Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading doi:10.1016/S2222-1808(14)60619-8

Evaluation of antioxidant and antimicrobial potential of different leaves crude extracts of Omani *Ficus carica* against food borne pathogenic bacteria

Afaf Mohammed Weli, Afaf Ali Mohammed Al-Blushi, Mohammad Amzad Hossain*

School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P. O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman

PEER REVIEW

Peer reviewer

Prof. Dr. Huge Dougle, Institute of Natural Products, University of Yale, UK.
E-mail: hugedu@gmail.com

Comments

The present study on antioxidant and antimicrobial activity of various leaves crude extracts of *F. carica* is giving the valuable brief and scientific information about this plant.
Details on Page 16

ABSTRACT

Objective: To prepare different polarities crude extract from the leaves of *Ficus carica* and to evaluate their antioxidant and antimicrobial potential against food borne pathogenic bacterial strains.
Methods: The dried leaves were macerated in absolute ethanol for one week. The ethanol was evaporated and the crude extract was defatted with ethanol-water. The defatted hydro alcoholic crude extract was successively extracted with hexane, chloroform and ethyl acetate. The antioxidant potential was determined against 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Evaluation of antimicrobial potential of different crude extracts against selected Gram positive and Gram negative bacteria by agar disc diffusion method.
Results: The total extraction yield was 2.2%. The highest extraction yield was in chloroform and the lowest in hexane. The antioxidant results were found in the order of hydro alcoholic>ethyl acetate>hexane>chloroform. Hydro alcoholic crude extract and its derived fractions display moderate antimicrobial potential against the selected bacterial strains such as *Staphylococcus aureus, Escheichia coli and Pseudomonas*, in the range of 0%-13%.
Conclusions: It is concluded that the hydro alcoholic and ethyl acetate crude extracts of *Ficus carica* possess very good antioxidant and antimicrobial potential.

KEYWORDS

Ficus carica, Al-Teen, Maceration method, DPPH, Antioxidant, Antimicrobial potential

1. Introduction

Ficus carica (*F. carica*) is a medicinal plant constituting one of the largest genera with about 750 species. It is a woody plants, trees and shrubs. Primarily it is found in subtropical and tropical regions throughout the world[1]. The genus is remarkable for the large variation in the habits of its species. It is commonly referred as fig. *F. carica* grows well up to a height of 6.9-10 m, with smooth grey bark. It is well known for its large and fragrant leaves. The leaves are 12-25 cm long and 10-18 cm across, and deeply lobed with three or five lobes[2-4]. The complex inflorescence of the common fig consists of a hollow fleshy structure called the syconium, which is lined with numerous unisexual flowers. The edible fig fruit is the mature syconium on the outside and numerous one-seeded fruits on the inside. The fruit is 3-5 cm long, with a green skin, sometimes ripening towards purple or brown. *F. carica* has milky sap. It is rich in vitamins, mineral elements, water, and fats. Figs are one of the highest plant sources of calcium and fiber[5]. The chemical constituents in the leaves of *F. carica*

*Corresponding author: Dr. Mohammad A. Hossain, School of Pharmacy, College of Pharmacy and Nursing, University of Nizwa, P. O. Box 33, Postal Code 616, Nizwa, Sultanate of Oman.
Tel: +96892327578
Fax: +96892877745
E-mail: hossainabi@gmail.com
Foundation Project: Supported by University of Nizwa, Nizwa, Sultanate of Oman (Grant No. 507/SOP/OB/1/2013).

