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A STUDY ON PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF *FICUS CARICA* FRUIT EXTRACT USING DIFFERENT *IN-VITRO* METHODS

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ABSTRACT

Ficus carica L. is one of the first plants grown by humans, because of their consumption as much as a dry and fresh fruit. It is used in our traditional medicine for the healing of various diseases. Since antiquity, phenolic compounds produced by plants were known as free radical scavengers and as powerful antioxidants. Huge interest has been made by researchers to the traditional uses of medicinal plants against illnesses related to oxidative stress. *F. carica* L. has three figs yields, early fig stays on the tree; late fig of autumn or figs flowers carries from August to winter and is locally known as Bakor and the green or winter figs. This study aims at performing a preliminary phytochemical analysis to evaluate the phytochemical composition and antioxidant activity of *Ficus carica* fruit extract. Preliminary phytochemical analysis of *Ficus carica* extract was done. *Ficus carica* extract was found to contain phytochemicals like flavonoids, glycoside, protein, terpenoids, saponin, alkaloids, tannins. Antioxidants potential was also estimated by Fenton reaction method. Figs are a good source of many enriching vitamins, antioxidants, and minerals. Improving overall health, balances skin nutrition and helps improve circulation. Further research is required to know the extract mechanism of action of fig as an antioxidant.

KEYWORDS

Ficus carica, Phytochemical, Fenton reaction, Fig and *In-vitro* methods.

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INTRODUCTION

India is very rich in large variety of plants which grow in different parts of the country. India is rich in all the three levels of biodiversity that is species diversity, generic diversity and habitat diversity (Baytop, 1984)[1] today the demand for traditional medicine as well as the drugs obtained from plants has increased rapidly. Since so many years plants

have been an important source for medicines (Joseph, *et al*, 2011)[2]. Ficus (Moraceae) is one of the largest genera in angiosperms with 800 species (Bucic-Kojic, *et al*, 2011)[3]. It is an important genetic resource due to its high economic and nutritional values (Mawa, *et al*, 2013)[4]. *Ficus carica L*. It is an important member of the genus *Ficus*. It is commonly referred to as fig. It is native to southwest Asia and the eastern Mediterranea (Tous, *et al*, 1996[5], Solomon, *et al*, 2009[6]) and (Patil, *et al*, 2011[7]) and it is one of the first plants that were cultivated by humans. The dried fruits of F. carica have been reported as an important source of vitamins, minerals, carbohydrates, sugars, organic acids, and phenolic compounds. The fresh and dried figs also contain high amounts of fibre and polyphenols. It is used to treat gastrointestinal (colic, indigestion, loss of appetite, and diarrhoea), respiratory (sore throats, coughs, and bronchial problems), and cardiovascular disorders and as anti-inflammatory. Ficuscarica plays many physiological roles as it contains phenolic compounds (Mawa, *et al*, 2013)[4]. Antioxidants in food are of interest for four major reasons: they can protect the food itself against oxidative damage, they can exert antioxidant effects in the human gastrointestinal tract, they can be absorbed and exert antioxidant effects in other body tissues, and they may be used in plant extracts, or as pure compounds, as therapeutic agents, (Halliwell, *et al*, 1999)[8] and tyrosin. Ficusin, bergaptene, stigmasterol, psoralen, taraxasterol, beta-sitosterol, rutin, sapogenin, Calotropenyl acetate, lepeolacetate and oleanolic acid sistosterol are present in the leaf. The plant also contains arabinose, β-amyrins, β-carotines, glycosides, βsetosterols and xanthotoxol16-18. Umbelliferone, campesterol, fucosterol, fatty acids 21, 6-(2- methoxy- Zvinyl)-7-methyl-pyranocoumarin and 9, 19-cycloarlanetriterpenoid as an anticancer 22 and 6-O- acyl-β-Dglucosyl -β-sitosterol 23, calotropenyl acetate, and lupeol acetate 24 as an antiproliferative agent (Joseph, 2011)[9]. Viewing the biological properties of *Ficus carica* fruits, our study focuses on the correlation between phytochemicals contents and antioxidant capacity of ripped fruits methanolic extracts.

MATERIAL AND METHODS

Source of Data

Experiment will be performed as described in the standard bibliography, literatures and text books. The reputed journals and publications are obtained from library and through web search.

Chemicals

All solvent used were of HPLC grade. Methanol was acquired from Merck Limited (Mumbai, India).

Plant Material

Ficus carica were procured from local market of Raipur and authenticated at Central Laboratory Facility, Chhattisgarh Council of Science and Technology, Raipur, Chhattisgarh.

