INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

THE COMPARATIVE FREE RADICAL SCAVENGING EFFECT OF *FICUS RELIGIOSA*, *AEGLE MARMELOS* AND *BUTEA MONOSPERMA*

Sourabh Jain[1], Prof. D. Kishore[1], Prof. Sanjay B. Kasture[2]
[1]*Department of Pharmacy, Banasthali University, Banasthali Rajasthan.*
[2]*Pinnacle Biomedical Research Institute (PBRI), Bhopal M.P.*

ARTICLE INFO

Article history
Received 30/07/2016
Available online
28/02/2017

Keywords
Ficus religiosa,
Aegle marmelos and
Butea monosperma.

ABSTRACT

Natural products have played crucial role in drug discovery and development. They are capable ofcombating free radicals mediated diseases. The most important of these bioactive constituents of natural products or plants are phenols, flavonoids, alkaloids, terpenoids, tannins, glycoside and saponins. Phenols, flavonoids are known to possess wide range of biological activities like antimicrobial, antioxidant and anti-inflammatory properties. The present study was performed to evaluate the *in vitro* antioxidant capacity of *Ficus religiosa*, *Aegle marmelos* and *Butea monosperma* against free radical damage using different standard methods such as DPPH (1,1-diphenyl-2-picrylhydrazyl), hydrogen peroxide scavenging assay and reducing power assay. Results of present study showed that *F.religiosa* extract had higher DPPH radical scavenging activity (IC50: 36.48 µg/ml) followed with *A. marmelos* (IC50: 40.41 µg/ml) and *B. monosperma* (IC50: 55.45 µg/ml) respectively. Similarly, in case of H_2O_2 radical scavenging assay, highest antioxidant activity was found in *F. religiosa.* . The total phenolic content of extract was found to be higher in *Ficus religiosa* (213.0±0.721 mg/gm GAE) then *Aegle marmelos* and *Butea monosperma* (191.6±1.058 and 174.7±1.007).The results support local claims of their therapeutic uses in folklore medicine. In conclusion, all three i.e. *F. religiosa*, *A. marmelos* and *B.monosperma* plant extracts could serve as free radical scavengers, can be considered as potent antioxidants. Their activity may be attributed to high phenolic and flavonoid contents.

Corresponding author
Sourabh Jain
Department of Pharmacy,
Banasthali University,
Banasthali Rajasthan.
9425042457,
sourabh294@gmail.com

*Please cite this article in press as **Sourabh Jain** et al. The comparative free radical scavenging effect of Ficus religiosa, Aegle marmelos and Butea monosperma .Indo American Journal of Pharmaceutical Research.2017:7(02).*

INTRODUCTION

Ayurveda is one of the conventional therapeutic frameworks with a set up history of numerous hundreds of years. Besides known as ayurvedic prescription, this old Vedic learning is thought to be one of the most seasoned recuperating sciences and has made due until the present era over numerous hundreds of years of custom. Begun in India a large number of years prior, ayurveda is known as the "Mother of All Healing"[1].

Drug formulation in ayurveda depends on two standards: Use as a solitary medication and utilization of more than one medications, in which the latter is known as poly herbal formulation. Generally, the ayurvedic writing "Sarangdhar Samhita" dated hundreds of years prior in 1300 A. D. has highlighted the idea of polyherbalism in this antiquated therapeutic framework [2].

Ficus religiosa has been used in traditional medicine for a wide range of ailments. Its bark, fruits, leaves, roots, latex, and seeds are medicinally used in different forms, sometimes in combination with other herbs[3].Fruits of the plant were used in asthma and as laxative and digestive. Seeds of the plant were used as refrigerant and laxative and latex was used in neuralgia, inflammations, and hemorrhages[4].*Ficusreligiosa* comprises asgaragine, tyrosine, undecane, tridecane, tetradecane, (e)-β-ocimene, α-thujene, α- pinene, β-pinene, α-terpinene, limonene, dendrolasine, dendrolasine α-ylangene, α-copaene, β-bourbonene, β- caryophyllene, α-trans bergamotene, aromadendrene, α-humulene, alloaromadendrene, germacrene, bicyclogermacrene, γ- cadinene and δ-cadinene[5]. Leaves contain campestrol, stigmasterol, isofucosterol, α-amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tryosine, methionine, valine, isoleucine, leucine, nnonacosane, n-hentricontanen, hexa-cosanol and n-octacosan[6,7].

