Plant Archives Vol. 20, Supplement 1, 2020 pp. 2669-2673 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

COMPARISON OF EXTRACTION YIELD, PHYTOCHEMICALS AND *IN VITRO* ANTIOXIDANT POTENTIAL OF DIFFERENT EXTRACTS OF TWO *FICUS SPECIES*

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Abstract

Ficus religiosa (L.) and *Ficus benghalensis* (L.), belonging to the family Moraceae, are two popular species of the genus Ficus. Various parts of both the trees like bark, fruit, leaves and seeds are widely used in indigenous system of medicine. The present study was conducted to compare extraction yield, phytochemicals and antioxidant activity of various solvent extracts of leaves of *F. religiosa* and *F. benghalensis.* Water extract of leaves (11.225±1.14%) of *F. religiosa* and methanolic extract of leaves (4.53 ± 0.28%) of *F. benghalensis* showed highest extraction yield. Chloroform extract of *F. religiosa* (4.76±0.15 µg/ml) showed highest % DPPH radical scavenging activity, whereas, water extract of *F. religiosa* (0.14±0.04 µg/ml) showed highest % ABTS radical scavenging activity. In case of *F. benghalensis,* chloroform extract (4.69±0.26 µg/ml) showed highest %DPPH radical scavenging activity, whereas, water extract (0.19 ± 0.11 µg/ml) showed highest %ABTS radical scavenging activity. Both the plants showed significant antioxidant activity in chloroform and water extract of leaves of both the trees. The study showed that leaves of both the plants can be exploited as natural source of antioxidants.

***Keywords*:** *Ficus religiosa*, *Ficus benghalensis,* Cold maceration, Extraction yield, Antioxidant activity.

Introduction

During recent era, several plants, especially medicinal plants, have been gained enormous importance due to their antioxidant property. Medicinal plants act as valuable therapeutic agents, and most of modern drugs are either plant-derived natural products or their derivatives (Kinghorn *et al*., 2011; Newman and Cragg, 2012). Several reports have highlighted the benefits of consumption of antioxidant compound containing plants, because of their role in down-regulating several degenerative processes and can effectively lower the incidence of cancer and cardio-vascular diseases (Arabshahi-Delouee and Urooj, 2007). The recovery of antioxidant compounds from plants through extraction techniques are based upon chemistry and uneven distribution of plant matrix. The soluble phenols are present in higher amount in the outer epidermal and sub-epidermal layers of fruits and grains as compared to mesocarp and pulp of fruits (Antolovich *et al.,* 2000). Isolation of plant antioxidant compounds is generally done using the solvent extraction method. But yield and antioxidant activities of plants mainly depend upon the type of solvent used for extraction as various chemical compounds show different solubility with the polarity of the solvent.

The genus Ficus (Moraceae) is one among the largest genera of angiosperms comprises of more than 800 species and 2000 varieties of Ficus genus, occurring in most tropical and subtropical forests worldwide (Hamed, 2011). *Ficus religiosa* and *F. benghalensis* are the two commonly occurring members of the genus Ficus (Sawarkar *et al*., 2011). All Ficus species act as source of latex-like material providing protection and self-healing to the plant (Sirisha *et al*., 2010). Several studies have highlighted the importance of Ficus species in the management of various types of diseases like respiratory disorders, sexual disorders, central nervous system disorders (CNS), cardiovascular disorders (CVS), gastric problems, skin infections, and diabetes, etc (Sirisha *et al*., 2010; Vinutha *et al*., 2007). *F. religiosa* has been extensively used in traditional medicine its bark, fruits, leaves, adventitious roots, latex and seeds are used as a medicine in combination with other herbs. The bark of *F. religiosa* is important Ayurvedic formulations like "Pancha Valkaladi Tailum and Pancha Valkala Kashaya" (Panda, 2005; Singh and Panda, 2005). *F. religiosa* modifies pitta and kappa hence prescribed an Ayurvedic remedy for the disorders associated with their imbalance like respiratory disorders, ulcers, stomatitis, hiccup, arthritis, gout, skin disease (Singh *et al*., 2011). Traditionally in India, Stem latex of *F. benghalensis* is applied topically on heel crack and the young stem is used as a toothbrush to treat dental problems (Muthu *et al*., 2006). This plant has antipyretic activities, analgesic effects (Vikas *et al*., 2010), antitumor activities (Shukla *et al*., 2004), and anti-inflammatory (Patil *et al*., 2009) activities. The bark of this plant has anti-inflammatory and analgesic properties in animal models (Thakare *et al*., 2010) and it is useful for burning sensation, ulcers, and painful skin diseases (Jaiswal and Ahirwar, 2013). Owing to its various pharmacological properties, the present study was performed to study the effect of solvent on extraction yield, and antioxidant activity of leaves of *F. religiosa* and *F. benghalensis.*

