April 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.1 • 354-360

EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACT OF *FICUS FISTULOSA LEAVES*: AN UNEXPLORED PHYTOMEDICINE

Sabreena Chowdhury Raka [1]*, Arifur Rahman [2,3] and Md. Kabir Hasan Kaium [2,3]

[1]Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka-1207, Bangladesh.
[2]Computational Biology Research Lab, Department of Pharmacy, Jagannath University, Dhaka-1100, Bangladesh.
[3]FDCC Testing & Research Lab, Department of Pharmacy, Jagannath University, Dhaka-1100, Bangladesh.

* raka.pharmacy@diu.edu.bd

Abstract

Ficus fistulosa is a traditional medicine to use in the remedies of diarrhea, diabetics, malaria. However, the antioxidant activity and antimicrobial activity of leaves extract of *Ficus fistulosa* have not been previously studied. Therefore, the objective of this study is to find out whether the extracts of *Ficus fistulosa* plant has antioxidant and antimicrobial properties or not. These results could then be used to carry out further experiments to find out the active phytochemicals and work with them. Furthermore, it can be seen that the demand for both antimicrobials and antioxidants is increasing in recent years and to be able to find both these agents in one plant will definitely help us save time and cost. The standard procedures were followed for measuring the antioxidant activity and the ability of the plant extract to scavenge DPPH. As for the antimicrobial activity, disk diffusion method using cultures of *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosae*, E. coli, E. coli mutant were carried out. The positive control ascorbic acid of which IC50 value is 11.93 µg/ml. On the other hand, the methanol extract showed promising DPPH free radical scavenging activity with IC50 value 16.66 µg/ml. As an antimicrobial agent, the water extract showed an effect at 0.4 mg/well concentration and the methanolic extract showed inhibitory activity at 0.4, 0.04 and 0.004 mg/well concentration. The *Ficus fistulosa* plant extract possessed antioxidant activity and also antimicrobial activity, where the potency depended on the type of extract. The methanolic extract showed better and wider inhibitory effect.

Keywords: *Ficus fistulosa*, Antioxidant activity, Antimicrobial activity, Phytochemical, IC50 value

Introduction

In today's world, the number of people falling ill is increasing, due to non-communicable diseases or infections. Thus, it has become necessary for us to look for alternative solutions and what better way than to look for it in Mother Nature. The use of herbs as medicines has played an important role in nearly every culture on earth, including Asia, Africa, Europe and America [1-4]. The World Health Organization (WHO) estimates that eighty percent (80%) of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care "Traditional medicine" and Bangladesh is an example of this [5]. *Ficus fistulosa* is a plant well known for its various uses. The root of the plant can be boiled and the infusion took for 3 days as a diaphoretic and it is used for post-natal treatments as well [6, 7]. The leaves when combined with opium, has a narcotic effect on the body. Other species of Ficus such as *Ficus septica, Ficus sycomorus, Ficus benjamina, Ficus religiosa, Ficus racemosa, Ficus pumila, Ficus vasta, Ficus thonningii* and *Ficus capensis* have shown antimicrobial activity and so an attempt has been made to evaluate the antimicrobial potency of *Ficus fistulosa* as well. At the same time, researchers have been testing out fruits, vegetables, herbs, leaves, and other foods to find out the nature of chemicals present in them and see their activities [8-15]. Many of these plant species contain antioxidants and they are capable of deactivating free radicals. These free radicals are very harmful and in recent studies, it has been found that free radicals are the main cause of aging and other degenerative diseases such as cancer, immune system decline, brain dysfunction, cataracts and heart diseases [16-21]. Therefore, antioxidants are essential for maximizing cellular and systemic health and overall functioning of an individual [22-25]. As a result, an experiment was also conducted to see the free radical scavenging activity of the phytochemicals obtained from *Ficus fistulosa*.

Material and Method

Plant material and extraction

The leaves of the plant *Ficus fistulosa* were collected, dried and crushed into powder form. Then 30 mg of it was dissolved in 30 ml of distilled water with a few drops of dimethyl sulphoxide to help in dissolution. Furthermore, 5 mg was dissolved in 5 ml of ethanol as the alcoholic extract.

Phytochemical investigation

Ficus fistulosa was tested for different phytochemicals like Tannins, Alkaloids, Saponins, Flavonoids, Glycoside, Reducing Sugar and Gum using the standard procedures.