Aegle marmelos is extensively described in the Vedic literature for the treatment of various diseases. *Aegle marmelos* is traditionally used to treat jaundice, constipation, chronic diarrhea, dysentery, stomachache, stomachic, fever, asthma, inflammations, febrile delirium, acute bronchitis, snakebite, abdominal discomfort, acidity, burning sensation, epilepsy, indigestion, leporsy, myalgia, smallpox, spermatorrhoea, leucoderma, eye disorders, ulcers, mental illnesses, nausea, sores, swelling, thirst, thyroid disorders, tumors, ulcers and upper respiratory tract infections[8].

Butea monosperma is extensively used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. Commonly *Butea monosperma* is used as tonic, astringent, aphrodisiac and diuretics. Roots are useful in night blindness, piles, ulcer and tumours. Flowers are useful in diarrhoea, astringent, diuretic, tonic, skin diseases, gout, thirst, burning sensation[9,10].

Considering the importance and cultural values of *Ficus religiosa*, *Aegle marmelos* and *Butea monosperma*, a need was felt for scientific investigations and study of various pharmacognostic and physico-chemical parameters of*Ficus religiosa*, *Aegle marmelos* and *Butea monosperma*which form the objectives of the present studyand to find out their potential for industrial applications.

MATERIALS AND METHODS

Reagents and laboratory wares

All reagents used were analytical grade were purchased from Hi-Media and Merck.

Collection of Plant Material

Fresh leaves of *Ficus religiosa*, *Aegle marmelos* and *Butea monosperma*were collected from local area of Bhopal district, M.P., India in the month of Aug-Oct. India.Herbarium of plant was prepared and submitted to Department of Botany, Safia College of Science, Bhopal (M.P.) for authentication. Plants were authenticated by Dr. Zia-Ul-Hasan, HOD Department of Botany.Voucher specimen numbers were 427/Bot/Saifia/13,447/Bot/Saifia/13 and 448/Bot/Saifia/13.

Physiochemical Examination[11,12]

Foreign matter

Foreign matter of *Ficus religiosa*, *Aegle marmelos* and *Butea monosperma* were perform using procedure described as per WHO[13,14]

Loss on drying[14]

Placed about 10 g of drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish, dried at 105° for 5 hours, and weighed. Continued the drying and weighing at one hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent.

Ash value[14]

Total ash

It depicts the total amount of material produced after the complete incineration of the ground drug above 500°C to remove all the carbon atoms. 2-4g of powdered drug was weighed and placed in the crucible and heated at about 500-600°C. The crucible was cooled in desiccator for 30 min and the % of the total ash of the crude drug was calculated in mg per g.

Acid insoluble ash

Total ash obtained was dissolved in 1N HCl solution and heated for 5 min. The insoluble matter was filtered in whatman filter paper; the filter paper was further dried at 70°C and then cooled. The residue was weighed and the percentage of insoluble ash of the crude drug w.r.t. the air dried sample of crude drug was calculated in mg per g.

Water soluble ash

To the total ash crucible, 25ml double distilled water was added and boiled for about 5min. Insoluble matter was collected on an ash less filter paper in a crucible, washed with hot water and ignited for about 15min above 45°C. The weight of the residue is subtracted from the weight of the total ash. Content of water soluble ash in mg per g of the air dried material was calculated.

Sulphated ash

The sulphated ash test is an analytical test for determining the inorganic content of a sample by weight. The ash powder was moistened with 1 ml of H_2SO_4 and ignited to 800 + 25ºC until it reaches a constant weight.