Article history:
Received 8 Apr 2014
Received in revised form 18 Apr, 2nd revised form 6 May, 3rd revised form 15 May 2014
Accepted 12 Jun 2014
Available online 11 Jul 2014

were protein (67.6%), fat (4.3%), fiber (1.7%), total ash (4.7%), nitrogen free compounds (5.3%), pentoses (16.4%); carotene (3.6%), bergaptene, stigmasterol, sitosterol, and tyrosine[6]. The sap of the fig's green parts is an irritant to human skin[5]. All parts of this plant such as bark, leaves, tender shoots, fruits, seeds, and latex are medicinally important[2]. The fig is a very nourishing food and used in industrial products. The leaves of this plant have anti-diabetic properties and reduce the amount of insulin needed by diabetics. The leaves have also the ability to inhibit the growth of cancer cells and to prevent colon cancer[7]. Figs are a good source of potassium and it is very important mineral to help control blood pressure[8]. The fiber of figs also helps to reduce weight and is recommended for obese people[8]. The ethanol crude extract of *F. carica* at doses of 100, 200 and 300 mg/kg showed significant dose-dependent reduction in normal body temperature and yeast provoked elevated temperature. Its crude extracts showed high acute toxicity with hemorrhagic enteritis. In addition, the crude extracts showed a weak anthelmintic efficacy. The plant crude extracts and their mixture decreased the level of mutations induced by N-methyl-N′-nitro-N-nitrosoguanidine in viciafaba cells, demonstrating the ability to decrease the genotoxicity of environmental mutagens[9]. The main objective of the present study was to determine the antioxidant and antimicrobial potential of different concentrations and different polarities of leaves crude extracts of *F. carica* against selected food borne pathogenic bacterial strains such as *Staphylococcus aureus* (*S. aureus*), *Escheichia coli* (*E. coli*) and *Pseudomonas*.

2. Materials and methods

2.1. Chemicals

The chemicals such as ethanol, chloroform, ethyl acetate, methanol and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich Chemical Company Limited. The food borne pathogenic bacterial strains *S. aureus*, *E. coli* and *Pseudomonas aeruginosa* (*P. aeruginosa*) were obtained from Microbiology Laboratory, Department of Biological Sciences, College of Arts and Sciences, Nizwa University, Sultanate of Oman. Filter paper discs of diameter 5 mm were obtained from Whatman Company. Nutrient agar and plastic Petri dishes were purchased from Sharlau Chemie Company. Deionized water was used throughout the experiment. Shimadzu1800 UV-visible spectrophotometer was used for analysis.

2.2. Plant samples

The leaves of *F. carica* sample were collected from Izki on 23 October, 2012 in the afternoon at 4.00-6.00 pm. The collected leaves samples were transported to the lab for processing.

2.3. Preparation of crude extracts

The whole leaves samples were separated from the affected one and washed with water. The fresh leaves samples were placed on newspapers and dried under shade. After complete drying, the leaves were grinded using kitchen grinder. The powdered leaves samples (349.31 g) were taken in a three liter beaker and added 95% ethanol (1.5 L) for one week. After the complete extraction, the solvent was decanted out and filtered under vacuum using Buchner apparatus to give clear solution. The ethanol was evaporated at low pressure using rotary evaporator to obtain crude ethanol extract. The crude extract was defatted with water and extracted successively with hexane, chloroform and ethyl acetate.

2.4. Radical scavenging potential by DPPH method

Free radical scavenging potential of different crude extracts was estimated as described by Blois[10,11]. Four concentrations (12.5, 25, 50, 100 μg/mL) were prepared from different crude extracts such as hexane, chloroform, ethyl acetate and hyrdo alcoholic. Four milliliter of each concentration were placed in the separate test tube. One milliliter of freshly prepared DPPH solution was added and shaken vigorously. After that, all the test tubes were placed at room temperature in dark place for 45 min. The control was prepared in the same way without adding any crude extract. The absorption of the samples was measured using UV spectroscopy at 517 nm. The inhibition percentage was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_{control} - A_{extract}}{A_{control}} \times 100$$

2.5. Antibacterial assay

The evaluation of antibacterial test was carried out by the agar disc diffusion method[12]. Four concentrations of each extract was prepared using serial dilution method with dimethyl sulphoxide to obtained 2000, 1000, 500, and 250 μg/mL solution. Filter paper discs were macerated with each concentration and placed on previously prepared agar gel plate. All the plates were incubated with microorganism at 37 °C for 24 h. Amoxicillin was used as a positive control. The calculation of antibacterial activity was determined by measuring the diameter of the zone of inhibition against the tested food borne pathogenic bacterial strains.

