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EFFECTS OF DIFFERENT SOLVENTS ON ANTIOXIDANT ACTIVITY OF FICUS RELIGIOSA L. LEAF EXTRACT

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ABSTRACT In the present investigation, *Ficus religiosa* leaves were assayed for their phenolic contents and screened for their DPPH free radical scavenging activity using three different solvents namely, acetone, methanol and water. The results revealed that methanol extract contained the highest amount of total phenolics (3.2) µg GAE/g fwb) and DPPH free radical scavenging activity of the *Ficus religiosa* leaf extracts varied widely and it increased with increase of concentration levels. Methanol extract exhibited the highest DPPH free radical scavenging activity with IC_{50} value (59.8 µg /mL) .

Keywords *Ficus religiosa*, phenolics, antioxidant activity.

INTRODUCTION

Plant secondary metabolites are the interest subject of research, but their extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed throughout the solvent extraction process[1]. During the extraction of plant material, it is important to minimize interference from compounds that may co-extract with the desired phytochemicals, and to avoid contamination of the extract as well as to prevent decomposition of metabolites or artifact formation as a result of extraction conditions. Extraction of phytochemicals from a plant material can be carried out using different solvents because of diversity of chemical nature and often unique distribution of these compounds in the plant matrix[2-3]. Various solvents are being frequently used for extraction of plant antioxidant compounds. However, the extract yields and antioxidant efficacy of the resulting extracts is strongly affected by polarity of the solvent as well as the chemical nature of the extracted compounds[3-4].

Medicinal plants have been used an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value[5]

Ficus religiosa is an important medicinal tree species belonging to the family *Moraceae.* It is commonly known as the Peepal tree and is one of the most reversed trees in Asia due

to its mythological and traditional background. It one among the four sacred trees "Nalpamara" (Ksirivarkas) meant to be planted around the home and temples[6]. The leaves contain 9.63% crude protein, 26.84% crude fibres,2.53% calcium oxalate and 0.4% phosphorous[7]. In Ayurvedic medicine, the *Ficus religiosa* were found to be effective wound healing medicine and tested in various experimental models. *Ficus religiosa* aqueous extract showed high antimicrobial activity. High activity was found against Bacillus subtilis and Pseudomonas aeruginosa, (multi-drug resistant)[8] The therapeutic utilities of plant have been indicated in traditional systems of medicine like Ayurveda,Unani,Siddha.[9] *Ficus religiosa* is a source of bioactive molecules that have antidiabetic properties[10].First time an antidiabetic biomolecules named stigmasterol was isolated from its leaves[11]. *Ficus religiosa* was also found to have anticancer[12],antiulcer[13],anti-asthmatic[14],dermatoprotective[15],antihelmenthic[16],and antioxidant properties[17] Therefore, the objective of present study was to evaluate the efficacy of solvents towards extracting of phenolic content from *Ficus religiosa* and also to assess their antioxidant activity.

RESULTS AND DISCUSSION

Total phenolic content

Total phenolic content of *Ficus religiosa* leaf extracts in three solvents varied widely. On fresh weight basis, methanol extract of *Ficus religiosa* contained the highest total phenolic content i.e. 3.2 µg GAE/g fwb followed by water (2.7 µg GAE/g fwb) and acetone (1.0 µg GAE/g fwb) extracts (Table 1).

Table 1: Total phenolics of *Ficus religiosa* leaf extract prepared using different solvents

Solvent	Total phenolics content (µg GAE/g fwb)
Acetone	1.0 ± 0.01
Methanol	3.2 ± 0.02
Water	2.7 ± 0.01

DPPH free radical scavenging activity

2,2'-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical (purple colour) and it transforms to non radical form (yellow colour) by abstracting one electron and hence, it is widely used as a measure for the electron donation capacity of antioxidants under assay conditions. In present studies, DPPH free radical scavenging activity (%) of the acetone, methanol and water extracts of *Ficus religiosa* leaf extract varied widely and it increased with increase of concentration levels (Table 2). It ranged from 14.1 to 90.2% (methanol extract), from 8.2 to 78.1% (water extract) and from 6.21 to 59.8% (acetone extract) at different concentration levels ranging from 15 to 250 µg /mL.

The IC_{50} value of methanol extract was lowest i.e. 59.8 µg /mL followed by 102.7 µg /mL of water extract and 151.3 µg /mL of acetone extract thereby showing that methanol extract has highest activity followed by water and acetone extracts.

Table 2: DPPH free radical scavenging activity (%) of different solvents of *Ficus religiosa* leaf extract.

