH, Well Diffusion Method

Introduction

For promoting the use of medicinal plants as useful sources of antioxidant and antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their uses.

Thousands of plants are there, that are well known in traditional medicine system for their medicinal and therapeutic potentials worldwide. Similarly, *Ficus carica* is also one of the medicinally important plant that belongs to the mulberry tree (Moraceae) and also one of the oldest fruits in the world. *Ficus carica* is commonly known as Anjir [India], figs [UK], teen [Arabic]. Figs (*Ficus carica* Linn.) are cultivated for over 11,000 years according to literature survey and can almost certainly predate for human use. Figs are the earliest cultivated plants [1] and used in our traditional system of medicine for healing various diseases like, diabetes, ulcer, cancer, fever etc., [2]. Its strong antioxidant and biological properties are also known to diffuse the toxic free radical. It can also be used as a possible food additive or in nutraceutical and biophar, also used as a digestion promoter and a cure for ulcerative inflammation and eruption in Korea [3]. For the evaluation of the anti-pyretic effect of an ethanol extract of leaves of *Ficus carica* Linn, a study was carried out at normal body temperature and yeast-induced pyrexia, in albino rats [4].

Leaves of *Ficus carica* shows high degree antimicrobial activity of methanol extract against oral bacteria. The combination effects of MeOH extract with ampicillin or gentamicin are synergistic against oral bacteria [3]. The methanol extracts made from bark, fruits and leaves of *F. carica* consists strong antioxidant activity demonstrated by the four different methods including DPPH and ABTS+free radicals scavenging, PMS-NADH system superoxide radical scavenging and β -carotene-linoleic acid system. Psoralen and bergapten (5-methoxy-psoralen) are two photoactive furanocoumarins reported in *F. carica* leaves. All but especially psoralen are used along with UV radiation in the treatment of skin diseases, such as skin depigmentation, mycosis fungoides, polymorphous dermatitis and eczema. Latex extracts of *F. carica* contain strong anti-angiogenic and anti-proliferative activities. It means that latex extract could be used as a potential agent for the

prevention of angiogenesis in cancer and other chronic disorders [5]. Immunomodulatory effect of ethanolic extract of the leaves of *Ficus carica* (Moraceae) was investigated in mice, which shows promising immunostimulant properties [6].

Ficus carica latex also has been studied for its antimicrobial activity in this Methanolic, hexanoic, chloroformic and ethyl acetate extracts of

properties against many bacteria and fungi [7]. Antimicrobial activity of methanol (MeOH) extract of fig leaves against methicillin-resistant *Staphylococcus aureus* (MRSA) isolated in clinic and leaves could be employed as a natural antibacterial agent in MRSA infection care products [3]. Leaves contain bergapten, 4',5'-dihydro-psoralen, rutin, 24-methylene cycloartenol umbelliferone, marmesin, stigmasterol, β -sitosterol, ficusogenin, lupeol, psoralen ψ -taraxasterol ester and tyrosine moisture, protein, Umbelliferone tyrosin fat, beta-sitosterol, crude fiber, ash, N-free extract Ficusin, pentosans, carotene on a dry weight basis [1-3,6,8-10] introduced significant hepatoprotective activity with the petroleum ether (60-80°C) leaf extract of *Ficus carica* Linn. with rifampicin induced hepatic damage on rats [11]. Its pharmacological actions include antibacterial, antioxidant, anti-inflammatory, vulnerary, antitumor, anticancer, antispasmodic, immunobalancing/immunoharmonizing, antidiabetic, antihelminthic, antiplatelet, hepatoprotective, hypocholesterolemic activity, Antispasmodic activity Haemostatic effect and antipyretic activities [12-18].

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Material and Methods

Chemicals

Methanol (merck india), Thin layer chromatography (TLC) plates, silicagel (merck india), DPPH (Merck india) peptone (Merck India), beef extract, agar, NaCl (Merck India). All other chemicals and reagents are of AR grade.

Test microorganism

Test microorganism which were used in this experiment are: *Klebsiella pneumoniae*, *B. cereus*, *E. aerogens*, *B. subtilis*, *S. epidermidis*, at different concentration (30, 40, 50, 60 µg/ml) concentration was used as a standard. All tested bacteria cultures were obtained and authenticated from Department of Bioscience, Integral University Lucknow, India.