Extractive value[14]

Ethanol soluble extractive value

Macerated 5 g of the air dried drug, coarsely powdered, with 100 ml of alcohol the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing standing for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dried, to constant weight and weighed. Calculated the percentage of alcohol soluble extractive with reference to the air-dried drug.

Water soluble extractive value

Method adopted was same as done in alcohol soluble extractive value for the determination of alcohol-soluble extractive, using chloroform-water instead of ethanol.

Processing and extraction of plant material

Collected leaves were washed under running tap water and kept in shade for drying. Dried plant materials were pulverized using mechanical grinded and observed for colour, odour, and texture, and were packed and labelled in air tight container for further proceedings. Extraction was performed using continuous hot percolation 'Soxhlation'[14]. Pulverised dried leaves (total 1000 gm, in different batches) were placed in thimble of Soxhlet apparatus. Extraction was performed at 40°C using petroleum ether at first. Exhausted plant material (marc) were dried and afterward extracted with (70 % Methanol). This procedure was continued till no colour was observed and completion of extraction was confirmed till solvent from the siphon tube does not left any residue when evaporated. Obtained extracts were evaporated using rotary vacuum evaporator at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formulae:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \times 100$$

Solubility Testing

The solubility of all extracts of *Ficus religiosa, Aegle marmelos* and *Butea monosperma* were observed in different solvent system.

Preliminary Phytochemical Studies.

Preliminary qualitative phytochemical screening was done for the presence of different group of active components such as alkaloids, flavonoids, saponins, tannins, sterols, carbohydrates, and glycosides [15,16] .

***In vitro* antioxidant assay**

DPPH Assay[17,18]

A solution of 0.1mM DPPH (4mg/100ml) in methanol was prepared. Different concentration of test sample with methanol was prepared. 2ml of test sample and 1ml of DPPH solution was added to 2ml of different dilution of test samples and allowed to react at at room temperature for 10 min. The absorbance values are measured at 515 nm against blank (methanol). % Inhibition was calculated using following formulae:-

% Inhibition = [(AC 515 nm- AS 515 nm/ AC 515 nm) x 100].

Hydrogen peroxide Scavenging Assay [19]

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1ml of the extracts and standards was prepared in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extracts in PBS without hydrogen peroxide. % Inhibition was calculated using following formulae:-

% Inhibition = [(AC 230 nm- AS 230 nm/ AC 230 nm) x 100].

Reducing Power Assay[18]

A spectrophotometric method was used for the measurement of reducing power. For this 0.5 ml of each of the extracts was mixed with 0.5ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide (10 mg/ml). The reaction mixture was incubated at 50 ºC for 20 min separately, and then rapidly cooled, mixed with 1.5 ml of 10% trichloroacetic acid and centrifuged at 6500 rpm for 10 min. An aliquot (0.5ml) of the supernatant was diluted with distilled water (0.5ml) and then ferric chloride (0.5ml, 0.1%) was added and allowed to stand for 10 min. the absorbance was read spectrophotometrically at 700 nm. Ascorbic acid (AA) was used as standard for construction of calibration curve.

Reducing Power (%) = (As / Ac) × 100

Here, Ac is the absorbance of control (AA) and As is the absorbance of samples (extracts) or standards.

Total Phenolic Content Estimation[20,21]

The amount of total phenolic in extracts was determined with the FolinCiocalteu reagent. Concentration of (10-100 µg/ml) of gallic acid were prepared in methanol. Concentration of 100 µg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced in to test and mixed with 2.5ml of a 10 fold dilute folin Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 765 nm spectrometrically. Galic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE).

Total Flavonoid Content Estimation[22]

Different concentration of rutin(10 to 100 µg/ml) was prepared in methanol. Test sample of near about same polarity (100 µg/ml) were prepared. An aliquot 0.5ml of diluted sample was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a 5% NaNO2 solution. After 6 min, 0.15 mL of a 10% AlCl3 solution was added and allowed to stand for 5min, and then 2 mL of 4% NaOH solution was added to the mixture. The final volume was adjusted to 5ml with distilled water. Absorbance was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutine/g DW), through the calibration curve of Rutin.

