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ORIGINAL ARTICLE

Bioactive Compounds and Their Antioxidant Activities of *Ficus religiosa* Extracts

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ABSTRACT
Ficus religiosa is a well-known medicinal plant with diverse applications since ancient days, but lacking scientific literature on the biochemical composition and their antioxidant properties. The present study aimed to investigate the levels of bioactive compounds (total phenolics, terpenoids, flavonoids, tannins, and alkaloids) and their antioxidant and free radical scavenging activities(ABTS, DPPH, H_2O_2, -OH free radical scavenging abilities, and reducing power) in the bark, fruit, and leaf extracts of Ficus religiosa. Bark exhibited highest levels total phenolics (100.94 ± 12 mg of GAE/gm), terpenoids (682.03 ± 13 mg LE/gm), and flavonoids (4.02 ± 0.21 mg RE/gm) as compared fruit and leaf extracts along with their enhanced ABTS, DPPH, H_2O_2, -OH free radical scavenging activities at the lowest concentration (IC_{50}) of extract. Observed a strong correlation between totalphenolics and ABTS, H_2O_2, and -OH free radical scavenging activities, whereas terpenoids displayed the highest correlation with DPPH and reducing power assay. These results concluded that the bioactive (phenolic, terpenoid, flavonoid) rich bark extract of Ficus religiosa exhibiting highest antioxidant potential with enhanced free radical scavenging activities at lowest concentration. Further studies are empowered for the isolation and characterization of these natural antioxidants to use as a source of drugs prevention of neural diseases.
Keywords: *Ficus religiosa, bioactive compounds, total phenolics, terpenoids, antioxidants, free radical scavenging activities.*

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INTRODUCTION

Plants have acknowledged as a significant source of phytochemicals to cure various diseases due to their healing benefits[1]. Phytochemical screening in medicinal plants is helping us to know the category of chemical compounds produced by plants. Identification and purification of these bioactive compounds having great benefits to humans in the discovery of drugs [2]. Phenolic compounds, tannins, flavonoids, alkaloids, tannins, terpenoids, steroids, saponin, etc. of bioactive compounds are essential in recognizing the new sources of industrial necessary and therapeutic drugs[3]. These phytochemicals can react with unstable free radicals and act as antioxidants. The equilibrium between free radicals and antioxidants in our body is essential for good health; if not, imbalance created and lead to oxidative stress and promotes several diseases includes cancer, neurodegenerative diseases, diabetes, aging, atherosclerosis, and cardiovascular disease. Therefore, the external supplementation of antioxidants needed for the body's antioxidant protection system for better management from oxidative stress and its related diseases[1].
Ficus religiosa is a widely used plant in Ayurveda, Siddha, Homeopathy, and Unani as indigenous traditional medicine systems. *Ficus religiosa* (Moraceae family) also perceived as a Bodhi tree and considered being a sanctified tree for both Buddhists and Hindus, which has tremendous traditional and pharmacognosy practices [4]. In early investigations reported that the *Ficus religiosa* and its six parts, i.e., bark, leaves, seeds, fruits, root, and latex have been using in folk medicine to treat various diseases like amnesia, gonorrhea, wound healing, cervical cancer, epilepsy, inflammation, ulcers, infection diseases, anti-cholinergic, anti-bacterial, anti-viral, and anti-anxiety activity [5–8].It is also reported that bark

extract shows the anti-HIV I protease activity[9], acetylcholinesterase inhibitory activity [10], the anti-lipid peroxidative effect [11], and anti-diabetic activity [7]. The bark extract inhibits the entry, viral attachment, andproduction of herpes simplex virus type 2 [12]. The bark also protects the liver against paracetamol-induced hepatotoxicity[13], and promotes wound healing[5, 14]. The fruit extract potentiated the bronchoconstriction induced by both acetylcholine and histamine on guinea pig tracheal chain preparation [15]. *Ficus religiosa* exhibited anti-amnesic against scopolamine-induced anterograde and retrograde amnesia in mice in a dose-dependent and also shows memory improvement[16].Root extracts of *Ficus religiosa* protect mice from seizures in a dose-dependent manner against strychnine, picrotoxin, pentylenetetrazole, and isoniazid[17].The aqueous leaf extracts exhibitedan antibacterial effect on *P.aeruginosa, Salmonella typhi, Bacillus subtilis,and E.coli* [18].Although several studies attempted to report its traditional uses without its scientific knowledge. No literature is available on the biochemical characterization of *Ficus religiosa* plant in detail. By focusing on this, the present investigation was carried out for evaluation of biochemical composition of *Ficus religiosa* extracts of bark, leaves, and fruits, and also determined their antioxidant and free radical scavenging activities.