Antibacterial activity

For this study, disk diffusion method was conducted using the cultures of Bacillus subtilis, *Staphylococcus aureus, Pseudomonas aeruginosae* which were the Gram-positive bacterial species and E. coli, E. coli mutant, Klebsiella pneumonia which was the Gram-negative bacterial species. The disks were prepared by using roundly cut filter papers which were then impregnated with test samples of known concentrations using a micropipette. The dried disks were then placed on Petri dishes containing agar medium seeded with the microorganisms. These disks were left for 12 hours at 40°C.

DPPH free radical scavenging Assay

The standard procedures were followed here and so, at first 10 mg extract of *Ficus fistulosa* was mixed with 10 ml of methanol (99-100%) to prepare 1000 µg/ml solution of extract as a stock solution. Ten more were made with concentrations of 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.81 µg/ml, 3.90 µg/ml, 1.95 µg/ml, 0.97 µg/ml. In the same way, various concentrations (500 µg/ml–0.97 µg/ml) of ascorbic acid solutions were prepared. 2 mg DPPH powder was measured by electronic balance and mixed with 100 ml of methanol (99-100%) to prepare 20 µg/ml DPPH solution. It should be kept in a cool, dry and dark place. 2.0 ml of a methanol solution of the sample (Control/extractives) at a different concentration from 500.0 to 0.977µg/ml were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ ml). After 30 minutes' reaction period at room temperature in a dark place, the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer.

Inhibition of free radical DPPH in percent (1%) was calculated as follows-

$$\{(A_o - A1)/A_o\} \times 100$$

Where, A_o is the absorbance of the control reaction (containing all reagents except the test material), and A1 is the absorbance of the extract/standard. Extract/standard concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract concentration.

Result and Discussion

Results of the phytochemical investigation

After completing a wide range of chemical test for the identification of major classes of therapeutically important compounds, the followings were found. The table below will give us a broad idea about phytochemicals present in *Ficus fistulosa*. **[Table-1]**

Antibacterial activity

After 12 hours the agar nutrient dishes were taken out of the incubator and the zone of inhibition measured in millimeters. The table below shows the data.

Ec – Escherichia coli, Ecm – E. coli mutants, Sa – Staphylococcus aureus, Bs – Bacillus subtilis, Kp – Klebsiella pneumoniae, Pa – Pseudomonas aeruginosae.

From the collected data, it can be seen that aqueous extract of the plant possesses inhibitory activity at 0.4 mg/well concentration tested against all bacterial strains. However, no inhibition was observed at lower concentrations. The methanolic extract possesses inhibitory activity at 0.4 mg/well and 0.004 mg/well concentration against all bacterial strains. At the least concentration used (0.004 mg/well), the extract shows activity against all except E. coli and Bacillus subtilis. **[Table-2]**

DPPH Free Radical Scavenging Activity

The DPPH test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. Practically, the reaction brings about the reduction of DPPH radicals to the corresponding hydrazine, which is manifested by a color change from violet to yellow, which is monitored spectrophotometrically. It is evident from the table that the % scavenging of DPPH radical was found to rise with increasing concentration of the samples. The positive control ascorbic acid of which IC50 value is 11.93 µg/ml. On the other hand, the methanol extract showed promising DPPH free radical scavenging activity with IC50 value 16.66 µg/ml. **[Table-3], [Table-4], [Figure-1], [Figure-2]**

Conclusion

Through this experiment, it was seen that there were many phytochemicals present in the extract of *Ficus fistulosa* sample and it is because of one or many of these compounds that the antioxidant properties of the plant could be seen. The IC50 value for *Ficus fistulosa* was 16.66 µg /ml. If the compound responsible for the antioxidant activity would be specifically located, then it can be used instead of synthetic drugs. On the other hand, the research showed promising results for the antimicrobial activity of *Ficus fistulosa* and the antimicrobial agent s can be further modified as phytomedicine for infectious diseases. Furthermore, they gave result against both Gram-positive and negative bacteria and so the plant extract can be used on a wide variety of pathogens. It was also seen that the methanolic extract showed better potency. In conclusion, the plant extract showed the positive result as both an antioxidant and antimicrobial agent, therefore opening up an array of opportunities to work with this plant species.

Reference

[1] Wargovich, M. J., Woods, C., Hollis, D. M., & Zander, M. E. (2001). Herbals, cancer prevention, and health. The Journal of nutrition, 131(11), 3034S-3036S.