RESULTS AND DISCUSSION

The present study aimed to do a comparative study about the pharmacognostical, Physicochemical, phytochemical and their antioxidant activity of three plants. The antioxidant activity of polyphenols is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching oxygen, or decomposing peroxides. Indeed the antioxidant activity of the plants extracts is highly correlated with the total phenol content.

The therapeutic effects, derived from several medicinal plants, have been attributed to the presence of phenolic compounds such as flavonoids, phenolic acid, proanthocyandins and tannins[23]. In this study, the phytochemical constituents and free radical scavenging activity of medicinal plants, were evaluated. The result of phytochemical screening test of methanol extracts of *Ficus religiosa*, *Aegle marmelos* and *Butea monosperma*revealed the presence of medically active compounds including terpenoids, reducing sugars, saponins and alkaloids.

Table 5 and 6 shows the total phenolic and flavonoid contents of methanol extracts of *Ficus religiosa*, *Aegle marmelos* and *Butea monosperma*. Total phenolic content was expressed as mg gallic acid equivalent/g of extract by reference to a standard curve (y = 0.005x + 0.065; R2 = 0.975). The total flavonoid content were expressed as mg rutin equivalent/g of extract by reference to a standard curve (y = 0.001x + 0.120; R2 = 0.988), respectively. The total phenolic content of extract was found to be higher in *Ficus religiosa* (213.0±0.721 mg/gm GAE) then *Aegle marmelos* and *Butea monosperma* (191.6±1.058 and 174.7±1.007).

A number of methods are available for the determination of antioxidant capacity but the assay involving the stable 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) has received the maximum attention due to its ease of use and its convenience[24]. The DPPH radical scavenging ability of the extract of *Ficus religiosa* showed maximum activity with IC50 of 36.48 µg/ml when compared with the *Aegle marmelos* and *Butea monosperma* counterpart (IC50 of 40.41 µg/ml and 55.45 µg/ml). However, the standard reference compound, ascorbic acid gave a better DPPH scavenging ability than all the three extracts with a lower IC50 of 14.38 µg/ml. (Figure 1) Similarly, the hydrogen peroxide radical scavenging assay of *Ficus religiosa* showed maximum activity with IC50 of 49.16.48 µg/ml when compared with the *Aegle marmelos* and *Butea monosperma* counterpart (IC50 of 68.54 µg/ml and 86.55 µg/ml). However, the standard reference compound, ascorbic acid gave a better H2O2 scavenging ability than all the three extracts with a lower IC50 of 30.77 µg/ml (Figure 2).

The reducing ability of the compound usually depends on the reductants, which have been exhibited antioxidative capacity by breaking the free radical chain, donating a hydrogen atom. The reducing power of all the three plant extract was found to be significant and increased with the increasing concentrations (Figure 3).

Table 1: Organoleptic evaluation of powder.

S.No.	Plant material	Colour	Odour	Texture
1	*Ficus religiosa*	Yellowish Green	Odourless	Rough
2	*Aegle marmelos*	Yellow	Odourless	Gritty powder
3	*Butea monosperma*	Green	Aromatic	Rough

Table 2: Physiochemical Examination of powder.

S. No.	Parameter	Result		
		Ficus religiosa	*Aegle marmelos*	*Butea monosperma*
01.	Foreign matter (%)	2.3	0.76	1.45
02.	Loss on Drying	0.1	6.89	0.59
03.	Total ash	6.8-7.86	8.2	9.25
04.	Water Soluble ash	3	2.86	0.75
05.	Acid Soluble ash	0.41-3.9	0.14	9.01
06.	Water Soluble Extractive Value	15.76	14.67	10.8
07.	Ethanol Soluble Extractive Value	7.21	11.36	7.20
08	Percentage yield	5.0	8.8	8.5

Table 3: Phytochemical investigation.