MATERIAL AND METHODS

Chemicals and Reagents

All routinely used chemicals and reagents were purchased from Merck Specialties Pvt. Ltd, Mumbai, India, and Thermo Fisher Scientific (India) Pvt. Ltd, Mumbai, India. Gallic acid, Linalool, Rutin, Atropine, Catechin, Trolox, Ascorbic acid, Linalool, ABTS (2,2'-azion-bis (3-ethylbenzothiazoline-6-sulfonic acid)), andDPPH (2,2-diphenyl-1-picrylhydrazyl)were purchased from the Sigma-Aldrich, St. Louis, MO, USA.

Collection of plant material

Plant parts leaf, bark, and fruit collected from the botanical garden of Yogi Vemana University campus, Kadapa, Andhra Pradesh, India, and voucher specimen with a number (YVUH-1973) have been deposited at the Herbarium, Department of Botany, Yogi Vemana University, Kadapa-516003, India. The leaf, bark, and fruits were air-dried at room temperature and ground into fine powder in a blender and sewed using 0.2 mm sieve and stored until further use at room temperature in a sealed cover.

Preparation of *Ficus religiosa* extract

All extractions were done under cold conditions. Two grams of plant material (leaf, bark, and fruit) was dissolved in 20 mL of ice-cold 80% ethanol in 50 ml flask with the sample to solvent ratio 1:10 and kept on a magnetic stirrer for overnight at 4°C under constant speed. The supernatant was obtained after centrifugation at 6000 rpm for 10 minutes. A fresh 20 mL solvent was added to the pellet and incubated for 3 h, and further, it was repeated two times so that all the bioactive compounds present in the **plant material** will be dispersed effectively into the extraction solvent. The obtained supernatants were combined and concentrated in the Rota evaporator (Heidolph Rotary Evaporator, Germany) in a vacuum under reduced pressure. Concentrated extracts were collected, aliquoted, and stored at -20 °C until use.

Analysis of bioactive compounds

Determination of total phenolics

Total phenolic content(TPC) in ethanolic extract of *Ficus religiosa* was quantified as per the method described by Singletonet al [19]with minor changes in 96 well plate. Briefly, to 140 µL of extract added 600 µL of Folin-Ciocalteu reagent, incubated for 5 min, and then added 450 µL 7.5% sodium carbonate. The total contents were incubated for 30 min in the dark at 45°C, followed by one-hour incubation at room temperature and read absorbance at 760 nm against the blank. The results were expressed as mg gallic acid equivalents per gram plant material (mg GAE/g).

Determination of terpenoids

Total terpenoid content was evaluated through the method described by Ghorai *et al* [20].To 200 µL of extract, added 1.5 mL chloroform, 100 µL Conc. H_2SO_4 and incubated for 90 min in the dark. Red precipitate was collected and dissolved in 80% methanol and read absorbance at 538 nm. Linalool used as a standard, and results showed as mg of linalool equivalents per gram (mg LE/g) of plant material.

Determination of flavonoids

The amount of flavonoids was estimated by the aluminum chloride colorimetric method adopted byPourmorad et al[21]. To 25 µL of extract, added 75 µL of ethanol followed by 5 µL 10% aluminum chloride and 1M potassium acetate, final volume made up to 260 µL with distilled water, incubated for 40 min and measured absorbance at 415nm. Rutin was used as the standard for expressing results as mg of Rutin equivalent per gram (mg RE/g) of plant material.