Preparation of plant powder

Fresh *Ficus carica* fruits were washed thoroughly in tap water followed by distilled water and were then shade dried until all the water content was lost completely. Dried plants were crushed and powdered using blender. Fine powder was obtained after sieving and stored in airtight container until further use.

Preparation of experimental plant extracts

The plant powder was extracted with methanol solvent with an increasing polarity. The successive extraction was done by a cold maceration process for seven days with regular agitation. After seven days of cold maceration process it was filtered through sterile muslin cloth and the solvent was evaporated using soxhlet apparatus. The residues obtained after evaporation were stored at -20ºC until used for experimentation.

Procedure of Phytochemical

Test for Alkaloids

Small amount of each extract were taken in the test tubes and few drops of dilute hydrochloric acid was added. Then the sample was filtered and treated with Mayer's reagent. Orange precipitate was obtained which shows the presence of Alkaloid.

Test of Saponins

0.5gm of each extract was taken in the test tubes and 5ml of distilled water was added. Then the

sample was kept in water bath for few minutes and filtered with the help of filter paper. The filtrate was mixed with 2.5ml of distilled water and the sample was shaken vigorously. Then 3 drops of olive oil was added and shaken well. Formation of emulsion was observed, which shows the presence of saponins.

Test for Glycosides

Small amount of each extract were taken in the test tubes and hydrolysed with 3ml of hydrochloric acid. Then the sample was kept in water bath for few hours. After few hours 2ml of Fehling solution (1ml of Fehling solution A and 1ml of Fehling solution B) was added and boiled for few minutes. Red colour precipitate was obtained, which shows the absence of Glycosides.

Test for Proteins

Small amounts of each extract were taken in the test tubes and 3ml of distilled water was added. Then a small amount of nitric acid was added, the sample mixture was heated for 1min and cooled under tap water. Then 40% of NaOH was added and orange precipitate was obtained, which shows the presence of protein.

Test for Phytosterols

Small amounts of each extract were taken in the test tubes and 1ml of sulphuric acid was added. All the samples were allowed to stand for 5minutes and shaken well. Golden yellow colour was obtained, which shows the presence of phytosterol.

Test for phenolic compounds

Small amount of each extract were taken in the test tubes and 5ml of distilled water was added. Then each sample was treated with ferric chloride. Violet colour is obtained, which shows the presence phenolic compound.

Test for Flavonoids

Small amount of each extract were taken in the test tubes and treaded with 2-3 drops of concentrated sulphuric acid. Orange colour was obtained, which shows the presence of Flavones. Hence Flavonoids are present.

Test for Terpenoids

5gm of each extract were taken the test tubes and 2ml of chloroform was added. Then 3ml of concentrated sulphuric acid was added from the side of the wall. Reddish brown colour was obtained, which shows the presence of terpenoids.

Test for Tannins

0.5gm of each extract was taken in the test tubes and 1ml distilled water was added. Then the sample mixture was boiled for few minutes and filtered. Then few drops of 0.1% ferric chloride were added to the filtrate. Brownish green colour was obtained, which shows the presence of tannins.

Procedure of *in-vitro* anti-oxidant activity

The OH- radical scavenging activity of Calendula officinal is bark extract (10-100ug/ml) was determined according to the deoxyribose method reported of Halliwell *et al*, (1987)[10]. In the protocol the presence of 100lM EDTA. FeCl3, H2O and ascorbic acid were prepared in degassed H2O prior to use. The reaction tube contained (final concentrations) 3.6mM deoxyribose, 100lM EDTA, 1mM H2O2, 100lM L- ascorbic acid, 100lM FeCl3, H2O in 25mM phosphate buffer, pH 7.4 in 1.0ml total volume. Samples was kept in incubation at 38ºC, 1 hrs, 1.0ml 1.0% TBA in 0.05M NaOH and 1.0ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbances were read at 532nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Abs: 532nm Control Abs. - 532nm sample Abs.} \times 100}{\text{532nm Control Abs}}$$

Antioxidant capacity of test compounds was expressed as IC50, the concentration necessary for 50% inhibition concentration of TBARS.

Statistical analysis of data

Experiments were carried out in triplicates. The results were expressed as the mean+ standard deviation. Raw data were imported to Microsoft Excel program for graphical representation. One way ANOVA was used to check the data significance.

