

Research Article

Antimicrobial, antioxidant, phytochemical and pharmacognostic study of the leaf powder of *Ficus carica* L.

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Citation

Hussan Ara Begum, Muhammad Hamayun, Mamoona Rauf, Humaira Gul, Kishwar Ali, Waqar Khan, Mark Schulze and Mohib Shah. Antimicrobial, antioxidant, phytochemical and pharmacognostic study of the leaf powder of *Ficus carica* L. Pure and Applied Biology. Vol. 9, Issue 1, pp999-1008.

<http://dx.doi.org/10.19045/bspab.2020.90105>

Received: 04/09/2019

Revised: 01/12/2019

Accepted: 02/01/2020

Online First: 18/01/2020

Abstract

Ficus carica L., (Moraceae), commonly found in tropical and subtropical areas, known for its ethno-medicinal uses. The current work aimed to study macro and microscopic features and screen the plant ethanol extract for its antioxidant and antimicrobial potential. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was used for antioxidant and Agar-well-diffusion method for screening antimicrobial activity. The results revealed that powdered drug possess unicellular, uniseriate covering trichomes, parenchymatous tissue containing spiral vascular strands, anomocytic stomata and calcium oxalate crystals. Phytochemically, the extract possesses reducing sugars, polysaccharides, oxalic acid, amino acid and proteins. The antioxidant activity of *Ficus carica* leaves ethanol extract, using various concentration (125, 250, 500, 750 and 1000 µg/ml) gave the results of 21.42±01, 29.65±03, 53.15±03, 57.00±01 and 62.99±05, respectively. The leaves ethanol extract used for antibacterial activity have concentrations of 200 and 500 mg/ml, both of which were found effective against the selected bacterial strains. *K. pneumonia* was inhibited (18 & 28mm), *E. coli* was found (20 & 26 mm) susceptible, *Staphylococcus aureus* was inhibited with (24 and 26mm) and *Pseudomonas aeruginosa* was inhibited by (22 and 28mm). It is concluded from the present study that leaf ethanol extract of *Ficus carica* has good antioxidant constituents and possess positive antimicrobial chemical metabolites. These metabolites can be used to treat various infectious and chronic diseases caused by microbes. This work is important in order to assess the significance of *Ficus carica* used locally. More research is required to isolate these metabolites to establish clinical trials for human benefits.

Keywords: Antimicrobial; Antioxidant; *Ficus Carica*; Medicinal Plants; Phytochemicals

Introduction

Plants are recognized as the primary source in traditional care systems due to their curative potency. Likewise, *Ficus carica* L.,

a member of the mulberry tree family (Moraceae) is medicinally important and the oldest known fruit tree to humans [1]. *Ficus carica* is known with various names in

different languages, e.g. (Anjir-Urdu), (figs - English), (teen-Arabic), collective (fig-tree), (Hindi-angir), (Sanskrit-angira), (Pashto-Inzir), (Punjabi-fagar) [2, 3].

Ficus has been used as a common medicinal plant for various ailments. *Ficus* sap has been used in many intoxicants of superstitions by Peruvian Shamans [4]. The Genus *Ficus* comprises more than 800 species of the family Moraceae. Several *Ficus* types comprise of several selections, vital in genetic variety and outstanding biological actions [5]. It is believed that the plant has been imported from western Asia to the Mediterranean. The plant is predominantly cultivated as a cash crop in characteristically slightly cold weather with dry hot season in countries like USA, Brazil, Turkey, and some Middle Eastern countries [6].

Ficus carica is globally cultivated for its edible fruits. Its fruits can be consumed fresh, dehydrated, preserved, or in other conserved forms. *Ficus* is one among the therapeutic vegetation principally arising in sub-tropical and tropical areas around the globe [7]. Various parts of the plant like wood, green leaves, soft branches, berries and sap are used for therapeutic and medicinal purposes. The fruit of the plant is nutritious and is of high commercial value. Vitamins, minerals, calcium, fibres, fats and water are in abundance in the fruit. Conferring to United State Department for Agriculture (USDA) the Task Diversity, dehydrated fruits are rich in fibre, copper, manganese, magnesium, potassium, calcium and vitamin K, absolute to man requirements, possess lesser quantities of several supplementary nutrients. The plant is cathartic and comprises various antioxidants, flavonoids and polyphenols [8]. Certain bioactive complexes such as arabinose, β -amyryns, β -carotenes, glycosides, B-sitosterol and xanthotoxol have been found in the plant [9, 10]. The dehydrated fruits have a substantial rise in plasma antioxidant dimensions and used in