Determination of alkaloids

Total alkaloid content was determined with slight modification in the spectrophotometric method ofNovelli et al[22]. To 1.0 mL extract, 5 mL Bromo Cresol Green (BCG) reagent added, followed by added 5 mL of 0.1 M phosphate buffer pH 4.7 and shaken vigorously in separating funnel. Later 5 mL of chloroform added and turns the entire solution yellow. The colored solution collected and read absorbance at 470 nm against blank having chloroform and BCG reagent without extract. Atropine used as a standard and expressed results as mg atropine equivalents per gram of plant material (mg AE/g).

Determination of tannins

Total tannins were estimated using a procedure described by Sun et al[23]by mixing 1 mL of the extract with 3 mL acidic methanol and followed by adding 6 mL Vanillin reagent. Read absorbance at 500 nm in a spectrophotometer against reagent blank. Catechin was chosen as the standard and expressed results as mg of catechin equivalents per gram of plant material (mg CE/g).

Analysis of antioxidant properties

ABTS antioxidant assay

ABTS free radical scavenging activity of ethanolic extracts of *Ficus religiosa* was determined by following a protocol described by Re et al [24]. ABTS reagent (290 µL) mixed with 10 µL extract and incubated for 30 min in room temperature. The ABTS solution's discoloration was measured at 734 nm against blank using Microplate Reader (Biorad 680, USA). Trolox was used as a standard, and results expressed as mg of Trolox equivalents per ml (mg TE/ml) and IC_{50} shown in µg GAE/mLl.

DPPH antioxidant activity

Antioxidant activity of ethanol extracts was assessed through DPPH radical scavenging action by the method of Paz et al[25]. Fifty µL of the extract was added to 250 µL of DPPH reagent (2.45 mg DPPH radical in 50 mL of 80% methanol) and incubated for 90 min at room temperature. The decreased purple color was measured at 517 nm against blank using Microplate Reader (Biorad 680, USA). The Ascorbic acid is used as standard and expressed results as Ascorbic acid equivalents per ml (AsAE/mL) and IC_{50} shown in µg GAE/mL.

Total antioxidant assay by Ammonium molybdate method

The total antioxidant activity can be quantified by the development of the phosphomolybdenum complex, as described by Prieto *et al* [26]. To 100 µL of an extract, added 1 mL of reagent solution (mixed equal volume of 0.6 M H_2SO_4, 28mM Sodium phosphate, and 4mM Ammonium molybdate) and incubated in boiling water bath at 95^0c for 90 min. After cooling, read the absorbance at 695 nm upon a blank having a reaction solution without extract in a spectrophotometer (Shimadzu UV-1800). The results expressed as mg gallic acid equivalents per gram plant material (mg GAE/g) and EC_{50} values of the extracts shown in µg GAE/mL.

Reducing power assay

Reducing power assay was performed as described by Ferreira et al[27]. Aliquots of extract (10 – 40 µL) were taken in tubes, and added 400 µL of distilled water, 500 µL of 0.2 M phosphate buffer pH 6.6, and 500 µL 1 % potassium ferricyanide, and kept in a water bath for 20 min at 50 ^oC. After incubation, taken out tubes and added trichloroacetic acid to stop the reaction and centrifuge the contents. Collected 0.5 ml of upper layer and mixed with 0.5 mL distilled water and 100 µL 0.1% ferric chloride. Read absorbance at 700 nm against the blank. Ascorbic acid was used as a standard and results were shown as EC_{50} (µg GAE/mL), where EC_{50} stands for effective concentration at which absorbance was 0.5.

Free radical scavenging activities

Hydrogen peroxide scavenging assay:

The strength of ***Ficusreligiosa*** extracts to scavenge hydrogen peroxide was determined according to the method described by Ruch et al[28]with insignificant modifications. A 43 mM hydrogen peroxide was prepared in 1 M phosphate buffer (pH 7.4) and added various concentrations of extracts to 0.6 ml of 43mM hydrogen peroxide solution. The absorbance of hydrogen peroxide at 230 nm was determined following 10 min against blank having phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a standard. The free radical scavenging activity was ascertained by evaluating in % of inhibition.