Materials and Method

Chemicals and solvents

The chemicals such as 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were obtained from Sigma-Aldrich Co. LLC, Mumbai. The solvents such as n-hexane, methanol and chloroform used were procured from Loba Chemie Pvt. Ltd., Mumbai.

Collection of plant materials and Extract preparation

Leaves of *F. religiosa* and *F. benghalensis* were collected in the month of March (2019) from Bilaspur, Himachal Pradesh, India. Leaves from both the trees were washed twice and allowed to dry at 40 °C. The dried plant

material was converted into powder using electric grinder. Cold macerated method was used to prepare extracts of different solvents (n-hexane, methanol, chloroform and water) (Kumar *et al*., 2016; Kumar *et al*., 2018; Chandel *et al*., 2019). The dried extracts were stored at 4 °C in airtight bottles till further use.

Comparison of % extraction yield in various solvent extracts of *F. religiosa and F. benghalensis*

The % age extraction yield of each solvent extract of leaves of *F. religiosa and F. benghalensis* was calculated using the following equation-

$$\% \text{ Extraction yield} = \frac{\text{amount (g) of dried crude extract obtained} \quad \text{amount (g) of finely grounded powder} \times 100}{\text{plant material used (g)}}$$

Qualitative phytochemical analysis in various solvent extracts of *F. religiosa and F. benghalensis*

All the solvent extracts of *F. religiosa and F. benghalensis* were examined for the presence of various secondary metabolites such as phenolic compounds, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate as described by Khandelwal, 2008 and Guleria *et al*., 2016.

In vitro* antioxidant activity in various solvents extracts of leaves of *Ficus religiosa* and *Ficus benghalensis

Antioxidant activity of various solvent extracts of leaves of *F. religiosa* and *F. benghalensis* was determined using methods such as DPPH, and ABTS method. To analyze the antioxidant potential of various extracts of leaves of *F. religiosa* and *F. benghalensis,* all the extracts were dissolved at a concentration of 1 mg/ml in ethanol and then diluted in order to prepare different concentrations (10-80 µg/ml) for antioxidant assays. Ascorbic acid was used as standard antioxidant compound for comparative analysis in all assays.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of various extracts of leaves of *F. religiosa* and *F. benghalensis* was measured by the method described by Barros *et al*. (2007) and Rolta *et al*. (2018). The capability of scavenging DPPH radical was calculated using the following equation:

$$\%\text{DPPH radical scavenging activity} = \{A_{(control)} - A_{(sample)} / A_{(control)}\} \times 100$$

Where $A_{(control)}$- Absorbance of control and $A_{(sample)}$ - absorbance of the test/standard.

ABTS scavenging assay

ABTS scavenging activity of various extracts of leaves of *F. religiosa* and *F. benghalensis* was calculated using method described by Re *et al.* (1999). Percentage ABTS scavenging activity was calculated as-

$$\textbf{ABTS radical scavenging activity (\%)} = [(A_{control} - A_{sample})] / (A_{control})] \times 100$$

where $A_{control}$ is the absorbance of ABTS radical + methanol; A_{sample} is the absorbance of ABTS radical + sample extract /standard.