many ailments like abdominal, breathing, provocative, cardiac problems, ulcers and cancers [11, 12]. In curing leucoderms and ringworm diseases, root is used locally while fruits possess antipyretic, purgative, aphrodisiac characteristics and relieving in irritation [13]. *Ficus carica* has been described as a good antioxidant, effective antiviral, antibacterial, antidiabetic, beating cancer and anthelmintic [14, 15]. Locally the plant is used in different illnesses like stomach problems, throat problems and normal tummy pain due to indigestion [16]. Research work has been done on dry and fresh fig fruit and leaves [17]. Various biological activities have been carried out on different parts of *Ficus carica* [18]. The current research aims at the antibacterial, antioxidant activities, phytochemical analysis and pharmacognostic examination of leaf powder of the medicinal plant *Ficus carica*.

Distribution

The Fig tree is widely dispersed, found in Asia to the Americas. Countries like Turkey and Spain are the key exporters of the fruit in Europe. Parts of the United States including California and other Western coastal states have plenty of commercial orchards of the plants. Arabia, Persia, India, China and Japan are exporter the plant to a moderate level. In India, Punjab and Uttar Pradesh are the bigger producers of the Fig fruits [19].

Morphology

The tree of *Ficus carica* L. is 15-20 ft. tall, with scattering branches and stalk of 7 ft. in diameter. Plants have creamy white latex which chiefly comprises of ficin (a protein digesting enzyme). The roots are typically shallow and spreading [20]. The leaves are wide, oval or lobbed, upper surface is coarse above and juvenile underneath. Fruit is axillary, different in mass and pigment. Ripened fruit is juicy and sugary, unripe fruit is gummy [21, 22]. Seeds are of different sizes and vary from 30 to 1600 in number in

each fruit. The seeds are the real fruits in figs [23]. 26 are the normal diploid numbers of chromosome of different species are analogous to one another in appearance in all plants. The genome size of the fig is very small, less than three times that of *Arabidopsis sp.* [24, 25].

Materials and methods

Plant material collection

Experimental material was collected locally and identified with the help of available literature and experts at Abdul Wali Khan University Mardan. The leaves were separated from the plant stem, washed, cleaned and dried in an oven at 45°. After drying, the plant material (Leaves) was powdered for further use. Ethanol was added to the powdered material and kept for 3 days to get the plant extracts.

Pharmacognostic Analysis

The leaf morphology was studied under the microscope. The dried-out leaf powdered material was used for this purpose and the extracts were used for the determination of phytochemical constituents [26].

Phytochemical tests

Benedict's test for reducing sugars: To a clean and dry test tube containing 3 ml of sample solution, 2-3 ml of Benedict's reagent was added and then boiled for 5 minutes.

Iodine test for non-reducing sugars

To about 2-3 ml of the sample solution, a couple of drops of iodine dye were added.

Tannic acid test for polysaccharides

About 60ml of 20% tannic acid was added to 3-4 ml of sample solution.

Tests for Oxalic acid

1) Calcium chloride test: To the sample solution, a few drops of 5% Calcium chloride solution were added.

2) Lead acetate test: To the sample solution, a few drops of 5% Lead acetate solution were added.

Test for Amino acid

Test for cysteine: About 5ml of sample solution was boiled with a little of 40%

NaOH and a few drops of 10% Lead acetate solution.

Test for Proteins

1) Biuret test: To about 3ml of sample solution, 1 ml of 4% NaOH solution and a few drops of 1% of Copper Sulphate (CuSO₄) solution were added.

2) Test for sulphur containing protein: About 5ml of sample solution was boiled after the addition of few millilitres of 40% NaOH solution and a drop or two of 10% Lead acetate solution.

Tests for Tannins

1) A few drops of 5% Ferric chloride solution were added to the extracted solution.

2) A few drops of Acetic acid (CH₃COOH) were added to the extracted solution.

3) Potassium dichromate drops were added to the extracted solution.