% of inhibition = [(control – test)/control] *100

Hydroxyl radical scavenging assay:

Hydroxyl radical scavenging activity of *Ficus religiosa* extract was assayed by the process described by Smirnoff and Cumbes[29]. Thereaction mixture contains 0.7 mL of hydrogen peroxide (6 mM), 1.0 ml of $FeSO_4$(1.5 mM), 0.3 mL of sodium salicylate(20 mM) and varied concentrations of ethanolic extracts in total volume 3.0 mL. Following 1h incubation at 37°C, the absorbance of the hydroxylated salicylate was read at 562 nm. The hydroxyl radical scavenging activity was determined by the following.

Hydroxyl radical scavenging assay: $[1-(A_1-A_2)/A_0] *100$

A_0 = absorbance of control (without extract)
A_1 = absorbance in the presence of extract
A2 = absorbance without sodium salicylate

Statistics

The results obtained were represented as mean±SEM of five independent experiments. Graph-pad software version 6.0 was used in the study. Statistical significance was calculated using one-way ANOVA with Turkey's multiple comparison post-hoc test to determine whether the compared groups are distinct at the significance level at $p<0.05$.The correlation coefficient (r^2) was used to correlate the results with each other.

RESULTS

Bioactive compounds obtained from plant origin are having strong antioxidant potential to resist oxidative stress besides encouraging health-promoting characteristics. In this connection, ethanolic extracts of *Ficus religiosa* bark, fruit, and leaf are using in present study for evaluation of bioactive compound composition (total phenolics, flavonoids, tannins, terpenoids, alkaloids, etc) and their antioxidant and free radical scavenging activities.

Analysis of bioactive compounds

The total phenolic content was measured in the bark, fruit, and leaves of *Ficus religiosa,* and results depicted in Table **I**. Significant variation was noticed in the levels of total phenolics among the bark, leaves, and fruit. Bark showed the highest levels of total phenolic contents (100.94 ± 12 mg of GAE/g), followed by leaves (17.15 ± 0.62 mg of GAE/g) and fruits (8.76 ± 0.65 mg of GAE/g), respectively. The levels of terpenoids in different parts of *Ficus religiosa* showed a remarkable variation as like total phenolics. Higher levels of terpenoids seen in bark (682.03 ± 13 mg LE/g) followed by fruit (394.30 ± 24 mg LE/g) and tiniest levels in leaves (5.04 ± 0.27 mg LE/g). Lower levels of flavonoids, alkaloids, and tannins were observed in bark, fruit, and leaves of *Ficusreligiosa* and summarized their values in Table **I**. The results obtained were indicated that the bark of *Ficusreligiosa* displayed the highest levels of total phenolics, terpenoids, and flavonoids as compared to fruit and leaves.

Table I: The levels of bioactive compounds present in the extracts of *Ficusreligiosa*.

S.No.	Bioactive compound	Bark	Fruit	Leaves
1	Total Phenolics (mg GAE/gm)	100.94 ± 12^a	8.76 ± 0.65^b	17.15 ± 0.62^c
2	Terpenoids (mg LE/gm)	682.03 ± 13^a	394.30 ± 24^b	5.04 ± 0.27^c
3	Flavanoids (mg RE/gm)	4.02 ± 0.21^a	0.26 ± 0.08^b	1.23 ± 0.05^c
4	Tannins (mg CE/gm)	0.528 ± 0.023^a	0.117 ± 0.014^b	0.006 ± 0.0001^c
5	Alkaloids (mg AE/gm)	0.032 ± 0.001^a	0.001 ± 0.00001^b	0.751 ± 0.06^c

GAE–Gallic acid equivalents; LE-Linalool equivalents; RE–Rutin equivalents; CE–Catechin equivalents; AE–Atropine equivalents. The data represented as mean ± SE for five independent determinations and the mean value bearing different alphabets (shown in superscript) in the same row are significantly different at $p<0.05$ according to ANOVA test.