Plant material

The fresh leaf of plants *Ficus carica* were collected from the wild sources herbal garden Pharmacy block integral university Lucknow, India, in the month of January and were authenticated by Faculty of Pharmacy, Integral University, Lucknow, India.

The dried leaf powder (20 gm) of plant *Ficus carica* was extracted successively with 200 ml each of methanol in a soxhlet extractor for 24 hrs. The solvent was evaporated at 30-35°C. The methanolic extracts of *F. carica* yield a dark brownish solid residue weighing 2.44 gm (12.2% w/w).

Antioxidant assay

Each sample was dissolved in 95% methanol at a concentration 1µg/ml and then diluted to prepare the series concentrations for antioxidant assays. Reference chemicals were used for comparison in all assays, all steps done in triplicate. DPPH radical scavenging activity assay: The DPPH assay was done according to the method of Brand-Williams, Cuvelier, and Berset with some modifications [19]. The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and then stored at 20°C until needed. The working solution was obtained by diluting DPPH solution with methanol to obtain an absorbance of about 0.980 (± 0.02) at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 ml of the fractions at varying concentrations (25–250 µg/ml). The solution in the test tubes were shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The scavenging activity was estimated based on the percentage of DPPH radical scavenged using the following equation: Scavenging effect (OD of control absorbance-OD of sample absorbance)/OD of control absorbance×100: EC50 value is the effective concentration that could scavenge 50% of the DPPH radicals. Ascorbic acid was used as standard.

Partial purification of the extract

The methanolic extracts of the plant were subjected to TLC (0.25 mm, 20×20 cm, SiO₂ GF254, Merck India) and developed in a solvent system of methanol:chloroform (4:1) of 150ml. The TLC of *F. carica* extracts showed 2 different bands, respectively [20,21].

Agar well diffusion method

Find the extracts from the plants for studying their antibacterial activity. A loop full of bacterial strain was inoculated in of Nutrient broth in a conical flask and incubated for 72 hrs. After solidification 0.25 ml of test strains were inoculated in the media separately and cared

for proper homogenization. The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (5 mm). Then extract compound was introduced into the well and plates were incubated at 37°C for about overnight. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of zone of inhibition.

Results and Discussion

The scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples, including plant extracts. The measured DPPH radical scavenging activity is shown in table 1 and figure 1. The *F. carica* leaves extract scavenging antioxidant activity was significantly increased with increasing concentration of the extract. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. Although the DPPH radical scavenging abilities of the extracts were significantly lower than those of ascorbic acid, it was evident that the extracts did show some proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The quality of the antioxidants in the extracts was determined by the percent inhibition values shown in table 2 and figure 2. Although the percent inhibition values of *F. carica* leaves extract were not much greater than the reference antioxidant. Lack of hydrogen donor bioactive constituents in the extract, slow rate of the reaction between DPPH and the substrate molecules resulting in low readings for antioxidant activity probably might explain the low DPPH antioxidant activity of the *F. carica* leaves extract [22-25].

Sample concentration (µg/ml)	Ascorbic acid(OD)	<i>F. carica</i> (leaves) (OD)
25 µg/ml	1.269 ± .001	1.285 ± 0.15
50 µg/ml	1.212 ± .005	1.225 ± 0.18
100 µg/ml	1.048 ± .008	1.10 ± 0.14
150 µg/ml	0.884 ± .009	1.04 ± 0.13
250 µg/ml	0.571 ± .015	1.00 ± 0.09

Table 1: Absorbance of *F. carica* methanolic leaf extract at various concentrations in DPPH mediated free radical scavenging system.

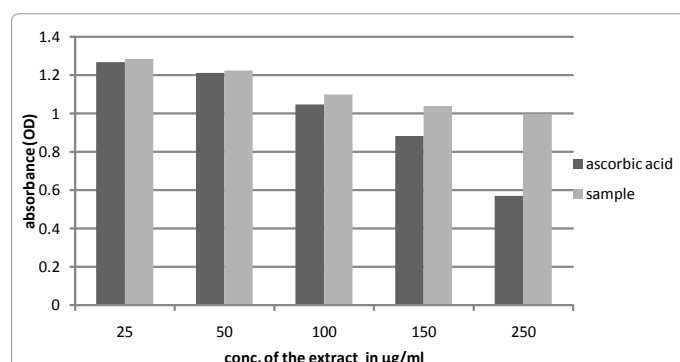


Figure 1: Absorbance of *F. carica* metabolic leaf extract at various concentrations in DPPH mediated free radical scavenging system.