Extracts / Conc. (µg /mL)	DPPH Free Radical Scavenging Activity (%)					IC_{50} (µg /mL)
	250	125	60	30	15	
Acetone	59.8±0.2	42.3±0.11	28.3±0.42	19.2±0.15	6.21±0.17	151.3
Mehanol	90.2±0.15	75.2±0.15	47.3±0.18	34.2±0.16	14.1±0.25	59.8
Water	78.1±0.22	54.3±0.40	36.4±0.36	23.1±0.15	8.2±0.29	102.7

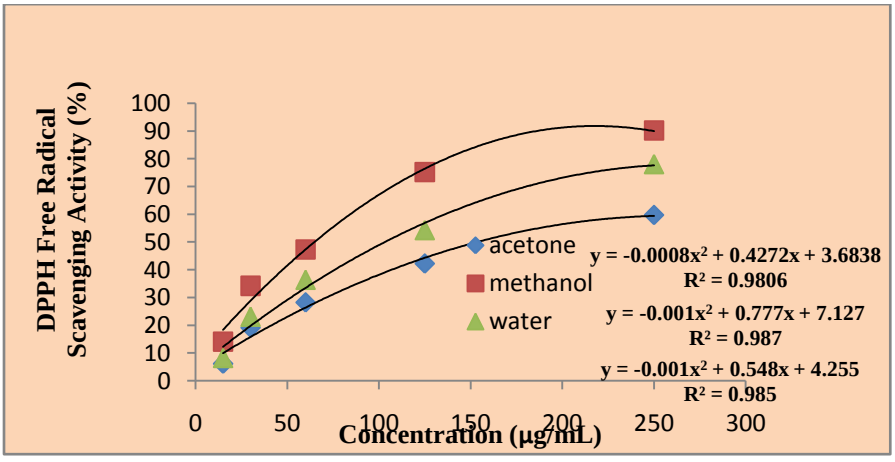

Fig. 1: Quadratic regression equations for IC_{50} values of different solvents of *Ficus religiosa* leaf extract.

EXPERIMENTAL

Plant material and extraction

Fresh and fully mature leaves of *Ficus religiosa* were procured from the campus of CCSHAU,Hisar. Ficus religiosa leaves were homogenized in Waring blender to make pulp. Ten gram of homogenized samples were extracted with 60 mL of solvents (acetone, methanol and water) in conical flasks by shaking on a mechanical shaker for 2 hr. Extracts were filtered and residues were again extracted twice (each shaking time 1 hr) with 40 and 30 mL respective solvents. Filtrates of each solvent from three extraction steps were pooled and their volumes were noted. Extracts were then used for estimation of total phenolics content and for evaluation of antioxidant activity.

Chemicals

The commercially available chemicals from Sigma-Aldrich, Qualigens, Merck and Hi-Media of highest purity, were used for various experimental procedures.

Estimation of total phenolics content

Total phenolics content of extracts was determined using Folin-Ciocalteu method[18]. Aliquots of 0.2 ml of extracts were mixed with 1 ml of 1 mol/L Folin-Ciocalteu reagent. After that, 2.0 ml of 20% (w/v) sodium carbonate solution was added. The solutions were mixed and volume was made up to 10.0 ml with distilled water. The absorbance was measured at 730 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using gallic acid as standard. Results were expressed as mg GAE/g on fresh weight.

DPPH free radical scavenging activity

The antioxidant activity of the extracts was evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method[19]. Acetone, methanol and water extracts were dried up completely and the weight of dry mass was noted. The dry mass of acetone and methanol extracts was redissolved in appropriate amount of methanol to make the stock solution (50 mg/mL). Since, the dry mass of water extract was not soluble in pure methanol, hence, it was redissolved in 50% (v/v) methanol : water to make the stock solution. From stock solution, different concentrations (15-250 µg/mL) were made by

appropriate dilutions with respective solvents (i.e. methanol for acetone and methanol extracts and with methanol : water for water extracts). For evaluation of antioxidant activity, in 0.2 mL of extracts (various concentrations), 3.0 mL of 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH; 0.1mM in 100 % methanol) was added and mixed thoroughly for 5 min. For antioxidant activity in water extracts (various concentrations), DPPH stock solution was prepared in 50 % (v/v) methanol : water and remaining procedure was same. A control was also made containing 0.2 mL of each solvent instead of extract. The absorbance of the sample as well as control was measured at 517 nm after 30 min of incubation in dark at room temperature using the UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank containing respective solvent. Three replications were carried out for each sample. A graph was drawn by plotting per cent DPPH free radical scavenging activity (y-axis) against extract concentration (x-axis). Then using Microsoft Excel Software, quadratic regression equation ($y= ax^2+bx+c$) was obtained and using the quadratic equation IC_{50} was calculated. The percentage of DPPH scavenged (% $DPPH^*_{sc}$) was calculated using:

$$\%\,DPPH^*_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample.