3. Results

3.1. Crude extracts from the leaves of F. carica

The powdered leaves samples were extracted with ethanol for one week. The ethanol was evaporated at low pressure using rotary evaporator to obtain crude ethanol extract. The crude extract was defatted with water and extracted successively with hexane, chloroform and ethyl acetate. The total yield was 2.7%. The highest extraction yield was in chloroform and the lowest in hexane and the order was chloroform>ethyl acetate>hydro alcoholic>hexane.

3.2. Antioxidant potential

The antioxidant potential was determined by agar gel diffusion method. The results of antioxidant potential for

hexane, chloroform, ethyl acetate and hydro alcoholic extract against DPPH radical are shown in Figure 1. All crude extracts from *F. carica* inhibited the DPPH radical. Hydro alcoholic crude extract showed radical scavenging of more than 90% at all concentrations. The lowest inhibition was shown by chloroform.

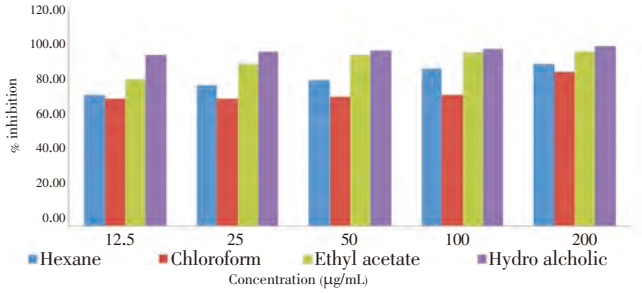

Figure 1. Antioxidant potential of different crude extracts against DPPH.

3.3. Antimicrobial potential

The determination of antibacterial potential of hexane, ethyl acetate, chloroform, hydro alcoholic crude extracts of *F. carica* against three food borne pathogenic bacterial strains were calculated the presence or absence of inhibition zones. The exhibition of antibacterial potential of four crude extracts of *F. carica* were shown against *S. aureus*, *E. coli* and *P. aeruginosa* bacterial strains at the concentrations of 2000, 1000, 500 and 250 μg/mL with dimethyl sulphoxide. Almost all crude extracts of *F. carica* were showed moderate potential of antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* bacteria at the concentrations of 2000, 1000, 500 and 250 μg/mL (Figure 2). Amoxicillin was used as a positive control.

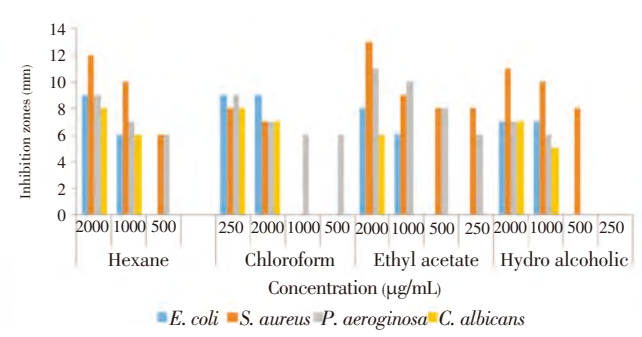

Figure 2. Antimicrobial potential of different leaves crude extracts of *F. carica* against *E. coli*, *S. aureus* and *P. aeroginosa*.

4. Discussion

The powdered leaves samples were extracted with ethanol and defatted with water and extracted successively with hexane, chloroform and ethyl acetate. The total extraction yield was 2.7%. The highest yield was obtained from chloroform and lowest from hexane. This result indicated that the chloroform crude extract contained high percentage of non and semi polar organic compounds. The antioxidant potential was determined by well established DPPH method. The principle of DPPH method based on production of free radical[13]. The hydro alcoholic crude extracts produced more free radical and chloroform crude extracts produced less free radical. Therefore, the highest antioxidant potential was obtained hydro alcoholic crude extract among the other crude extracts and the lowest was chloroform. The antioxidant results was found in the order of hydro alcoholic>ethyl acetate>hexane>chloroform (Figure 1). The hydro alcoholic extract showed highest potential and the ethyl acetate also showed high potential compared to hexane and chloroform crude extracts. The variation of antioxidant potential might be poly phenolic chemical compounds in the crude extracts[14,15]. It was observed to have the highest antioxidant potential in DPPH assay, which is in agreement with previous study[11].

