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Antioxidant and Anti-inflammatory Evaluation of Ficus carica Whole Fruit Extracts from Kalar Kahar, Pakistan

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[AM] Conception and design, Collection and assembly of data, [HM] Analysis and interpretation of the data, Statistical expertise, [SK, SM] Final approval and guarantor of the article

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A B S T R A C T

Background: Ficus carica, or fig, is the most widely consumed edible fruit. The plant is having rich history of use in traditional medicine due to its innumerable health benefits including anti-inflammatory, antioxidant, anticancer and antidiabetic properties. Fig having high amount of phenolic compounds (flavonoids, anthocyanins), prenylated isoflavone derivatives that exhibit strong medicinal properties.

Objectives: This study focused on the Black Mission fig variety, which is well grown in Pakistan. The research aimed to explore its antioxidant and anti-inflammatory properties from different organic solvent extracts of ripened fig fruit.

Methodology: Fresh figs were sourced from a farms Kalar kahar, and four organic solvents (hexane, ethanol, butanol, ethyl acetate and distilled water extracts were prepared from oven-dried crushed fruit. The extracts were assessed for total phenolic content using the Folin-Ciocalteu reagent. Their antioxidant activity was assessed using DPPH free radical scavenging assay against Ascorbic acid as reference, while inflammatory controlling potential was evaluated through inhibition of egg proteins' denaturation against diclofenac sodium (standard drug).

Results: Phenolic compounds analysis of all extracts revealed that ethyl acetate extract possessed high phenolic compounds content (368.7 GAE/gm) suggested the presence of abundant amount of these compounds. Among all selected extracts, ethyl acetate extract (25 µg/mL) exhibited the highest antioxidant activity (77.36%) in comparison to ascorbic acid (84.12%) by DPPH assay. Ethyl acetate (10 µg/mL) inhibited the inflammatory response (77.5%) in comparison to diclofenac sodium (89.9%) using protein denaturation assay. Strong antioxidant and anti-inflammatory response of local variety of fig fruit was observed in ethyl acetate extract due to potential phenolic molecules and supporting its therapeutic potential.

Conclusion: All of these findings point to the need for further investigation of the extracted compounds from ethyl acetate extract using advanced biological techniques and wet lab experiments to comprehend the biological functions of these molecules in medicinal field.

Key words: Ficus carica, anti-oxidant, anti-inflammatory, organic extracts, phenolic compounds.

Introduction

Free radicals play an important role in destroying pathogens by phagocytosis but increased concentration can cause oxidative stress leading to degenerative diseases such as cancer, heart disease, cataracts, impaired immunological function, and cell lysis.[1] Antioxidants can reduce the frequency of infections and illnesses by preventing damage caused by free radicals, boosting immunity, and shielding cells death. Antioxidant and reactive oxygen species (ROS) including hydrogen peroxide, nitric oxide, superoxides, organic hydroperoxides and hydroxyl redicals.[2] Among the ways these compounds can act as antioxidants and lessen cell degeneration and diseases progression are reducing agents, free radical scavengers, hydrogen donators, and singlet oxygen quenchers.[3] A large portion of the antioxidant defense system in humans is derived from the diet because natural herbal compounds are a big source of antioxidants and safe for human ingestion.[4]

Sterile inflammation, is a condition in which tissue damage or trauma cause inflammation without the involvement of a pathogen.[5] Numerous health disorders, including diabetes, asthma, heart disease, and cancer, are significantly impacted by it. According to recent research, flavonoids have the ability to block transcription factors or regulatory enzymes that are necessary for regulating inflammatory mediators.[6] Certain other pharmaceutical approaches, such as anti-histamines and steroids (e.g., hydrocortisone, betamethasone, prednisone), are associated with a range of side effects.[7] As a result, safe anti-inflammatory drugs made from natural sources are required.

Ficus carica, a black mission fig (anjeer in urdu), is the most popular edible fruit variety also widely used in traditional medicine systems, Ayurveda, Homeopathy and Siddha, as well as a functional food plant used to prepare many commercial food products.[8] *F. carica,* belongs to family Moraceae with largest plant genera and around 800 species.[9] Fig is cultivated worldwide that extends from South West Asia, Middle East, and Eastern Mediterranean region including Turkey, Egypt, Algeria and Morocco due to their warm climate.[10, 11] This delicious fruit possess variety of bioactive constituents including flavonoids, furanocoumarin derivatives (psoralen, isopsoralen, and bergapten), various acids derivatives (chlorogenic, carboxylic etc), terpenoids, alkaloids, carbohydrates, anthocyanins derivatives as analyzed by mass spectrometry.[12, 13, 14]