Sample concentration(µg/ml)	Ascorbic Acid (%scavenging inhibition)	<i>F. carica</i> (leaves) (% scavenging inhibition)
25	7.1	0.1822
50	13.1	1.522
100	28.3	4.111
150	40.3	8.101
250	60.4	10.222

Table 2: Percent Inhibition of DPPH mediated free radical generation by *F. carica* methanolic leaf extract at various concentrations.

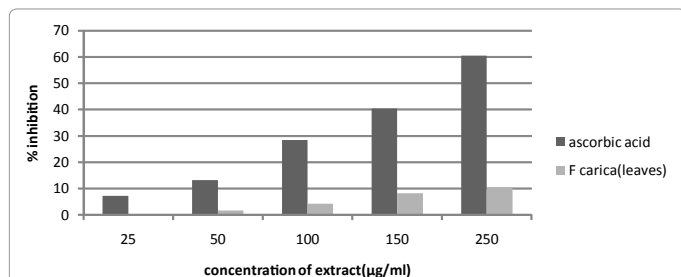


Figure 2: Percent Inhibition of DPPH mediated free radical generation by *F. carica* methanolic leaf extract at various concentrations.

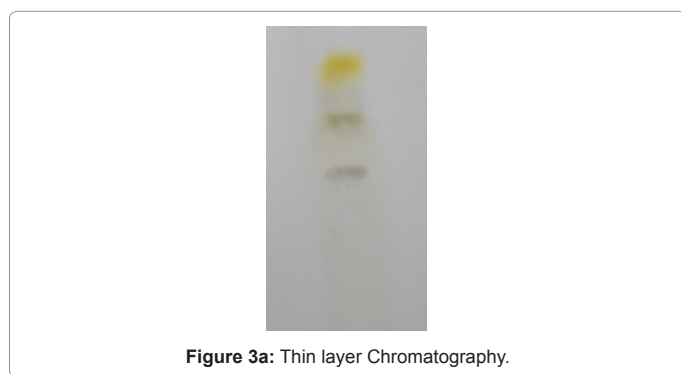


Figure 3a: Thin layer Chromatography.

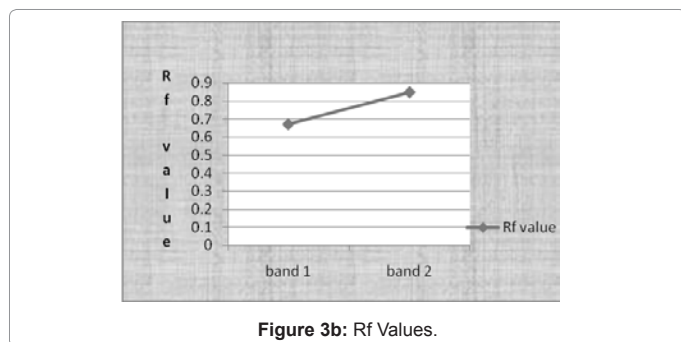


Figure 3b: Rf Values.

Thin layer chromatograph (TLC)

The TLC of methanolic extract for *F. carica* plant is shown in figure 3a and 3b with their R_f values. Based on solvent system used for TLC, silica gel column will be executed. There are two clear band was found. From the figure it is evident that there are many compounds or components that are responsible for the antioxidant activity. Hence, further investigations are required to isolate, purify and characterized those compounds which are responsible for this antioxidant activity.

Antimicrobial activity

Antimicrobial activity was determined by using agar well diffusion assay. Our results indicated a potent antioxidant and antimicrobial activity of methanolic leaf extract of *F. carica*. In the present study methanolic extracts of plant have been tested against resistant bacteria. The antimicrobial activity of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter (Table 3).