There are several studies of antimicrobial potential of *F. carica* leaves crude extracts. Jung reported that the methanol crude extract from the leaves of *F. carica* exhibited strong potential against *E. coli* but weak potential against *S. aureus*[14]. Another good study demonstrated that the variation of antimicrobial potential was due to some flavonoid compounds in the leaves of *F. carica*[15]. Ahmad *et al.* reported that antimicrobial potential of methanol leaves crude extract of *F. carica* against five bacterial strain *Bacillus cereus, Enterobacter aerogens, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus epidermidis* at different concentrations was found in the following decreasing order *Staphylococcus epidermidis>Klebsiella pneumoniae>Bacillus subtilis>Bacillus cereus>Enterobacter aerogens*[16]. The antibacterial potential by agar disc diffusion assay showed that methanol crude extract of *F. carica* exhibited potential against pathogenic as well as non-pathogenic test bacteria. The authors also mentioned that significant effect on growth inhibition of Gram positive and Gram negative bacteria[16].

In this present study, highest strong potential was obtained from ethyl acetate against *E. coli* and *P. aeroginosa* at all applied concentration but exhibited moderate potential against *S. aureus* at the concentration 2000 and 1000 μg/mL (Figure 2). However, the concentration at 500 and 250 μg/mL did not show any microbial potential against *S. aureus*. Chloroform crude extracts showed moderate potential against *E. coli* at all applied concentration but the other pathogenic bacterial strains showed moderate potential only at the concentrations of 2000 and 1000 μg/mL. However, 500 and 250 μg/mL did not show any activity against *S. aureus* and *P. aeroginosa*. Hexane crude extract showed moderate potential against all applied bacteria at the concentration of 2000, 1000, and 500 μg/mL. Hydro alcoholic extract also showed potential against all pathogenic bacteria at concentration 2000 and 1000 μg/mL.

In conclusion, the leaves of *F. carica* were found to possess strong antioxidant and moderate antimicrobial activity. Gram negative bacteria were found to be more susceptible than Gram positive bacteria indicating that active ingredients in the studied extracts are inhibiting growth of bacteria via unusual mechanism. It will be thus interesting to isolate these compounds and further investigated their antimicrobial properties. Antioxidant compounds are known to possess both anticancer and neuro protective characteristics. Hence, it is suggested to extend phytochemical investigation of *F. carica*

from Oman in order to evaluate further its pharmacological potentials.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to Prof. Dr. Nafsiah Binti Shamsudin, Dean, College of Pharmacy and Nursing, University of Nizwa, Sultanate of Oman for her continuous encouragement during the work and all laboratory facilities. The authors are also grateful to University of Nizwa, Nizwa, Sultanate of Oman for providing all chemicals and other expenses from their internal fund to carry out this project (Grant No. 507/SOP/OB/1/2013). Thanks also go to Qasim Al-Riyami, Assistant Dean, Training, School of Pharmacy, University of Nizwa for his continuous encouragement and Khaloud Ali Said Al-Alawi and Ahlam Rashed Alabri, Lab Technicians, Natural Product Lab, University of Nizwa for their continuous help during the experiment. The authors wish to express sincere gratitude to the Central Instrument Laboratory, College of Agriculture and Marine Sciences, Sultan Qaboos University, Sultanate of Oman where the tests were confirmed.

Comments

Background

F. carica plant is a medicinal plant constituting one of the largest genera with about 750 species. All parts of this plant such as bark, leaves, tender shoots, fruits, seeds, and latex are medicinally important. The fig is a very nourishing food and used in industrial products. The leaves of this plant have anti-diabetic properties and reduce the amount of insulin needed by diabetics.