Analysis of antioxidant properties

The antioxidant and free radical scavenging activities were measured in *Ficus religiosa* extracts (bark, fruit, leaves), and depicted the results in Table II**.** The bark extract exhibited the highest antioxidant and free radical scavenging activity (899±50 mg TE/ml) at the lowest concentration of extract (IC_{50} - 0.34±0.02 µg GAE/ml) against ABTS free radicals as compared to the fruit (17±1.2 mg TE/ml; IC_{50} - 1.8±0.04 µg GAE/ml), and leaves (210±15 mg TE/ml; IC_{50} - 2.31±0.12µg GAE/ml), respectively. A similar trend was noticed in DPPH free radical scavenging activity with all *Ficus religiosa* ethanolic extracts. The bark shows the enhanced antioxidant property against DPPH free radical (8.4±0.4 mg AsE/ml) as compared to fruit (0.35±0.2 mg AsE/ml) and leaves (3.3±0.2 mg AsE/ml) with an IC_{50} value of 0.709±0.023 µg of GAE/ml followed by the fruit with IC_{50} value 1.017±0.07 µg of GAE/ml and leaves with 2.02±0.12 µg of GAE/ml, respectively. Further to know the peroxide (H_2O_2) and hydroxyl (–OH) free radical scavenging activities (critical free radicals in living cells), the extracts were subjected for assays and obtained results summarized in Table II**.** The ethanolic extracts of bark exhibited the highest H_2O_2 free radical scavenging activity with the lowest IC_{50} at the concentration (4.1±0.05 µg GAE/ml) as compared to leaves (8.62±0.54 µg GAE/ml) and fruit (11.36±1.34 µg GAE/ml), respectively. Similar trend was noticed in hydroxyl radical scavenging activities in all three extracts as like H_2O_2 scavenging activities

at the concentration of IC_{50} (bark - 1.92±0.027, fruit - 37 ± 1.48, and leaves 29±0.32µg GAE/ml), respectively. The results of free radical scavenging assays indicated that bark extract potentially scavenging all types of free radicals tested (ABTS, DPPH, H_2O_2, and –OH) at the lowest concentration as compared to fruit and leaf extracts.

Total antioxidant activity in *Ficus religosa* extracts revealed that the enhanced reduction activity was noticed in the bark extracts (73±2 mg GAE/ml) at lower concentration EC_{50} (1.97±0.2 µg GAE/ml) as compared to moderate activities in leaves (26±6 mg GAE/ml; EC_{50} 8.8 ±1.1 µg GAE/ml), and fruit extracts (6.4±1 mg GAE/ml; EC_{50} 2.25±0.2 µg GAE/ml), respectively. Further, the reducing power assay was performed for all three extracts to know the effective concentration, where exhibits higher reduction potential. Bark extract showed the highest reduction potential at the lowest concentration levels (EC_{50} 26.53±2.23 µg of GAE/ml) as compared to fruit (32.74±1.34 µg of GAE/ml) and leave extracts (56.24±3.23 µg of GAE/ml), respectively. These results indicated that the bark displayed antioxidant activities with enhanced reduction potential at the lowest concentration as compared to fruit and leaf extracts. Results summarized in Tables I & II revealed that the bark is enriched with phenolics, terpenoids, and flavonoids and exhibited the highest antioxidant potential with enhanced free radical scavenging activities at lowest concentration of extract.

Table II: The free radical scavenging activities, total antioxidant activities, and reduction potential of *Ficus religiosa* extracts.

	Free radical scavenging activities						Total antioxidant activity		Reduction potential EC_{50} (µg GAE/ml))
	ABTS		DPPH		H_2O_2 IC_{50} (µg GAE/ml)	-OH IC_{50} (µg GAE/ml)	mg GAE/ml	EC_{50} (µg GAE/ml)	
	mg TE/ml	IC_{50} (µg GAE/ml)	mg AsE/ml	IC_{50} (µg GAE/ml)					
Bark	899 ±50[a]	0.34 ±0.02[a]	8.4 ±0.4[a]	0.70 ±0.023[a]	4.11 ±0.54[a]	1.92 ±0.027[a]	73 ±2[a]	1.97 ±0.2[a]	26.53 ±2.23[a]
Fruit	17 ±1.2[b]	1.8 ±0.04[b]	0.35 ±0.02[b]	1.017 ±0.07[b]	11.36 ±1.34[b]	29 ±0.32[b]	6.4 ±1[b]	2.25 ±0.2[b]	32.74 ±1.34[b]
Leaves	210 ±15[c]	2.31 ±0.1[c]	3.3 ±0.2[c]	2.02 ±0.12[c]	8.62 ±0.054[c]	37 ± 1.48[c]	26 ±6[c]	8.8 ±1.1[c]	56.24 ±3.23[c]