CONCLUSION

Results of present study shows that solvent play a vital role in the extraction of the plant constituents. Methanol extract of *Ficus religiosa* contained highest total phenolic contents and also exhibited highest antioxidant activity as compare to other solvents.

REFERENCES

1. A. Ghasemzadeh, H. Z. E. Jaafar and A. Rahmat, *J. Med. Plant. Res.*, 2011, **5**, 1147.
2 M. Antolovich, P. Prenzler, K. Robards and D. Ryan, *Analyst*, 2000, **125**, 989.
3. B. Sultana, F. Anwar and M. Ashraf, *Molecules*, 2009, **14**, 2167.

4. G. Shabbir, F. Anwar, B. Sultana, Z. M. Khalid, M. Afzal, M. Q. Khan and M. Ashrafuzzaman, *Molecules*, 2011, **16**, 7302.
5. A.Nostro , MP.Germano , V.D'Angelo ,A. Marino , MA.Cannatelli , Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Letters in Appl. Microbiol.* 2000, 30(**5**):379
6. DNG Bakshi , DC Pal ,P Sensarma , *A Lexicon of Medicinal Plants in India*, Naya Prokash, 2001
7. Patil V.V. PRB, Patil V.R, Pharmacognostical Studies and Evaluation of Anti-inflammatory Activity of Ficus bengalensisLinn, *Journal of Young Pharmacists*,2009,1(**1**)
8. Preeti. R., Vimal Devanathan V and M. Loganathan. Antimicrobial and antioxidant efficacy of some medicinal plants against food Borne pathogens advances in Biological Research 2010; **4** (2): 122-125
9. D.Singh,B.Singh and R.K goel, Traditional uses, phytochemistry and pharmacology of *Ficus religiosa;* A review.*Journal of Ethnopharmacology*.2011**,134**,565-583.
10. S.Elavarasi,K.Saravanan and C. Renuka, A systematic review on medicinal plants used to treat diabetes mellitus.*IIJPCBS*.2013,3(**3**):554-556.
11. S.Vedulla,S.A. Parameswari and C.Gopinath, Isolation of active compound in *Ficus religiosa. International journal of Pharmacognosy and Phytochemical Research*,2013,(**5**):60-65.
12. V.Gulecha and T.Sivakumar,2011.Anticancer activity of *Tephrosia purpurea* and *Ficus religiosa* using MCF 7 cell lines.*APJTM*,2011,43 (**6**):526-529.
13. M.Gregory,B.Divya,A.R.Mary,M.M.H.Viji,V.K.Kalaichelvan and V.Palanivel, Anti-ulcer activity of *Ficus religiosa* leaf ethanolic extract.Asian Pac J Trop Biomel.2013,3(**7)**:554-556.
14. M.Kapoor,N.Jasani,N.Acharya,S.Acharya and V.Kumar, Phytopharmacological evaluation and anti-asthmatic activity of *Ficus religiosa* leaves.APJTM,2011,4(**8**):642-644.

15. M.Waheed, F.Muhammad,I.Javed,M.Akhtar,M.K.Saleemi.T.Khaliq,M.Awais and M.I. Anwar,Dermatoprotective effects of some plant extracts against experimentally induced toxicological insults in rabbits.Toxicology and Industrial Helth2013,pp1-8.
16. Z.Iqbal,Q.K.Nadeem,M.N.Khan,M.S. Akhtar and Waraich,F.N.,In vitro antihelmintic activity of Allium sativum,Zingiber officinate,Curcurbita Mexicana and *Ficus religiosa*.Int.J Agri Bios.2001**,3**.454-457.
17. B.Sultana,F.Anwar and M.Ashraf, Effect of extraction solvent on the antioxidant activity of selected medicinal plant extracts.Molecules ,2009,**14**,2167-2180.
18. V. L. Singleton and J. A. Rossi, *Am. J. Enol. Vitic.*, 1965, **16**, 144.
19. T. Hatano, H. Kagawa, T. Yasuhara and T. Okuda, *Chem. Pharm. Bull.*, 1988, **36**, 2090.