The results of antimicrobial activity of *F. carica* was encouraging and that the plant extract showed significant antimicrobial activity

S.No.	Strains	Concentrations			
		30µg/ml	40µg/ml	50µg/ml	60µg/ml
1	<i>K.pneumoniae</i>	1.2cm	1.25cm	1.3cm	1.35cm
2	<i>B.cereus</i>	1.15cm	1.20cm	1.25cm	1.3cm
3	<i>B.subtilis</i>	1.1cm	1.25cm	1.3cm	1.35cm
4	<i>S.epidermidis</i>	1.2cm	1.25cm	1.30cm	1.40cm
5	<i>E.aerogens</i>	1.05cm	1.1cm	1.2cm	1.25cm

Table 3: Zone inhibition by *F. carica*.

S.No.	Strains	MIC	MBC
1	<i>K. pneumoniae</i>	7µg/ml	11µg/ml
2	<i>B. cereus</i>	3µg/ml	6µg/ml
3	<i>S. epidermidis</i>	4µg/ml	7µg/ml
4	<i>B. subtilis</i>	6µg/ml	11µg/ml
5	<i>E. aerogens</i>	3.5µg/ml	8µg/ml

Table 4: MIC/MBC of various bacterial strains by *F. carica*.

against different bacterial strains. Antimicrobial activity of methanolic extract of *F. carica* against five bacterial strain *B. cereus*, *E. aerogens*, *K. pneumoniae*, *B. subtilis*, *S. epidermidis* at different concentration (30, 40, 50, 60 µg/ml) was found in the following decreasing order *S. epidermidis*>*K. pneumoniae*>*B. subtilis*>*B. cereus*>*E. aerogens* (Table 3).

Determination of antibacterial activity by agar well diffusion assay showed that methanolic extract of *F. carica* leaves exhibited the antibacterial effect against pathogenic as well as non-pathogenic test bacteria. Significant effect on growth inhibition of gram positive and gram negative bacteria was also noticed. It was noted that among all tested organisms: the gram positive bacterial strain *S. epidermidis* registered maximum susceptibility to the methanolic extract at 60 µg/ml of the entire *F. carica* leaves, with the maximum inhibitory zone of 1.40 cm. MIC/MBC of various bacterial strains by *F. carica* are shown in table 4.

Phytochemical analysis of the extract denoted the presence of flavonoids and phenols. The presence of these compounds was detected by Thin Layer Chromatography. The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to evaluate of possible synergism among extract components for their antioxidant and antimicrobial activity.

Conclusion

In conclusion, *Ficus* species are rich source of naturally occurring antioxidant and antimicrobial activity. Its compound play a vital role in preventing innumerable health disorders related to oxidative stress including cardiovascular diseases, neurodegenerative diseases and cancer. Our results indicated a significant increase in antioxidant activity of *Ficus carica* leaves extract. In addition, the extract also showed potent antimicrobial activity with all the bacterial strains with maximum inhibition against *S. epidermidis*. These results encourage the researchers to do further *in vitro* and *in vivo* research that will explore the role of bioactive constituents responsible for these activities. Hence, further studies are needed to evaluate the antioxidant activity and antimicrobial activity of their partially purified fractions. Further studies are also needed at molecular level.