Fig has highest concentrations of antioxidant polyphenols mostly found in the peel, and fruits where dark color peels have higher concentrations of them.[15, 16] These compounds are identified as bergapten, psoralen, furfural derivatives, benzene acetaldehyde etc. with a role in prevention of major health issues like cardiovascular diseases, respiratory disorders, hepatic and stomach cancer, diabetes, ulcer, skin diseases and neurodegenerative disorders, hemorrhoids, dysentery.[17] Similarly, antioxidant activity has been reported from methanolic extract of fig leaves[18], from fruits[19], from peel and pulp[20], and anti-inflammatory activity from leaves.[21]

In Pakistan, the two varieties of Fig are cultivated including *F. carica* (black mission fig) and *F. palmata* (brown turkey), which are abundantly planted in northern areas of the country with annual production about 875,000 hectares and average production rate is 8.23 tons per hectare.[22] In present work the total phenolic content along with free radicals anti-oxidant and anti-inflammatory potential of *F. carica* whole fruit were measured in four organic solvents (ethanol, ethyl acetate, butanol, and hexane) and distilled water through *in vitro* analysis. Previously, only methanolic extract was reported for the anti-oxidative potential of fig fruit.[13, 14]

Materials and Methods

It is a phytochemical based study on whole fruit of *F. carica* with the focus to compare and evaluate the anti-inflammatory and antioxidative activity of extracted compounds from four organic solvents and distilled water. The fruits of *F. carica* (1.5 kg, black mission variety with dark purple color) were collected manually from Ameer Farms, Kallar Kahar, Pakistan at its ripening season and identified by a botanist. Fruits were thoroughly cleaned with tap water and dried at 40 ºC in hot air oven (DHG-9053 A). Fruits powder was obtained by crushing and grinding dry fruits (986 gm) through grinder (MJ-M176P, Panasonic, Japan) and through 80-mesh sieve. The obtained fruit powder (667.2 gm) and residual material (312.7 gm) further stored in air tight jars at room temperature till further use.

Five solvents were used to prepare the powdered fruit extracts: ethanol, ethyl acetate, butanol, n-hexane, and distilled water. Fine grounded sample (3g) was added in each solvent, and mixture was shaken continuously at 120 rpm overnight at room temperature. After three sets of filtration (through whatman filter paper), the solvents were evaporated from collected filtrates within three days at room temperature, combining and condensing all of the filtrates. The percentage yield was then determined for each.

Ferric chloride was used for preliminary phenolic compounds contents in each extract, which led to the identification of phenolic chemicals presence through color changes (blue, green, red, or purple). The reaction mixture contained 1 mg fig extract in 1 mL deionized water, and one to two drops of $FeCl_3$. Reaction mixture was mixed well and allowed to settle at room temperature, differences in color change were noted.

Using Gallic acid (1 mg/mL in methanol) as the standard, Folin-Ciocalteu-reagent (FCR) based assay was used to determine the total phenolic content (TPC).[23] Assay reaction mixture contained 2 mL of each extract (1 mg/10 mL) with 5 mL of 10% FCR (in deionized water) were mixed well and incubated for two minutes at room temperature. The mixture was then mixed with of 0.7 M sodium carbonate (4 mL) and incubated for 30 minutes at 37 ºC. The reaction mixture turned blue by formation of phosphotungstic-phosphomolybdenum complex due to phenolic compounds. They were measured spectrophotometrically (UV-1600PC) at 765 nm using quartz cuvette. The total phenolic compounds were assessed in relation to standard gallic acid curve (1 mg/mL) against control (without any extract). One (1) gram of gallic acid per 100 g of dry sample were included in phenolic content.

Each prepared fruit extracts were further evaluated for their antioxidant activity using 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

based on free radical scavenging activity by method .[20] DPPH stock solution (0.1 mM) and fruit extract (1mg/mL) were prepared in methanol. Five different dilutions (25, 50, 125, 250, 500 µg/mL) of each extracts were mixed with freshly prepared DPPH solution (3mL) and incubated in dark for 30 mint and at 517 nm absorbance was taken to quantify the decrease in DPPH absorption against blank. Ascorbic acid (1 mg/mL) was used as the standard, and the percentage of DPPH radical scavenging activity was determined in comparison to a control sample that contained DPPH in methanol without any plant extract. Plant extracts and DPPH standard percentage inhibition were measured using formula[20]:

To measure this activity of prepared fruits extracts were assessed through thermal denaturation of egg white proteins, following the methods of Padmanabhan.[24] All fig fruit extracts were diluted to three concentrations of 10, 20 and 50 µg/mL using deionized water prepared from stock solutions (100 µg/mL). Solution of Diclofenac sodium (10 µg/mL), as a standard drug, was prepared from its crushed powder in deionized water. To conduct the assay, 1x saline phosphate buffer (pH 6.4) was prepared. An egg white solution (2 mL of egg proteins in 6 mL of saline phosphate buffer, pH 6.4) was also prepared.