[2] Patwardhan, B., Warude, D., Pushpangadan, P., & Bhatt, N. (2005). Ayurveda and traditional Chinese medicine: a comparative overview. Evidence-Based Complementary and Alternative Medicine, 2(4), 465-473.

[3] Bannerman, R. H. (1983). The role of traditional medicine in primary health care.

[4] Scartezzini, P., & Speroni, E. (2000). Review on some plants of Indian traditional medicine with antioxidant activity. Journal of Ethnopharmacology, 71(1-2), 23-43.

[5] World Health Organization. (1978). The promotion and development of traditional medicine. Report of a WHO meeting. The promotion and development of traditional medicine. Report of a WHO meeting. (No. 622). Geneva, Switzerland.

[6] Percival, M. (1996). Antioxidants. Clin. Nutr. Ins., 1, 1-6.

[7] Rice-evans, C. A., Miller, N. J., Bolwell, P. G., Bramley, P. M., & Pridham, J. B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free radical research, 22(4), 375-383.

[8] Almas, K., Al-Bagieh, N. H., & Akpata, E. S. (1997). In vitro antimicrobial effects of extracts of freshly cut and 1-month-old miswak (chewing stick). Biomedical Letters (United Kingdom).

[9] Fadulu, S. O. (1975). The antibacterial properties of the buffer extracts of chewing sticks used in Nigeria. Planta Medica, 27(02), 122-126.

[10] Reynold, J. E., & Martindale, K. B. (1996). The extra pharmacopeia 31st edition. Published by the council of the royal pharmaceutical society of Great Britain, 885.

[11] Lis-Balchin, M., & Deans, S. G. (1997). Bioactivity of selected plant essential oils against Listeria monocytogenes. Journal of applied microbiology, 82(6), 759-762.

[12] Deans, S. G., & Ritchie, G. (1987). Antibacterial properties of plant essential oils. International journal of food microbiology, 5(2), 165-180.

[13] Morris, J. A., Khettry, A., & Seitz, E. W. (1979). Antimicrobial activity of aroma chemicals and essential oils. Journal of the American Oil Chemists' Society, 56(5), 595-603.

[14] Ross, S. A., El-Keltawi, N. E., & Megalla, S. E. (1980). Antimicrobial activity of some Egyptian aromatic plants. Fitoterapia, 51(4), 201-205.

[15] Yousef, R. T., & Tawil, G. G. (1980). Antimicrobial activity of volatile oils. Die Pharmazie, 35(11), 698-701.

[16] Anderson, D. (1996). Antioxidant defenses against reactive oxygen species causing genetic and other damage. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 350(1), 103-108.

[17] Islam, M. (2017). Evaluation of Antioxidant Activity and Determination of Total Phenolic Content of leaves extract of Heritiera littoralis (Sundari tree) (Doctoral dissertation, Daffodil International University).

[18] Van Poppel, G., & van den Berg, H. (1997). Vitamins and cancer. Cancer letters, 114(1-2), 195-202.

[19] Daniel, R. S., Devi, K. S., Augusti, K. T., & Nair, C. R. (2003). Mechanism of action of antiatherogenic and related effects of Ficus bengalensis Linn. Flavonoids in experimental animals.

[20] Obrenovich, M. E., Nair, N. G., Beyaz, A., Aliev, G., & Reddy, V. P. (2010). The role of polyphenolic antioxidants in health, disease, and aging. Rejuvenation research, 13(6), 631-643

[21] Krishnaiah, D., Sarbatly, R., & Bono, A. (2007). Phytochemical antioxidants for health and medicine a move towards nature. Biotechnology and Molecular Biology Reviews, 2(4), 97-104

[22] Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y. S. R., & De, B. (2010). Free radicals, antioxidants, diseases, and phytomedicines: current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research, 3(1), 91-100.

[23] Sies, H. (1997). Oxidative stress: oxidants and antioxidants. Experimental Physiology: Translation and Integration, 82(2), 291-295.

[24] Flora, S. J. S. (2007). Role of free radicals and antioxidants in health and disease. Cellular and Molecular Biology, 53(1), 1-2.

[25] Morrissey, P. A., & O'brien, N. M. (1998). Dietary antioxidants in health and disease. International Dairy Journal, 8(5-6), 463-472.

Table-1: List of phytochemicals found in Ficus fistulosa

Group Name	Name of the Test	*Ficus fistulosa*
Tannins	5% Ferric Chloride	+
Alkaloids	Mayer's Test, Dragendroff's Test	++, ++
Saponins	Test for Saponins	-
Flavonoids	Test for Flavonoids	-
Glycoside		-
Reducing Sugar	Fehling Sugar(Standard)	+
Gum		-

Table-2: Comparative Zone of Inhibition of various extracts at various conc.