Name of test	*Ficus religiosa*	*Aegle marmelos*	*Butea monosperma*
Carbohydrates	(-) ve	(+) ve	(+) ve
Alkaloids	(+) ve	(-) ve	(+) ve
Terpenoids	(-) ve	(+) ve	(-) ve
Flavonoids	(-) ve	(-) ve	(+) ve
Tannins and Phenolic compounds	(-) ve	(+) ve	(+) ve
Saponin	(-) ve	(+) ve	(+) ve
Protein and Amino acids	(-) ve	(-) ve	(-) ve
Glycosides	(-) ve	(-) ve	(-) ve
Fats and lipids	(-) ve	(+) ve	(-) ve

Table: 4 Inhibitory concentrations (IC_{50}) of *Ficus religiosa, Aegle marmelos, Butea monosperma* and Ascorbic acid.

S. No.	Test	*Ficus religiosa*	*Aegle marmelos*	*Butea monosperma*	Ascorbic acid
01	DPPH antioxidant activity	36.48	40.41	55.45	14.38
02	H_20_2 radical scavenging activity	49.16	68.54	86.55	30.77

***In vitro* antioxidant assay**

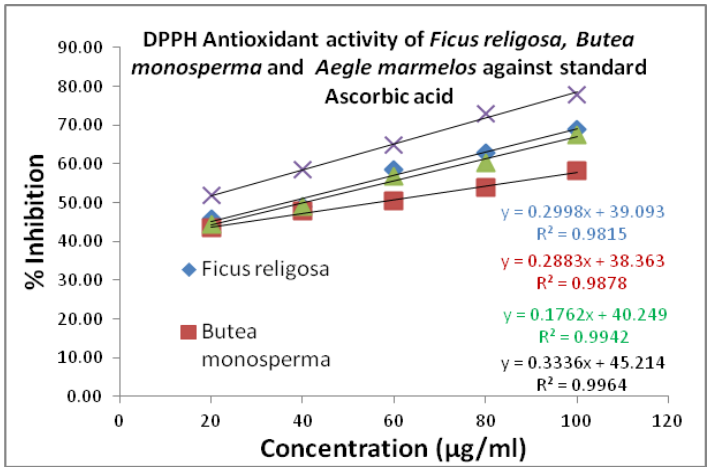

Figure 1: DPPH Antioxidant Activity.

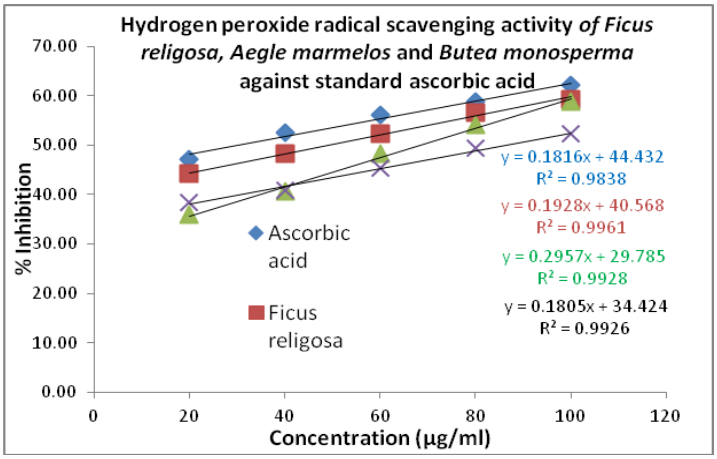

Figure 2: Hydrogen Peroxide Scavenging.

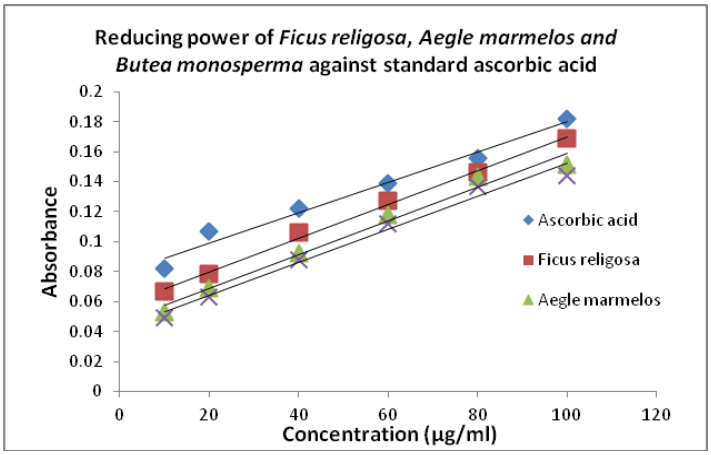

Figure 3: Reducing Power Assay.