Research frontiers

The aim of this study is to prepare various crude extracts using different polarities of solvent and to quantitatively evaluate antioxidant activity and antimicrobial activity of *F. carica* collected from Izki, Nizwa, Sultanate of Oman.

Related reports

According to the literature search, no work has been done on Omani *F. carica* by the researcher. The other parameters of this plant have been done by other researchers.

Innovations & breakthroughs

Although the experimental work done by the author is routine work, it gives the new information and data to the scientific community.

Applications

This plant is used worldwide as a traditional herbal medicine. According to the paper, there are so many bioactive compounds that can be used to prepare medicine.

Peer review

The present study on antioxidant and antimicrobial activity of various leaves crude extracts of *F. carica* is giving the valuable brief and scientific information about this plant.

References

[1] Kislev ME, Hartmann A, Bar-Yosef O. Early domesticated fig in the Jordan Valley. *Science* 2006; **312**(5778): 1372-1374.

[2] Salem MZ, Salem AZ, Camacho LM, Ali HM. Antimicrobial activities and phytochemical composition of extracts of *Ficus* species: an over view. *Afr J Microbiol Res* 2013; **7**(33): 4207-4219.

[3] Abdel-Hameed El-Sayed S. Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. *Food Chem* 2009; **114**(4): 1271-1277.

[4] Hada LS, Kakiuchi N, Hattori M, Namba T. Identification of antibacterial principles against *Streptococcus mutants* and inhibitory principles against glucosyltransferase from the seed of *Areca catechu* L. *Phytother Res* 1989; **3**(4): 140-144.

[5] Abdsamah O, Zaidi NT, Sule AB. Antimicrobial activity of *Ficus deltoidea* Jack (Mas Cotek). *Pak J Pharm Sci* 2012; **25**(3): 675-678.

[6] Adeshina GO, Okeke CL, Osuagwu NO, Ehinmidu JO. Preliminary *in vitro* antibacterial activities of ethanolic extracts of *Ficus sycomorus* Linn. and *Ficus platyphylla* Del. (Moraceae). *Afr J Microbiol Res* 2010; **4**(8): 598-601.

[7] Herre EA, Jandér KC, Machado CA. Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. *Ann Rev Ecol Evol Syst* 2008; **39**: 439-458.

[8] Wallis TE. *Textbook of pharmacognesy*. 4th ed. Boston: J. & A. Churchill; 2008.

[9] Ahmad S, Rao H, Akhtar M, Ahmad I, Hayat MM, Iqbal Z, et al. Phytochemical composition and pharmacological prospectus of *Ficus bengalensis* Linn. (Moraceae)-a review. *J Med Plants Res* 2011; **5**(28): 6393-6400.

[10] Bolis MS. Antioxidant determination by the use of a stable free radical. *Nature* 1958; **181**: 1199-1200.

[11] Hossain MA, Shah MD, Gnanaraj C, Iqbal M. *In vitro* total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant *Tetrastigma* from Sabah. *Asian Pac J Trop Med* 2011; **4**(9): 717-721.

[12] Ahlam AA, Hazaa AM, Afaf W, Sadri S, Amzad H, Sohail A. *In vitro* antioxidant, cytotoxic and antimicrobial screening of the leaves of Acridocarpous orientalis, native to Sultanate of Oman. *British J Pharm Res* 2013; **3**(4): 734-742.

[13] Hossain MA, Rahman SM. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. *Food Res Int* 2011; **44**(3): 672-676.

[14] Jung EK. Antimicrobial activity of extract and fractions from *Dryaria fortune* against oral bacteria. *J Bacteriol Virol* 2007; **37**(2): 61-68.

[15] Jeong MR, Cha JD, Lee YE. Antibacterial activity of Korean fig (*Ficus carica* L.) against food poisoning bacteria. *Korean J Food Cookery Sci* 2005; **21**: 84-93.

[16] Ahmad J, Khan I. Evaluation of antioxidant and antimicrobial activity of *Ficus carica* leaves: an *in vitro* approach. *J Plant Pathol Microbiol* 2013; **4**: 157.