Statistical analysis:

All analysis was done at least in duplicate/triplicates, and these values were then presented as average values along with their standard derivations. Subsequently, the IC_{50} values of the antioxidant assay were calculated from the linear regression method.

Results

Comparison of extraction yield among various solvent extracts of leaves of *F. religiosa* and *F. benghalensis*

The extraction with solvents of increasing polarity resulted in extraction of phytocompounds of a plant according to their degree of solubility. The yield percent of hexane, chloroform, methanol and water extracts obtained from leaves of *F. religiosa* and *F. benghalensis* was shown in Fig. 1. It was observed that among all extracts of leaves of *F. religiosa,* highest extraction yield was obtained with water (3.46±0.35%) followed by chloroform (1.36±0.20%), methanolic (1.01±0.06%) and hexane (0.73±0.06%). In case of *F. benghalensis,* methanolic extract (2.27±0.28%) showed highest extraction yield followed by hexane (0.93±0.08%), chloroform (0.58±0.13%) and water (0.37±0.09%) (Fig. 1).

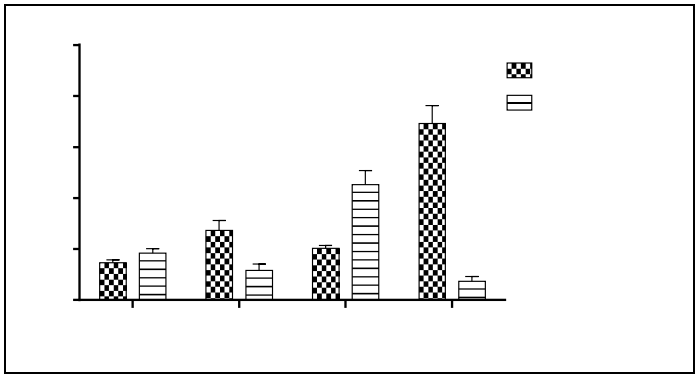

Fig. 1: Percentage extraction yield in various extracts of leaves of *F. religiosa* and *F. benghalensis.* HE-Hexane extract; CE-Chloroform extract; ME-Methanolic extract; WE-Water extract.

Comparative phytochemical screening of various solvent extracts of *Ficus religiosa* and *F. benghalensis.*

Qualitative phytochemical analysis showed that carbohydrates are present, while alkaloids are absent in all solvent extracts (hexane, chloroform, methanol and water) of *F. religiosa*. Methanolic and water extracts of leaves of *F. religiosa* showed the presence of same type of phytochemicals such as phenolic, flavonoids, tannins, terpenoids, carbohydrates and saponins. Hexane extract showed the presence of only carbohydrates, while chloroform extract showed the presence of glycosides along with carbohydrates. In case of *F. benghalensis,* terpenoids, carbohydrates and glycosides are found to be detected in hexane extract; while carbohydrates, glycosides and alkaloids are present in chloroform extract of leaves. Phenolic, flavonoids, tannins, alkaloids, terpenoids, carbohydrates and saponin were detected in methanolic and water extract of leaves of *F. benghalensis* (Table-1). Similar to present study, Prakash *et al*. (2017) also reported the presence of alkaloids, carbohydrates, saponins, phenolics, flavonoids, tannins and terpenoids in aqueous and methanolic extracts of *F. religiosa.* Etratkhah *et al*. (2019) have reported the presence of steroids, alkaloids, tannins and phenolic content, cardiac glycosides, anthraquinones and flavonoids in various fractions of aerial roots of *F. benghalensis*.

Table 1 : Qualitative analysis of extracts of *F. religiosa* and *F. benghalensis.*

Phytocompounds	*F. religiosa*				*F. benghalensis*			
	HE	**CE**	**ME**	**WE**	**HE**	**CE**	**ME**	**WE**
Phenolic compounds	-	-	+	+	-	-	+	+
Tannin	-	-	+	+	-	-	+	+
Flavonoids	-	-	+	+	-	-	+	+
Alkaloids	-	-	-	-	-	+	+	+
Terpenoids	-	-	+	+	+	-	+	+
Carbohydrates	+	+	+	+	+	+	+	+
Glycosides	-	+	+	+	+	+	-	-
Saponin	-	-	+	+	-	-	+	+

HE-Hexane extract, CE-Chloroform extract, ME-Methanol extract, WE-Water extract.
(+) indicate presence, while (–) indicate absence of phytocompounds.