TE–Trolox equivalents; GAE–Gallic acid equivalents; AsE–Ascorbic acid equivalents. The data represented as mean ± SE for five independent determinations and the mean value bearing different alphabets (shown in superscript) in the same column are significantly different at $p<0.05$ according to ANOVA test.

Table III: Correlation coefficients (r^2) for bioactive compounds and their antioxidant properties.

	Phenolics	Terpenoids	Flavonoids	Tannins	Alkaloids	ABTS	DPPH	Total antioxidant activity	H_2O_2	OH	Reduction potential
Phenolics	-	0.592	0.971	0.920	0.155	0.904	0.385	0.212	0.912	0.906	0.346
Terpenoids	0.592	-	0.424	0.843	0.797	0.874	0.956	0.846	0.292	0.290	0.939
Flavonoids	0.971	0.424	-	0.807	0.054	0.770	0.230	0.092	0.980	0.776	0.197
Tannins	0.920	0.843	0.807	-	0.405	0.998	0.666	0.477	0.687	0.998	0.628
Alkaloids	0.155	0.797	0.054	0.405	-	0.449	0.931	0.995	0.009	0.443	0.950
ABTS	0.904	0.874	0.770	0.998	0.449	-	0.707	0.522	0.645	1	0.672
DPPH	0.385	0.956	0.230	0.666	0.931	0.707	-	0.964	0.125	0.702	0.998
Total antioxidant activity	0.212	0.848	0.092	0.478	0.994	0.522	0.963	-	0.028	0.516	0.978
H_2O_2	0.912	0.292	0.980	0.687	0.009	0.645	0.125	0.028	-	0.652	0.100
OH	0.906	0.290	0.776	0.998	0.443	1	0.702	0.516	0.652	-	0.665
Reduction potential	0.346	0.939	0.197	0.628	0.950	0.672	0.998	0.978	0.1	0.665	-

as per Henseler *et al*. [30], r^2 with 0.25, 0.50 and 0.75 described as weak, moderate, and substantial, respectively.

Correlation between bioactive compounds and antioxidant activities

Linear correlations were performed for all the parameters performed in the study, including total phenolics, terpenoids, flavonoids, tannins, alkaloids, and their antioxidant activities (ABTS, DPPH, H_2O_2, -OH free radical scavenging and total antioxidant activity with reduction potential) among all the bark, fruit, and leaf extracts of *Ficus religiosa*. The results obtained were depicted in Table III. It is revealed that phenolics strongly correlated with ABTS (r^2 =0.904), H_2O_2 (r^2 =0.912), and -OH (r^2 = 0.906) free radical scavenging activities. However, the terpenoids displayed the highest correlation with DPPH free radical scavenging activity, total antioxidant activity and reduction potential. Flavanoids are positively correlated strongly with $H_2O_2$and –OH free radical scavenging activities. These correlation results indicated that the phenolic rich extracts exhibited the highest antioxidant and free radical scavenging activities at the lowest concentration level. It is pertinent to note that the bark extract of *Ficus religiosa* enriched with phenolics and terpenoids and exhibiting the highest antioxidant and free radical scavenging activities at the lowest concentration as compared to fruit and leaf extracts.