References

1. Kislev ME, Hartmann A, Bar-Yosef O (2006) Early domesticated fig in the Jordan valley. Science 312: 1372-1374.

2. Sirisha N, Sreenivasulu M, Sangeeta K, Madhusudhana Chetty C (2010) Antioxidant properties of *Ficus* species—A Review. Int J PharmTech Res 2: 2174-2182.
3. Jeong MR, Kim HY, Cha JD (2009) Antimicrobial activity of methanol extract from *Ficus carica* leaves against oral bacteria. J Bacteriol Virol 39: 97-102.
4. Patil VV, Bhargale SC, Patil VR (2010) Studies on immunomodulatory activity of *Ficus carica*. Int J Pharm Pharm Sci 2: 97-99.
5. Mostafaie A, Mansouri K, Norooznezhad AH, Mohammadi-Motlagh HR (2011) Anti-angiogenic activity of *Ficus carica* latex extract on human umbilical vein endothelial cells. Cell J 12: 525-528.
6. Patil VV, Bhargale SC, Patil VR (2010) Evaluation of anti-pyretic potential of *Ficus carica* leaves. Int J Pharm Sci Rev Res 2: 48-50.
7. Aref HL, Salah KB, Chaumont JP, Fekih A, Aouni M, et al. (2010) *In vitro* antimicrobial activity of four *Ficus carica* latex fractions against resistant human pathogens (antimicrobial activity of *Ficus carica* latex). Pak J Pharm Sci 23: 53-58.
8. Young-Soo L, Cha JD (2010) Synergistic antibacterial activity of fig (*Ficus carica*) leaves extract against clinical isolates of Methicillin-resistant *Staphylococcus aureus*. Kor J Microbiol Biotechnol 38: 405-413.
9. Li C, Bu PB, Yue DK, Sun YF (2006) Chemical constituents from roots of *Ficus hirta*. Zhongguo Zhong Yao Za Zhi 31: 131-133.
10. Patil VV, Patil VR (2011) Evaluation of anti-inflammatory activity of *Ficus carica* Linn. leaves. Indian Journal of Natural Product and Resources 2: 151-155.
11. Gond NY, Khadabadi SS (2008) Hepatoprotective activity of *Ficus carica* leaf extract on rifampicin-induced hepatic damage in rats. Indian J Pharm Sci 70: 364-366.
12. Chang MS, Yang YC, Kuo YC, Kuo YH, Chang C, et al. (2005) Furocoumarin glycosides from the leaves of *Ficus ruficaulis* Merr. var. *antaensis*. J Nat Prod 68: 11-13.
13. Perez C, Canal JR, Torres MD (2003) Experimental diabetes treated with *Ficus carica* extract: effect on oxidative stress parameters. Acta Diabetol 40: 3-8.
14. Joseph B, Raj SJ (2011) Pharmacognostic and phytochemical properties of *Ficus carica* Linn—An overview. Int J PharmTech Res 3: 8-12.
15. Rubnov S, Kashman Y, Rabinowitz R, Schlesinger M, Mechoulam R (2001) Suppressors of cancer cell proliferation from fig (*Ficus Carica* Linn.) resin: isolation and structure elucidation. J Nat Prod 64: 993-996.
16. Saeed MA, Sabir AW (2002) Irritant potential of triterpenoids from *Ficus carica* Linn. leaves Fitoterapia. J Nat Prod 73: 417-420.
17. Vinson JA (1999) The functional food properties of figs. Cereal Foods World 44: 82-87.
18. Wu PL, Rao KV, Su CH, Kuoh CS, Wu TS (2002) Phenanthroindolizidine alkaloids and their cytotoxicity from the leaves of *Ficus septica*. Heterocycles 57: 2401-2408.
19. Bondet V, Brand-Williams W, Berset C (1997) Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. LWT—Food Sci Technol 30: 609-615.
20. Canal JR, Torres MD, Romero A, Perez C (2002) A chloroform extract obtained from a decoction of *Ficus carica* leaves, improve the chlosterolaemia of rats with streptozocin-induced diabetes. Acta Physiol Hung 87: 71-76.
21. Innocenti G, Bettero A, Caporale G (1982) Determination of the coumarinic constituents of *Ficus carica* Linn. leaves by HPLC. II Farmaco Edizione Scientifica 37: 475-485.
22. Ahmed F, Khan RA, Rasheed S (1992) Study of analgesic and anti-inflammatory activity from plant extracts of *Lactuca scariola* and *Artemisia absinthium*. J Islamic Acad Sci 5: 111-114.
23. Richter G, Schwarz HP, Dörner F, Peter L (2002) Activation and inactivation of human factor X by proteases derived from *Ficus carica* Linn. British Journal of Haematology 119: 1042-1051.
24. Stepek G, Buttler DJ, Duce IR, Lowe A, Behnke JM (2005) Assessment of the anthelmintic effect of natural plant cysteine proteinases against the gastrointestinal nematode, *Heligmosomoides polygyrus*, *in vitro*. Parasitology 130: 203-211.
25. Gilani AH, Mehmood MH, Janbaz KH, Khan AU, Saeed SA (2008) Ethnopharmacological studies on antispasmodic and antiplatelet activities of *Ficus carica*. J Ethnopharmacol 119: 1-5.