Test Samples	Conc. (mg/well)	Zone of inhibition (mm)					
		E.c.	Ecm	S.a	Bs	K.p.	P.a.
Standard	0.4	34.33+ 0.56	37.5 +0.76	38.66+ 0.87	38.83+ 0.60	35.00 +0.58	38.83+ 0.83
Water extract	0.4	7.16+0.26	7.27+0.01	7.82+0.03	7.62+0.01	6.7+0.09	6.65+0.05
	0.04	--	--	--	--	--	--
	0.004	--	--	--	--	--	--
Methanol extract	0.4	10.35+0.15	11.38+0.1 3	10.26+0.18	9.43+0.11	8.3+0.11	11.7+0.13
	0.04	8.61+0.10	8.86+0.09	7.93+ 0.09	7.73+ 0.08	6.55 +0.07	8.9 +0.08
	0.004	--	6.61+0.11	6.56+ 0.12	--	6.65+ 0.11	6.65+ 0.13

Table-3: IC50 value of Ascorbic acid

Absorbance of control	Conc. (µg/ml)	Absorbance of Ascorbic Acid	Inhibition (%)	IC_{50} (µg/ml)
0.496	500	0.066	86.69	11.93
	250	0.07	85.89	
	125	0.071	85.69	
	62.5	0.074	85.08	
	31.25	0.079	84.07	
	15.625	0.096	80.65	
	7.813	0.212	57.26	
	3.906	0.307	38.11	
	1.953	0.43	13.31	
	0.977	0.45	9.27	

Table-4: the IC50 value of leaves extract of Ficus fistulosa.

Absorbance of control	Conc. (µg/ml)	Absorbance of Ascorbic Acid	Inhibition (%)	IC50 (µg/ml)
0.496	500	0.026	94.76	16.66
	250	0.039	92.14	
	125	0.067	86.49	
	62.5	0.098	80.24	
	31.25	0.122	75.4	
	15.625	0.274	44.76	
	7.813	0.329	33.67	
	3.906	0.396	20.16	
	1.953	0.441	11.09	
	0.977	0.474	4.36	

Figure 1: DPPH free radical scavenging activity of Ascorbic acid (ASA)

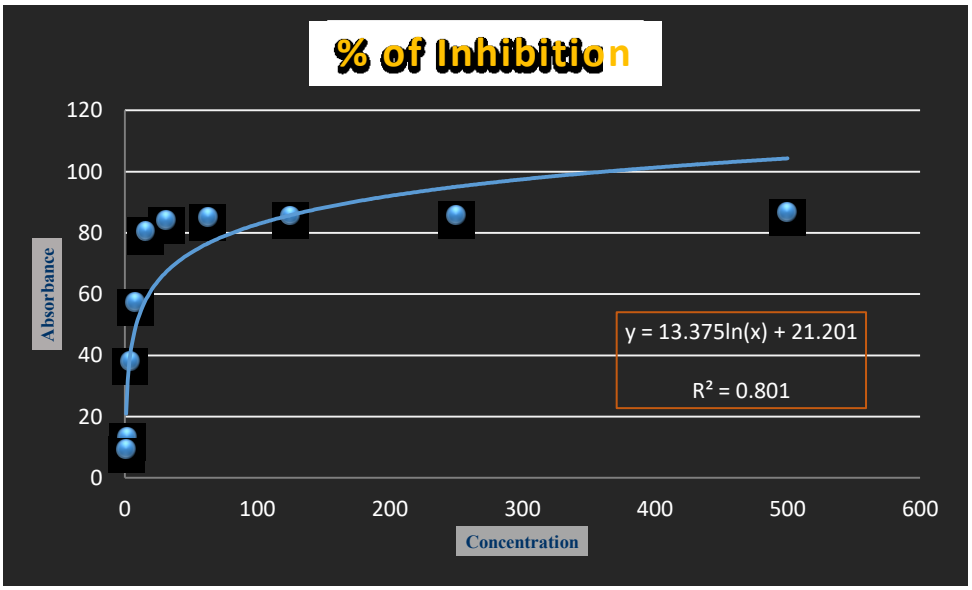

Figure-2: DPPH free radical scavenging activity of leaves extract of Ficus fistulosa at different concentration.