Comparison of antioxidant activity in various solvent extracts of leaves of *F. religiosa* and *F. benghalensis*

Antioxidant activity in various solvent extracts of leaves of *F. religiosa* and *F. benghalensis* was analyzed using DPPH and ABTS radical scavenging assay and expressed in terms of half maximal inhibitory concentration (IC_{50}). Antioxidant activity with both assay showed dependence on concentration, i.e. activity increases with increase in concentration of extracts. Ascorbic acid was used as control in both the assays. Concentration-dependent % DPPH radical scavenging activity of leaves of *Ficus religiosa* and *Ficus benghalensis* was shown in Fig. 2 (A&B). In case of *F. religiosa*, chloroform extract (4.76±0.15 µg/ml) showed highest % DPPH radical scavenging activity, whereas, water extracts (0.14±0.04 µg/ml) showed highest % ABTS radical scavenging activity. Least %DPPH radical scavenging activity was observed with hexane extract (20.11±1.10 µg/ml) and least %ABTS scavenging was identified in methanolic extract of leaf (1.52±0.29 µg/ml). Among all extracts of *F. benghalensis* chloroform extract (4.69±0.26 µg/ml) showed highest %DPPH radical scavenging activity, whereas, water extract (0.19±0.11 µg/ml) showed highest %ABTS radical scavenging activity. Least %DPPH radical scavenging activity was observed with hexane extract (21.57±0.96 µg/ml) and least %ABTS scavenging was identified in water extract of leaf (1.3±0.29 µg/ml) as show in table-1. Ascorbic acid showed IC_{50} value of 2.78±0.06 µg/ml with DPPH radical scavenging assay and 2.71±0.12 with ABTS radical scavenging assay (Table-1). Antioxidant nature of leaf of Ficus religiosa was also shown by various reports (Melinda *et al*., 2010; Charde *et al*., 2010; Kumar *et al*., 2011). Similarly, Pedgaonkar *et al*. (2019) have reported the antioxidant nature of leaves of *Ficus benghalensis.* Abusufyan *et al*. (2018) compare antioxidant nature of different solvent extracts (petroleum ether, benzene, chloroform, ethanol and distilled water) of leaves of Ficus species. All extracts of leaves of *F. benghalensis* showed comparatively high antioxidant nature as compared to that of *F. religiosa.*

Table 1 : Half maximal inhibitory concentration (IC_{50}) of leaves of *F. religiosa* and *F. benghalensis*. Values are expressed as mean ± S.D. of three independent experiments.

Plant	Assay	Half of inhibitory concentration (IC_{50}) (µg/ml)				
		AA	**HE**	**CE**	**ME**	**WE**
F. religiosa	DPPH	2.78±0.06	20.11±1.10	4.76±0.15	10.35±2.08	14.01±2.45
	ABTS	2.71±0.12	0.64±0.07	1.42±0.11	1.52±0.29	0.14±0.04
F. benghalensis	DPPH	2.78±0.06	21.57±0.96	4.69±0.26	17.69±1.6	13.13±3.7
	ABTS	2.71±0.12	1.05±0.12	0.58±0.12	0.19±0.11	1.3±0.29

AA-Ascorbic acid, HA-Hexane extract, CE-Chloroform extract, ME-Methanol extract, WE-Water extract