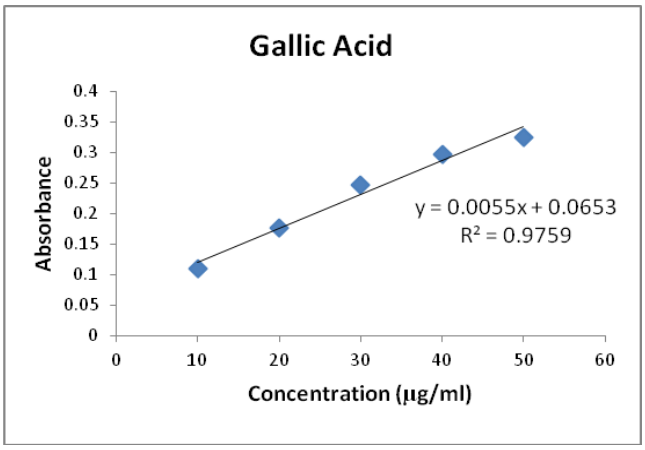

Figure 4: Standard curve of Gallic acid.

Table: 5 Total phenolic content in *Ficus religiosa, Aegle marmelos* and *Butea monosperma.*

S. No.	Extract	TPC (mg/gm) extract GAE
1	*Ficus religiosa*	213.0±0.721
2	*Aegle marmelos*	191.6±1.058
3	*Butea monosperma*	174.7±1.007

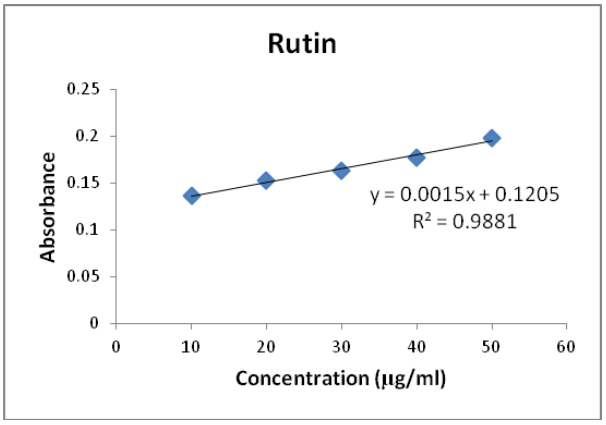

Figure 5: Standard curve of rutin.

Table 6: Total flavonoid content in *Ficus religiosa, Aegle marmelos* and *Butea monosperma.*

S. No.	Extract	TFC (mg/gm) extract RE
1	*Ficus religiosa*	148.7±2.517
2	*Aegle marmelos*	115.3±1.155
3	*Butea monosperma*	92.3±3.055

CONCLUSION

The present study concludes that the crude methanolic extract of *Ficus religiosa, Aegle marmelos* and *Butea monosperma* exhibited a significant antioxidant activity which may be relevant in the treatment of oxidative stress. The antioxidant activity was measured as free radical scavenging activity DPPH method, Reducing power determination method, Hydrogen peroxide method. All the methods show good response due to the presence of phenolic compounds and flavonoids in three species. Among all the three extracts tested, showed highest antioxidant activity with IC 50 value in the order of *Ficus religiosa> Aegle marmelos > Butea monosperma* when compared to the standards. The result reveals that all the extracts have the scavenging character in accordance with the standards. The further work has been developed for the isolation of particular phenolic compound for this activity, and also can be used for the new formulation development.