RESULTS AND DISCUSSION

Almost all of the *Ficus* species belonging to family *Moraceae* haven traditionally used as folk medicine to cure respiratory disorders and skin diseases. The roots of *Ficus* species are important to treat gout and gums diseases that have anthelmintic activity. Fruit of *Ficus* species, such as, *F. carica*, *F. hispida*, *F. microcarpa* and *F. sycomorus* has been found to be helpful improving digestion or treating vomiting.

Phytochemicals, including phenolics, flavonoids, flavanols, ascorbic acid, lignin, xanthones, stilbenes, etc., are plant-based secondary metabolites, which are associated with the protection of human health against chronic diseases. The antioxidant activity by various methods and the extract possessed a good antioxidant property due to the presence of vitamin -C, poly phenolic, flavonoid content. *Ficus carica* was evaluated for antioxidant activity by the OH radical scavenging activity using Fenton reactions. In result we found that all the different concentration extracts of *Ficus carica* and that of ascorbic acid which we have used to control solution inhibited the solution of the OH radicals.

The phytochemicals analysis is tested on *Ficus carica* extract and as follows: test for alkaloid, test for glycoside, test for protein, test for phytosterol, set for phenolic compound, test for tannin, test for terpenoids, test for flavonoids and test for saponin were carried out. Alkaloid, Protein, glycoside, Tannin, Terpenoids, Flavonoids and Saponin shows positive results as phytosterol and Phenolic compound shows a negative result, as presented in Table No.1.

Antioxidant analysis of *Ficus carica* extract

The result of the examined *Ficus carica* and control solution i.e.: ascorbic acid shows that they inhibited the production of OH^+ radicals. The percentage of free radical scavenging activity of the extract increases with an increase in concentration. Extent of hydroxyl radical scavenging was determined by the increase in intensity of light pink color which was determined at 532nm. The antioxidant activity was compared with the ascorbic acid which was taken as a positive control.

Discussion

The body's innate mechanism has many enzymes and nonprotein compounds that protect it from the free radicals and reactive oxygen species produced inside the body during normal metabolism and also due to external stimuli. Major compounds include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione, which also play a major role in detoxification and coordinate the body's antioxidant defense processes. The superoxide dismutase is a metalloprotein that scavenges superoxide anions. Catalase is a heme protein, localized in the peroxisome or the microperoxisome, which catalyzes the decomposition of H2O2 to water and oxygen and thus protects the cell from oxidative damage produced by H_2O_2. The glutathione peroxidase catalyzes the reaction of hydroperoxides, which reduces glutathione to form glutathione disulfide (GSSG) and the reduction product of the hydroperoxide. Glutathione reductase is involved in the regeneration of glutathione that has been converted to GSSG by oxidation and thiol transfer reactions. Glutathione, a major nonprotein thiol, is mainly involved in detoxification (Halliwell and Gutterridge, 1985)[11].

F. carica has been traditionally used for its medicinal benefits as metabolic, cardiovascular, respiratory, antispasmodic, and anti-inflammatory remedy. It is commonly referred to as "Fig". Leaves, fruits, and roots of *F. carica* are used in native medicinal system in different disorders such as gastrointestinal (colic, indigestion, loss of appetite, and diarrhea), respiratory (sore throats, cough, and bronchial problems), inflammatory, and cardiovascular disorders (Penelope, 1997[12], Veberic, 2008)[13]. Fruits of *F. carica* can be eaten fresh or dried or used as jam. Figs are used as an excellent source of minerals, vitamins, carbohydrates, and dietary fibre because it is fat and cholesterol free and contain high number of amino acids. It is also reported that figs have been conventionally used for their therapeutic benefits as

laxative, cardiovascular, respiratory, antispasmodic, and anti-inflammatory remedies (Solomon, 2006)[6]. Phytochemicals, including phenolics, flavonoids, flavanols, ascorbic acid, lignin, xanthones, stilbenes, etc., are plant-based secondary metabolites, which are associated with the protection of human health against chronic diseases. The antioxidant activity by various methods and the extract possessed a good antioxidant property due to the presence of vitamin -C, poly phenolic, flavonoid content. *Ficus carica* was evaluated for antioxidant activity by the OH radical scavenging activity using Fenton reactions. In result we found that all the different concentration extracts of *Ficus carica* and that of ascorbic acid which we have used to control solution inhibited the solution of the OH radicals.

The phytochemicals analysis is tested on *Ficus carica* extract and as follows: test for alkaloid, test for glycoside, test for protein, test for phytosterol, set for phenolic compound, test for tannin, test for terpenoids, test for flavonoids and test for saponin were carried out. Alkaloid, Protein, glycoside, Tannin, Terpenoids, Flavonoids and Saponin shows positive results as phytosterol and Phenolic compound shows a negative result, as presented in Table No.1.