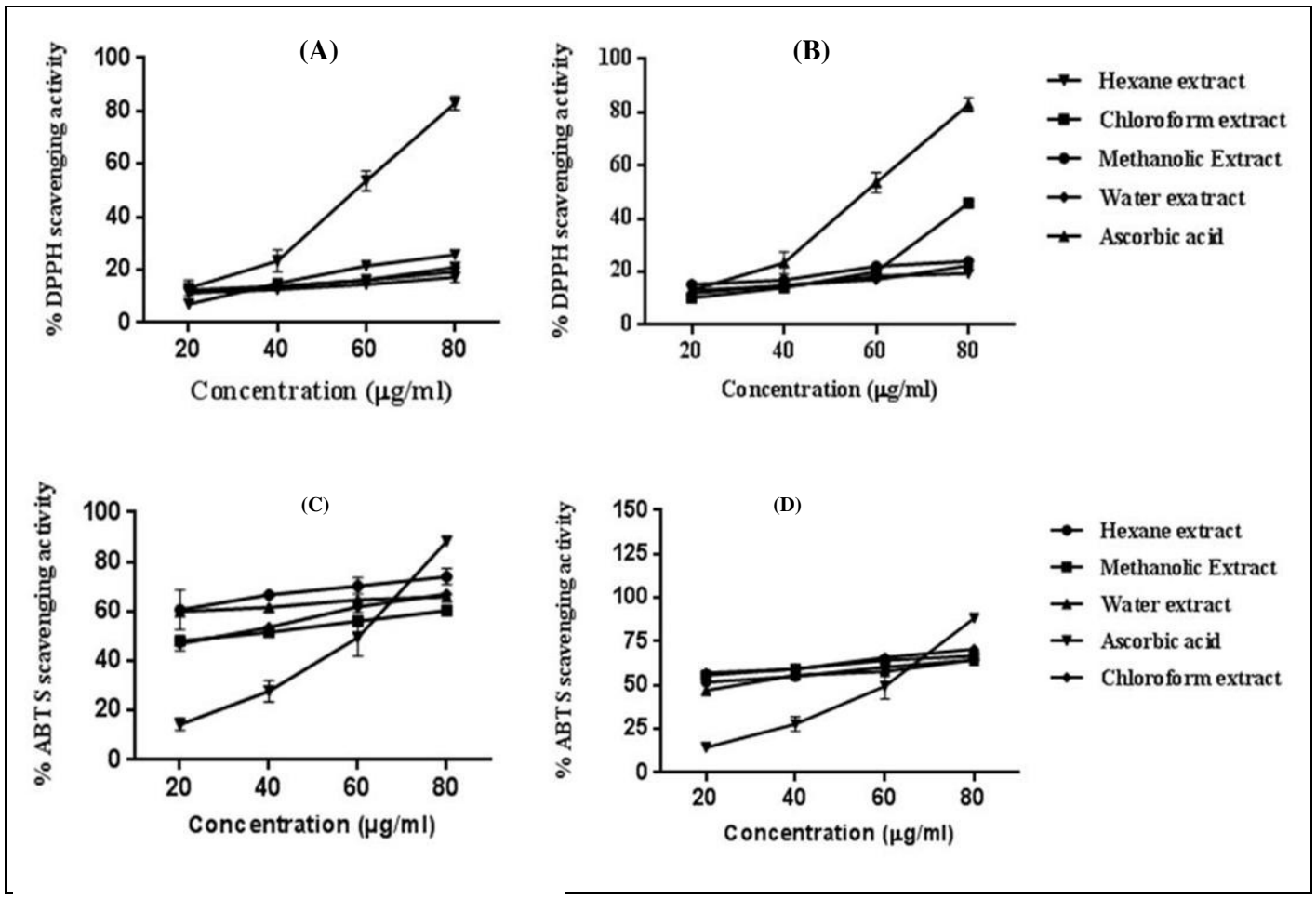

Fig. 2 : Concentration-dependent-radical scavenging of various solvent extracts of leaves of *F. religiosa* and *F. benghalensis* using DPPH (A-B) and ABTS assay (C-D).