In assay reaction mixture, each extract (2 mL) was added to separate test tubes. Freshly blended egg proteins (2 mL) was then added to each tube, following addition of 1x phosphate buffer (2 mL). The control consisted of egg white solution (2 mL), 2 mL buffer (pH 6.4), and 2 mL of respective solvent of each extract. The standard comprised 2 mL of 10 µg/mL Diclofenac sodium solution, 2 mL of egg white, and 2 mL of phosphate buffer (1x). All test tubes were heated under moist conditions at 70°C, 10 minutes. Later assay reaction mixture was kept at room temperature (10 minutes) following by centrifugation (12,000 rpm, 10 minutes) and optical density of clear supernatant was measured at 660 nm. Reactions were performed in triplicate and mean absorbance values were recorded for calculating the percentage inhibition (I%) in proteins' denaturation using the previously mentioned formula.

Results

In current study total 1.5 kg of harvested fig fruit was dried completely at 40 ºC, and 667.2 g of dried fig powder was obtained (Figure 1). The %age yield for each extract was calculated by dividing the weight of final dry extract to weight of dry fruit powder.

After preparing the extracts in each solvent, it was found that the water extract yield was highest (21.7%), followed by ethanol (17.93%), ethyl acetate (10.33%), and n-hexane (3.3%).

Figure 1: *Ficus carica* fruit used in the study. (a) dry form of fruit, (b) powder form of fruit.

Ferric chloride was used for preliminary phenolic compounds screening, and the reaction mixture's color change indicated the positive results. This test result indicated the presence of phenolic compounds all types of fig fruit extracts.

Gallic acid standard curve (using 10-50 µg/mL) was used for the analysis of total phenolic contents in all extracts. Using obtained standard curve equation, the absorbance of each extract (as unknown) was measured and their total phenolic content were measured. Comparative analysis showed that ethyl acetate extract had highest phenolic acid (368.76 mg GAE/gm), while the water extract had the lowest quantity of phenolic molecules (97.5 mg GAE/gm) in table I).

As a reference antioxidant, ascorbic acid was used in the DPPH

Table I: Measurement of total phenolic contents (TPC) of fig fruit.

Sr. no.	Extracts name (1 mg/10mL)	Absorbance (765 nm)	Total Phenolic Content (mg of GAE/gm)
1	Ethanol	1.43	120.4
2	Ethyl acetate	4.36	368.7
3	Distilled water	1.16	97.5
4	Butanol	1.87	157.4
5	Hexane	3.17	267.7

free radical scavenging experiment and its standard curve was prepared. At 517 nm, the absorbance of ascorbic acid and each extract of 25 and 50 µg/mL concentrations were measured. Maximum ant-oxidative %age inhibition was observed by ethyl acetate (78%) in comparison to Ascorbic Acid (84%) followed by Ethanol (65%) and Butanol in figure 2.

The protein denaturation inhibition assay was used to quantify the *in vitro* anti-inflammatory effect of extracted compounds. The highest percentage inhibition of proteins denaturation was found in ethyl acetate (89%) extract, followed by ethanol (69%) extract at 50 µg/mL concentration. Furthermore, the least denaturation inhibition of egg proteins was observed in non-polar solvent extracts. Standard medicine diclofenac sodium exhibited the highest percentage (89.9%) inhibition (figure 3).

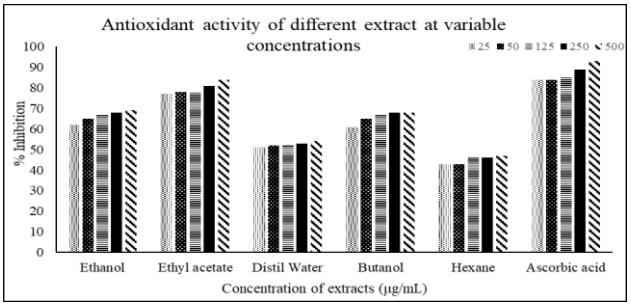

Figure 2: Graph illustrated the activity (antioxidant) comparison of all five extracts at variable concentrations.