DISCUSSION

Phytochemicals are plant-based non-nutritive bioactive compounds that impart protection against diseases [31, 32].These molecules are sources and lead molecules for drug discovery. As ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) passed phytochemicals have less side effects in comparison to the synthetic drug, there is always a quest to identify drug against various diseases from natural resources[33–36]. However, to determine the best possible drugs, it is highly essential to understand the phytochemical constituents of any plant. Considering this, in the present study the authors reported the biochemical composition of *Ficus religiosa.* T**he** bark is showing the highest levels of phenolics, terpenoids, and flavonoids contents with enhanced antioxidant activities as compared to leaves and fruits (Table I). Similar results were observed in C*innamomun zeylancium* [37] and a leaf of *Capparis spinose* [37]. On the contrary, inthe fruit of *Laurusnobilis* exhibiting moderately higher terpenoids compared to flavonoids[37].Tables I & II shown that phenolics and terpenoids have the highest antioxidant potential, particularly in the bark, as compared to fruit and leaves. The content of total phenolics are strongly correlated with ABTS (r^2 =0.916), H_2O_2 (r^2 =0.906), -OH (r^2 = 0.906) free radical scavenging activities and reducing power assay (r^2=0.929) (Table III). Similar linear correlations observed between antioxidant activity tests and total phenolic content as reported in previous literature [38–40]. However, the terpenoids have substantial correlation with DPPH (r^2 = 0.956) and total antioxidant assay (r^2=0.962). Chaouche *et al* reported a similar type of observation in *Haloxylona rticulatum, Echium pycnanthum, and Solenostemma oleifolium*[41]. And also seen the content of total flavonoids showed a good correlation as phenolics with the scavenging assays like H_2O_2(r^2= 0.9067), OH (r^2=0.9863. Luiza et al [42] reported results revealing that flavonoids have high correction with scavenging activities. Alkaloids strongly correlated with ABTS(r^2=0.999), total antioxidant assay(r^2=0.937) and reducing power assay (r^2=0.998).Gan *et al* [43]observed a similar type of study. The outcomes of the investigation imply that the plant extract comprises phytochemicals components that scavenge the free radicals by providing hydrogen and prevents the loss.

H_2O_2 and –OH free radicals are incredibly significant because of their capacity to pass through the biological membranes and causes cell death. H_2O_2 is not highly reactive by itself. However, this can seldom be deadly to cells because it may provide an advance to -OH radicals in the cells [44]. Phenolics present in the extracts will scavenge the H_2O_2, consequently counterbalancing into water[45]. The outcome of results indicated that all the extracts may stimulate the change of H_2O_2 to H_2O by the contribution of an electron by antioxidant compounds present in the extracts. The bark extracts of *Ficus religiosa* showed a similar trend in their scavenging action against H_2O_2 at the lowest IC_{50} value when compared with leaf and fruit. The oxidative destruction to protein, DNA, and lipids done by the most common reactive oxygen species called hydroxyl radical[46]. The scavenging of the hydroxyl radicals by *Ficus religiosa* extracts may be due to the bearing of hydrogen provided by a capacity of phenolic compounds.

Antioxidants (reductants) and its mechanism can be explained by redox reactions, wherever one reaction species diminished at the expense of the oxidation of the other, where reductants can inactivate the oxidants. Reducing power activities of *Ficus religiosa* extracts were significantly different and dose-dependent; as the concentration of extracts increases, the reducing power also increases and exhibits the highest correlation with bioactive compounds. It indicates that the extracts of *Ficus religiosa* possibly work as electron contributors and stop radical series reaction. Finally, the results of present study revealed that the bark extract of *Ficus religiosa* enriched with phenolics and terpenoids and exhibiting

the highest antioxidant and free radical scavenging activities at the lowest concentration as compared to fruit and leaf extracts.

CONCLUSIONS

In conclusion, the results revealed that the bark extract of *Ficus religiosa* is enriched with the highest levels of bioactive compounds (total phenolics, terpenoids, flavonoids) as compared to fruit and leaf extracts, and also exhibiting significant antioxidant and free radical scavenging activities at the lowest concentration and may be used as a drug source to alleviate oxidative stress caused diseases. Nevertheless, farther isolation of bioactive compounds would assist in ascertaining its strength and safety as a lead candidate for antioxidants for pharmaceutical uses.

CONFLICTS OF INTEREST

None

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