Tests for Alkaloids

1) Mayer's test: To about 3ml of extract, a few drops of Mayer's reagent were added.

2) Wagner's test: To about 3ml of extract, a few drops of Wagner's reagent were added.

Antioxidant activity

1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay

To measure the free radical scavenging activity of the powdered leaf extract, we used 1, 1-diphenyl-2-picryl hydrazyl (DPPH) assay following the method of [27]. Ascorbic acid was used as a standard solution to compare with. A 0.1 mm concentration solution of DPPH in methanol was prepared by adding 1 ml of this solution to 3 ml. of the crude extract in ethanol at a different concentration (100, 250, 500, 750 & 1000 µg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for an hour; using a spectrophotometer, absorbance was measured at 517 nm. The percent DPPH scavenging effect was considered by using the following calculations.

DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$.

Where A_0 was the Absorbance of control reaction and A_1 was the Absorbance in the presence of test or standard sample [28].

Antibacterial activity

Bacterial strains

Klebsella pneumoniae, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* bacteria were used for antibacterial activity to check the leaf ethanol extract.

Preparation of inoculum

Some selected bacterial strains from a 24-hour old culture were mixed with physiological normal saline solution to get a McFarland turbidity standard [10^6 colony forming unit (CFU) ml^{-1}]. The LB Ager medium was then inoculated with the prepared bacterial samples. Preparation of Agar plates followed the agar well diffusion method [29].

Concentration of extract for activity

Two concentrations were used for activity 1mg/1ml and 5mg/1ml dissolved in DMSO. Solution of a standard antibiotic (1 mg/1ml of *Cefotaxime*) were used as a positive and DMSO as negative controls.

Pouring of test solution, incubation and measurement of zone of inhibition

75 μl of test sample was poured into a labelled micropipette. At 37 °C, incubation was done for 24 hours and the diameter of clear zones were recorded and the bacterial growth around each well was measured. The average of inhibition zone and standard deviation was calculated from three trials.

Results and discussion

Medicinal uses

Common fig has laxative, calmative, cough relieving and analgesic properties. It is actively used in the research of making laxative syrups, along with Senna and other carminatives. A decoction of the fruit is second hand in case of chills, relaxing the mucous membranes of the respiratory tract. Fresh figs can be used superficially as an emollient and in dressings in the treatment of

boils and small swellings. Sap taken out from stems and leaves is used for treating warts.

Macroscopic Analysis

Leaves were green, odourless with a slight bitter taste and fracture was horney. The leaves varied from 7-9 cm in length and 4-6 cm in width. The upper surface of the leaf is rough white while the lower one is rough and green. Other peculiar features include oval apex, cordate base, and serrate margins and reticulate venation in the leaves.

Microscopic Analysis

The transverse section of the leaf reveals a single layered epidermis and a thin and smooth cuticle. The stomata are anomocytic, trichomes are non-glandular (sometimes unicellular and uniserrate) with two layers of palisade parenchyma of mesophyll and four layers of spongy parenchyma where Calcium oxalate crystals are present. There is a biconvex midrib, angular cells of collenchyma in about 8-10 rows and the collateral vascular bundles are entrenched in the ground parenchyma.

Powder analysis

1. Trichomes were abundant and have pointed at the apexes and thick bases.
2. Parenchyma tissue with spiral vascular strands.
3. Anomocytic stomata are present.
4. 7-10 microns in diameter prismatic Calcium oxalate crystals found as free and in fragments of parenchymatous cells.

Ficus carica has simple leaves, large shape, acute tip and subcordate base, irregular deeply cut palmate leaf, irregular margin, measuring 6–18 cm long and 5–15 cm wide, petiolate, rough lamina texture and multi-convergent reticulate venation.

Behaviour of the crude powdered drug with chemical reagents

Behaviour of the crude powder obtained from leaf was analysed with different chemical reagents to detect the presence of Phyto-constituents. The results were analysed with

the colour change under ordinary daylight (Table 1).