Conclusions

It can be concluded from the present study that extraction yield was highest in water and methanol solvent in *F. religiosa* and *F. benghalensis***,** respectively. Phytochemicals analysis showed that phytocompounds depend upon type of solvent used for extraction. The highest antioxidant activity was also shown by chloroform and water extract in leaves of both the trees, thereby supporting the exploitation of leaves of *F. religiosa* and *F. benghalensis* as a source of natural antioxidants.

Acknowledgements

The authors acknowledge Shoolini University, Solan, for providing infrastructure support to conduct the research work. Authors also acknowledge the support provided by Yeast Biology Laboratory, School of Biotechnology, Shoolini University, Solan, India.

References

Abusufyan, S.; Ibrahim, M. and Mohib, K. (2018). Comparative in vitro antidiabetic and antioxidant activity of various extracts of Ficus species. Pharmacogn. Res. 10.

Antolovich, M.; Prenzler, P.; Robards, K. and Ryan, D. (2000). Sample preparation in the determination of phenolic compounds in fruits. Analyst. 125: 989-1009.

Arabshahi-Delouee, S. and Urooj, A. (2007). Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. Food Chem. 102: 1233-1240.

Bhaskara Rao, K.V.; Ojha, V.; Preeti, G.K. and Karthik, L. (2014). Phytochemical composition and antioxidant activity of *Ficus benghalensis* (Moraceae) leaf extract. Journal of Biologically Active Products from Nature. 4: 236-48.

Charde, R.M.; Dhongade, H.J.; Charde, M.S. and Kasture, A.V. (2010). Evaluation of antioxidant, wound healing and anti-inflammatory activity of ethanolic extract of leaves of *Ficus religiosa*. Int. J. Pharm. Sci. Res. 19: 73-82.

Chaudhary, S.; Alok, S. and Verma, A. (2016). Phytochemical screening and chromatographic evaluation of *Ficus benghalensis* leaves. Int. J. Pharma. Sci. Res. 7: 3522-32.

Etratkhah, Z.; Ebrahimi, S.E.S.; Dehaghi, N.K. and Seifalizadeh, Y. (2017). Antioxidant activity and phytochemical screening of *Ficus benghalensis* aerial roots fractions. J. Rep. Pharma Sci. 8: 24.

Guleria, S.; Dev, K. and Khosla, P.K. (2016). Comparative analysis of phytochemicals and antioxidant activities of fruit and leaves of *Terminalia chebula* from Himachal Pradesh. IJBPAS. 5:1195-206.

Hamed, M.A. (2011). Beneficial effect of *Ficus religiosa* Linn. on high-fat-diet-induced hypercholesterolemia in rats. Food Chem. 129: 162-170.

Jaiswal, R. and Ahirwar, D. (2013). A literature review on *Ficus bengalensis*. Int. J. Adv. Pharm Res. 5: 57-61.

Khandelwal, K. (2008). Practical pharmacognosy. Pragati Books Pvt. Ltd.; 2008.

Kinghorn, A.D.; Pan, L.; Fletcher, J.N. and Chai, H. (2011). The relevance of higher plants in lead compound discovery programs. J. Nat. Prod. 74: 1539-1555.

Kumar, H.M.; Goswami, S.Y. and Rao, C.V. (2011). Evaluation of *In-Vitro* Antioxidant Activity in *Ficus religiosa* (L.) Leaves. International Journal of Research in Pharmacy & Science. 1: 102-110.

Kumar, V.; Dev, K.; Sourirajan, A. and Khosla, P.K. (2016). Comparative antioxidant potential of bark and leaves of *Terminalia arjuna* (Roxb) Wight & Arn from Himachal Pradesh. Int. J. Pharma Phytopharmacol. Res. 6: 27-33.

Kumar, V.; Sharma, N.; Sourirajan, A.; Khosla, P.K. and Dev, K. (2018). Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of *Terminalia arjuna* from north-western Himalayas. India. J Tradit Complement Med. 8: 100-106.