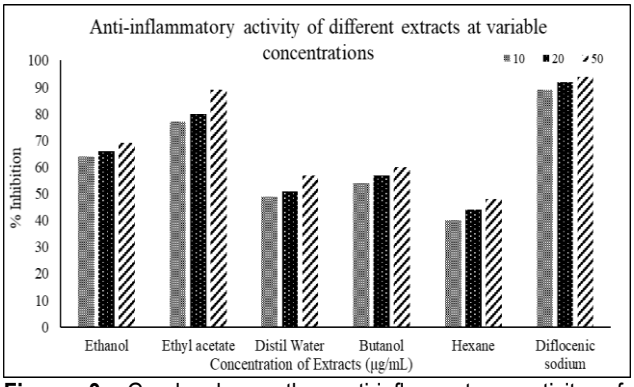

Figure 3: Graph shows the anti-inflammatory activity of different extracts at three concentrations (10, 20 and 50 µg/mL).

Discussion

Ficus carica fruit is the most edible, popular and delicious food with lots of health benefits which could work as weapons against multiple diseases. 125 potential chemicals have been identifying so far from its different plant sections.[25] According to phytochemical studies on figs, ficucaricones, which are prenylated isoflavone derivatives, may have anti-inflammatory and anti-proliferative properties.[26] The current study sought to determine the highest anti-inflammatory and antioxidant properties from different organic extracts of *F. carica* whole fruit through *in vitro* analysis. Ethyl acetate extract was identified as the most promising one through this study investigation.

For polyphenols extraction, the solid-liquid extract ratio was observed as 1:4 or 1:16 for efficient extraction.[14] In current study, this ratio was 1:10 which gave the 21.7% maximum yield with water followed by ethanol (17.93%) and ethyl acetate (10.33%). Various studies reported that sugar dissolution become high in the presence of water in fig while 100% pure solvent allowed the extraction of moderately polar and non-polar compounds.[27, 28] *F. carica* ethanol extract gave 17.93% yield which showed the maximum extracted polar and non-polar compounds in it. In this study the highest total phenolic compounds (369 mg/GAE/g) was obtained in ethyl acetate extract. Previously it has also reported the total phenolic contents of dried fig fruit varies between 80 and 458 mg GAE/g in aqueous and methanol extracts of three different fig species.[29]

According to study by Farooq et al., the dried fig hydroalcoholic extract has higher antioxidant activity than the aqueous extract due to the presence of flavonoids, saponins, and terpenoids class of compounds.[2] In current study, ethyl acetate extract had good free radical scavenging activity (84% using 50 µg/mL extract) in comparison to the standard ascorbic acid (93%). This indicated the remarkable antioxidant potential of *F. carica* fruit variety from Pakistan and the presence of potential flavonoid compounds in the extract. In another study the 65 to 88% inhibition in DPPH free radical-scavenging assay of *F. carica* fruit from Western Algeria was reported.[29] *F. carica* regulate the oxidative stress due to increase in lipo-peroxidation and malondialdehyde production upon prolong usage. It also increases the insulin production and improved the glutathione peroxidase and superoxide dismutase action.[30, 31]

In current study *F. carica* ethyl acetate extract (50 µg/mL concentration) showed the highest inflammatory activity (89%) and hexane extract showed the lowest (48%) at same concentration as compared to standard diclofanic sodium drug (94%) which suggested the high extraction of anti-inflammatory compounds could depend on solvent nature. Diclofanic sodium is the standard drug that controls inflammation by inhibiting the cyclooxygenase and lipoxygenase pathways. Prenylated flavonoids present in fig fruit have the remarkable anti-inflammatory effects by inhibiting the production of inflammatory mediators of different pathways including NADH oxidase, prostaglandin, cyclooxygenase which are involved in inflammation and platelet aggregation.[32] This suggesting its broad compounds-target intaeractions. Interleukin-6 is a pro-inflammatory cytokine which produce in response to infections, burns, traumas or cancer by immune mediated cells, various epithelial, endothelia, fibroblasts, and, osteoblasts cells. Recently, molecular docking analysis confirmed the potential phytocompounds from *F. carica* could effectively inhibit the IL-6 production. These include Cyanidin-3,5-diglucoside, Kaempferol-7-O-rutinoside, Cyanidin-3-rhamnoglucoside, and Rutin.[33] Therefore, *F. carica* fruit is the rich source of isoflavones derivatives as well as delicious fruit. These isolation and implementation of these medicinal compounds could be beneficial for multiple health issues.

Conclusion

The antioxidant and anti-inflammatory properties found in the whole fruit of *F. carica* could be beneficial for combating various diseases associated with oxidative stress and inflammation as a drugs replacement or working in combination of other drugs. To

further validate the health-related benefits of potential compounds of *F. carica* fruit, additional studies including clinical trials using animal models, cell lines, or molecular networking of compounds-target genes should be conducted in the future.

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