Table 1. Behaviour of the Ficus powder with chemical reagents

Reagents	Color/ppt	Constituents
FeCl ₃	No color	Tannins absent
Iodine	No color	Starch absent
KOH	Yellow	Anthraquinone glycosides present
AgNO ₃	Ppt	Protein present
NaOH	Yellow	Flavonoid present
H ₂ SO ₄	Reddish brown	Steroids and terpenoids present

Colour reaction of the powder with different solvents and acids

Different solvents and acids react with the powdered material of the leaf of the plant with a peculiar colour (Table 2). The

powdered material was treated with different solvents, reagents and acids which gave different colours corresponding to the presence of different constituents.

Table 2. Colour reactions of the Ficus powder with different solvents and acids

S. No.	Acids	Color
01	Phosphoric acid	Dark green
02	HCl	Dark green
03	H ₂ SO ₄	Black brown
04	HNO ₃	Reddish orange
05	Acetone	Yellowish green
06	Benzene	Olive green
07	Ether	Light green
08	H ₂ O	Light yellow

Phytochemical tests

We carried out phytochemical tests with the powdered material of the leaf for the detection of compounds like reducing and non-reducing polysaccharides, oxalic acids, amino acids, proteins, tannins and alkaloids. The powdered material of leaf origin of *Ficus carica* shows the presence of reducing sugar, poly saccharides, oxalic acid, amino acid and protein while the non-reducing sugar tannins and alkaloids were not detected in the material (Table 3). Different phytochemicals have been found to possess a wide range of activities which may help in protection against different diseases. For example, alkaloids have anti-inflammatory activities; terpenoids can reduce blood sugar level.

Local uses

Locally, the fruit is used in stomach problems, bowel problems and sore throats. The leaf is used as poultice over burns and pus-filled acnes. Wood is used for fire purposes. Traditional knowledge about medicinal plants reflects the interest of local people in plant sources. Although the modern medicines are extra-ordinary in their action against diseases, medicinal plants are vital in their culinary properties [30]. Different parts of *Ficus carica* are used to treat diseases. Flavonoids are said to be the most active chemical constituents in all biological activities [31]. This work revealed that the plants are important source of potentially useful phytochemicals like terpenoids, proteins,

glycosides, flavonoids, steroids, polysaccharides, etc. [32]. To conclude the present study, we have found that most of the biologically active phytochemicals are

present in the ethanolic extract of *Ficus carica* leaves as phytochemical research carried out on *F. carica* has led to the isolation of plant metabolites.

Table 3. Phytochemical tests of the Ficus leaf extracts

Test	Observation		Result
Benedict's test	for reducing sugars	Red color of solution	+ve
Iodine test	non-reducing sugars	No change	-ve
Tannic Acid Test	polysaccharides	Ppt formed	+ve
Calcium Chloride test	Oxalic acid	White ppt	+ve
Lead acetate test	Oxalic acid	White ppt	+ve
Test for cysteine	Amino acid	Black ppt	+ve
Biuret test	For proteins	Pink color	+ve
Test for sulphur containing Protein	For proteins	Black Brown color of solution	+ve
*Ferric chloride	Tests for Tannins	No change	-ve
*Lead acetate		No change	-ve
*Potassium dichromate		No change	-ve
Mayer's test	Alkaloids	No ppt	-ve
Wagner's test	Alkaloids	No ppt	-ve

Antioxidant activity of leaves of *Ficus carica*

Living organisms have their own defensive system which is active throughout their life. Medicinal plants are one of the rich sources of scavenging free radicles which either slow or stop oxidation [33]. The DPPH assay is widely used to check the potency of extracts to scavenge free radical and to determine the antioxidant activity in plant materials [34]. The antioxidant activity of the drug could be due to the presence of flavonoids and steroids [35]. Hazratullah [36] reported that the fruit of *Ficus carica* is a very good source of antioxidants and this might be due to the presence of several chemical groups like phenols flavonoids. The present study showed very good antioxidant activity of leaves extract of *Ficus carica* using different concentration obtained by serial dilution (125, 250, 500, 750 and 1000µg/ml) comparing with the standard using ascorbic acid each concentration showed potent activity. Superoxide dismutase changes radicals of superoxide into hydrogen-

peroxide in organisms. The leaf ethanol extract of *Ficus carica* possessed effective scavenging DPPH radical activity. The different concentrations gave good results in increasing order of concentration; the results are presented in (Table 4), which showed the potential antioxidant activity of *Ficus carica* that it has specific chemical constituents which are responsible for good result and confirms a dose dependent activity using different concentrations of the extract [37]. Evaluation of antioxidant and antimicrobial potential of different leaves crude extracts of Omani *Ficus carica* against food borne pathogenic bacteria [39].