ACKNOWLEDGEMENT

Authors are thankful to Pinnacle Biomedical Research Institute for providing necessary facilities during this research work.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. Pharmacognosy reviews. 2014; (16): 73.
2. Srivastava S, Lal VK, Pant KK. Polyherbal formulations based on Indian medicinal plants as antidiabetic phytotherapeutics. Phytopharmacology. 2012; 2(1): 1-15.
3. Aiyegoro OA, Okoh AI. Use of bioactive plant products in combination with standard antibiotics: implications in antimicrobial chemotherapy. Journal of Medicinal Plants Research. 2009; 3(13): 1147–1152.
4. Warrier PK. Indian Medicinal Plants—A Compendium of 500 Species. Orient Longman, Chennai, India. 1996; 3: 174-76.
5. Grison PL, Hossaert MK, Greeff JM, Bessière JM. Fig volatile compounds—a first comparative study. Phytochemistry. 2002; 61(1): 61-71.
6. Singh S, Jaiswal S. Therapeutic Properties of Ficus Religiosa. International Journal of Engineering Research and General Science. 2014; 2(5): 2091-2730.
7. Joseph B, Raj SJ. Phytopharmacological and phytochemical properties of three Ficus species-an overview. International journal of pharma and Bio sciences. 2010; 1(4): 246-53.
8. Sekar DK, Kumar G, Karthik L, Rao KB. A review on pharmacological and phytochemical properties of *Aegle marmelos* (L.) Corr. Serr.(Rutaceae). Asian Journal of Plant Science and Research. 2011; 1(2): 8-17.
9. Kirtikar KR. Indian Medicinal Plants. Lalit Mohan Basu, Allahabad, India. 1935; 2: 1527-28.
10. Ambasta BP. The useful plants of India. CSIR, New Delhi. 1994; 1-91.
11. Anonymous. The Ayurvedic Pharmacopoeia of India. Govt. of India, M.H & F.W, Dept. of ISM &H. 1999; I(II): 248-252.
12. Anonymous. The World Health Report 1998. Life in the 21st century: A vision for all - Report of the Director- General; WHO Library Cataloguing in Publication Data, World Health Organization Geneva. 1998; 02-10.
13. Anonymous.WHO Guidelines. A.I.T.B.S. Publishers and distributors, Delhi. 1998;1: 8-9.
14. Kokate CK. Practical pharmacognosy. VallabhPrakashan, New Delhi.1994; 4: 110-111.

15. Trease GE, Evans WC. Pharmacognosy. Bailliere Tindall, London. 1994; 11: 45-50.
16. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Niraliprakashan. 2006; 23: 493-497.
17. Gulçin I, Elias R, Gepdiremen A, Boyer L. Antioxidant activity of lignans from fringe tree (*Chionanthusvirginicus* L.). Eur Food Res Technol. 2006; 223: 759-767.
18. Jain R, Jain SK. Total Phenolic Contents and Antioxidant Activities of Some Selected Anticancer Medicinal Plants from Chhattisgarh State, India. Pharmacologyonline. 2011; 2: 755-762.
19. Jayaprakasha GK, Jaganmohan Rao L,Sakariah KK. Antioxidant activities of flavidin in different *in vitro* model systems. Bioorg. Med. Chem. 2004; 12(19): 5141-46.
20. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissue using Folin-Ciocalteu reagent. Nature Protocol. 2007; 2(4): 875-877.
21. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, *in vitro* antioxidant and anti inflammatory activity of flowers of *Moringaoleifera*. Asian pacific Journal of Tropical Biomedicina. 2013; 3(8): 623-627.
22. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999; 64: 555-559.
23. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenols, flavanoid contents of selected Iranian medicinal plants. Afr J Biotechnol. 2006; 5: 1142-1145.
24. Sanchez-Moreno C, Larrauri JA, Saura-Calixto FA. Procedure to measure the antiradical efficiency of polyphenols. J Sci Food Agric. 1998; 76: 270-276.

5487847845116075**5**