The another set of experiment the antioxidant activity of ascorbic acid shows that they inhibited the production of OH^+ radicals. The percentage of free radical scavenging activity of the extract increases with an increase in concentration. Extent of hydroxyl radical scavenging was determined by the increase in intensity of light pink color which was determined at 532nm. The antioxidant activity was compared with the ascorbic acid which was taken as a positive control.

The important antioxidant capacity of methanolic extracts of fig flowers fruits is impressive and probably it is the result of their exceptional richness in phenolic compounds. Such bioactive molecules reacting as natural antioxidants and then, they are well known to display a positive impact on human health and can be considered for future uses as antioxidant components in agro-food industries. The Algerian flora known for its high richness and biodiversity as well as Algerian folk medicine is also considered as an appreciable source of both new drugs and bioactive molecules since ancient times. Future studies will be needed to elucidate more and more medicinal plants and traditional preparations used for therapeutic purposes.

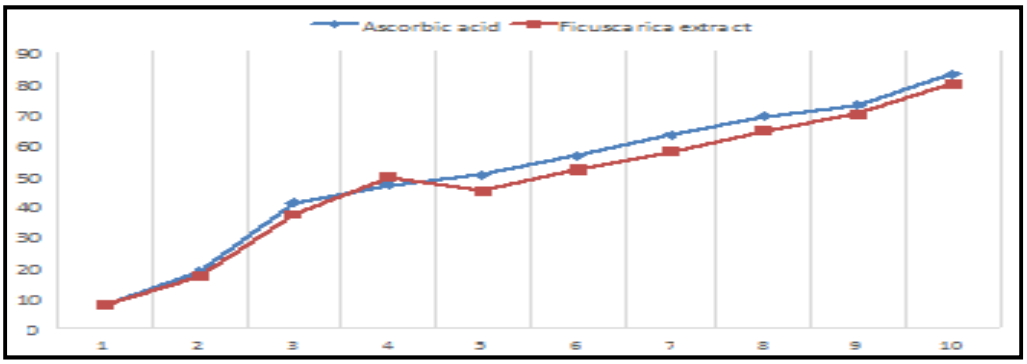
Table No.1: The study of phytochemicals analysis on *Ficus carica* Extract

S.No	Test	Result
1	Alkaloid	+ve
2	Glycoside	+ve
3	Protein	+ve
4	Phytosterol	-ve
5	Phenolic compound	-ve
6	Tannin	+ve
7	Terpenoids	+ve
8	Flavonoids	+ve
9	Saponin	+ve

+ =Positive, - = negative

Table No.2: Antioxidant activity ascorbic acid and extract of *Ficus carica*

S.No	Conc. (µg/ml)	Percentage of inhibition	
		Ascorbic acid (Mean ± SE)	*Ficus carica* extract (Mean ± SE)
1	10	7.52 ± 0.77	7.52±0.46
2	20	18.27± 0.56	16.8±0.48
3	30	41.28±0.66	37.09±0.84
4	40	46.98±0.69	41.50±1.86
5	50	50.21±0.38	44.94±1.13
6	60	56.3±0.56	51.82±1.54
7	70	63.11±0.76	57.74±1.17
8	80	69.2±0.46	64.40±0.57
9	90	72.69±4.55	69.88±1.19
10	100	82.68±0.75	79.73±0.57



Graph showing the antioxidant activity of ascorbic acid and extract of *Ficus carica*

CONCLUSION

Traditional medicine has been practiced in India for decades and is still widely practiced even today. The knowledge of medicinal plants is passed on based on indigenous knowledge system and orally by the traditional herbal practitioners form one generation to the next. The medicinal plants are extracted from trees and shrubs. The common practice is the use of the bark, roots and sometimes both. Medicinal plants have a wide range of pharmaceutical use in disease diagnosis etc. Experimental data revealed that there might be correlation between total phenolic and antioxidant capacity of different extracts of lemon grass.

Ficus carica extract was found to be a good source of flavonoids, glycoside, protein, terpenoids, saponin, alkaloids, tannins. The *Ficus carica* extract showed a good antioxidant potential also. Presence of Antioxidant potential indicates a significant therapeutic value. In future the extract can be tested for anti-cancer properties. The fruit is edible and does not require a tedious processing, thus can be used in drug formulation.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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