Melinda, K.P.; Rathinam, X.; Marimuthu, K.; Diwakar, A.; Ramanathan, S.; Kathiresan, S. and Subramaniam, S. (2010). A comparative study on the antioxidant activity of methanolic leaf extracts of *Ficus religiosa* (L.), *Chromolaena odorata* (L.) King & Rabinson, *Cynodon dactylon* (L.) Pers. and *Tridax procumbens* L. Asian Pac. J. Trop. Biomed. 3: 348-350.

Muthu, C.; Ayyanar, M.; Raja, N. and Ignacimuthu, S. (2006). Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. J. Ethnobiol. Ethnomed. 2: 43.

Newman, D.J. and Cragg, G.M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. J. Nat. Prod. 75: 311-335.

Panda, H. (2005). Herbs Cultivation and Medicinal Uses, second ed. National Institute of Industrial Research, Delhi, India, 302–303.

Patil, V.V.; Pimprikar, R.B. and Patil, V.R. (2009). Pharmacognostical studies and evaluation of anti-inflammatory activity of *Ficus bengalensis* Linn. JYP. 1: 49-53

Pedgaonkar, A.; Adithya, G.M.; Reddy, P.S.; Thanuja, K.; Divyashree, L.; Vasudha, B. and Narender, B. (2019). Assessment of anti-oxidant activity of *Ficus benghalensis* leaves: an *In-vitro* design. J. Drug Deliv. Ther. 9: 20-4.

Prakash, V.; Gandotra, S.; Kumar, P. and Singh N. (2017). Phytochemical Screening and Antimicrobial Activity of *Ficus religiosa*. J. Pharma. Sci. Res., 9(2):100.

Prakash, V.; Gandotra, S.; Kumar, P. and Singh, N. (2017). Phytochemical Screening and Antimicrobial Activity of *Ficus religiosa*. Int. J. Pharm. Sci., 9: 100.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol Med. 26: 1231-1237.

Rolta, R.; Sharma, A.; Kumar, V.; Sourirajan, A.; D.J. Baumler and K. Dev (2018a). Methanolic Extracts of the Rhizome of *R. emodi* Act as Bioenhancer of Antibiotics against Bacteria and Fungi and Antioxidant Potential. J. Med. Plant Res. 8: 74-85.

Sawarkar, H.A.; Singh, M.K.; Pandey, A.K. and Biswas, D.E. (2011). *In vitro* anthelmintic activity of *Ficus bengalensis*, *Ficus caria* & *Ficus religiosa*: a comparative anthelmintic activity. Int. J. PharmTech. Res. 3: 152-3.

Shukla, R.; Gupta, S.; Gambhir, J.K.; Prabhu, K.M. and Murthy, P.S. (2004). Antioxidant effect of aqueous extract of the bark of *Ficus benghalensis* in hypercholesterolaemic rabbits. J. Ethnopharmacol. 92: 47-51.

Singh, D., Singh, B. and Goel, R.K. (2011). Traditional uses, phytochemistry and pharmacology of *Ficus religiosa*: A review. J. Ethnopharmacol. 134: 565-583.

Singh, M.P. and Panda, H. (2005). Medicinal Herbs with their Formulations. Daya publishing house, Delhi, India, 404–405.

Sirisha, N.; Sreenivasulu, M.; Sangeeta, K. and Chetty, C.M. (2010). Antioxidant properties of *Ficus species*–a review. Int. J. Pharm. Tech. Res., 2: 2174-82.

Thakare, V.N.; Suralkar, A.A.; Deshpande, A.D. and Naik, S.R. (2010). Stem bark extraction of *Ficus bengalensis* Linn for anti-inflammatory and analgesic activity in animal models. Ind. J. Exp. Bio. 48: 39-45.

Vikas, V.; Patil, B.S.; Narkhede, S.B.; Jawle, N.M. and Patil, V.R. (2010). Analgesic and antipyretic activities of *Ficus benghalensis* bark. Int. J. Pharma Res., 2: 526.

Vinutha, B.; Prashanth, D.; Salma, K.; Sreeja, S.L.; Pratiti, D.; Padmaja, R. and Deepak, M. (2007). Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J. Ethnopharmacol. 109: 359-363.