Antimicrobial activity of leaves of *Ficus carica*

The results obtained from the present study revealed that the leaves ethanolic extract possess strong antibacterial activity against all tested microorganisms (Table 5). Ahmad *et al.*, [2] also reported the antimicrobial activity of methanol extract of *Ficus carica* which showed that the plants leaf extract is a potent antimicrobial agent. Rashid *et al.* [37]

reported the antimicrobial activity of latex and leaves of *Ficus carica* where the selected microbes were found less susceptible to extracts. *Ficus carica* has also been suggested as a natural oral antiseptic based on their report against oral bacteria [38]. The present study is comparable with the work of Weli *et al.* [39], reported the omani ficus which possess protective agents against food borne microbes. Environmental and edaphic factors might be involved for these different results of the same plant which alter the chemical composition of the medicinal plants. The difference in results may also be due to methodology adopted in various researches. Phenols and flavonoids are main constituents which are responsible for antioxidant activity. It has been found, for example that vitamin C, carotenoid and enzymes are mostly responsible for scavenging free radicles [40, 41]. Antimicrobial activity of *Ficus carica* showed favourable results against selected

bacterial strains which support its local uses. *K. pneumoniae* and *P. pseudomonas aeruginosa* were susceptible showing 28mm zone of inhibition at 500mg/ml. *Staphylococcus aureus* showed 24mm and 26 mm zone of inhibition at 200 and 500 mg/ml concentration respectively. *E. coli* was inhibited with 20mm and 26mm diameter at 200 and 500 mg/ml. These differences in the zone of inhibition of the bacteria is may be due to the cell wall composition of these strains. Chemistry of the plant is responsible for any type of biological activity. Previous research also supports these results where the *Ficus* leave extract were found effective against pathogenic bacteria [42]. Aref *et al.* [43] also investigated the antimicrobial activity of latex of *Ficus carica* where ethyl acetate fraction produced good results by inhibiting the bacterial strains and showed that latex is also a biologically active substance.

Table 4. Antioxidant activity of the leaf extracts of *Ficus carica*

Conc. of sample	Ascorbic acid	DPPH % inhibition
125 µg /ml	71.52±02	21.42±01
250µg/ml	72.18±02	29.65±03
500µg/ml	75.91±04	53.15±03
750µg/ml	83.01±06	57.00±01
1000µg/ml	84.11±01	62.99±05

K. pneumoniae was inhibited (18 & 28mm), *E. coli* was found (20 & 26 mm) susceptible, *Staphylococcus aureus* was inhibited with (24 and 26mm) and *Pseudomonas aeruginosa* was inhibited by (22 and 28mm)

Table 5. Antimicrobial activity of the leaf extracts of *Ficus carica*

S. No.	Bacterial strains	Extract concentration		+tive control	-tive control
		200mg/1ml	500mg/1ml	1mg/1ml	
1.	<i>K. pneumoniae</i>	18mm	28mm	34mm	Nil
2.	<i>E. coli</i>	20mm	26mm	29mm	Nil
3.	<i>Staphylococcus aureus</i>	24mm	26mm	30mm	Nil
4.	<i>Pseudomonas aeruginosa</i>	22mm	28mm	32mm	Nil

Conclusion

It is concluded that *Ficus carica* L. has potentials metabolites to be used as an alternative to antibacterial and antimicrobial drug. The plant has many important

chemicals present its crude form and that is might be the reason that it is used locally for treating various diseases in the ethnobotanical domain. This plant provides a potential source to be served as a medicine

due to its chemical constituents based on its biological activity. More work is needed to isolate its chemical for clinical trials to be used for humanity. It is suggested that the plant has much potential in the local ethnomedical market and therefore recommend that it should be medicinally exploited for its possible treatment in diseases.

Authors' contributions

Conceived and designed the experiments: M Hamayun & HA Begum, Performed the experiments: HA Begum, Analyzed the data: M Rauf & M Shah, Contributed materials/analysis/ tools: M Hamayun & H Gul, Wrote the paper: HA Begum, K Ali, W Khan & M Schulze.

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