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Research Article

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Phytochemical Screening of *Ficus religiosa* Seeds and Evaluation of its Antioxidant Potential

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Abstract

Ficus religiosa is one of the oldest trees portrayed in Atharvaveda, Arthasastra, Upanishads, Bhagavadgita, Buddhist literature, etc. The wide therapeutic usage of *F. religiosa* since ancient times is mentioned in Indian traditional medicine systems like Ayurveda, Unani, etc. Every part of the tree, leaves, bark, fruits, and seeds are used in herbal medicine and ayurvedic formulations. Based on the previous activities reported, the present study is carried out to validate the traditional medicinal use of *F.religiosa* seeds by identifying and quantifying the phytoconstituents and their antioxidant activities. Hexane, chloroform, ethyl acetate, methanol, and aqueous soxhlet extracts of *F.religosa* seeds were screened for their Phyto-constituents. Results revealed the presence of phenols, tannins, flavones, flavonones, flavonoids, quinones, coumarins, and cardiac glycosides. The quantitative evaluation of these extracts revealed that the highest amount of TPC (total phenolic contents) (6 ± 0.003), TTC (total tannins contents) (14.44 ± 1.78), and TFC (total flavonoid contents) (89.00 ± 0.089) in terms of mg/g was found in ethyl acetate extract. Nonenzymatic antioxidant assays, i.e., total antioxidant, FRAP, and DPPH assays were performed with all the solvent extracts, which demonstrated various qualities in agreement with the accessible standard as BHT and ascorbic acid. Of all the five extracts, ethyl acetate extract showed maximum antioxidant activity in FRAP and DPPH tests. The results of the present investigation demonstrated significant variations in the phytochemical and antioxidant activities among different solvent extracts of *F. religiosa* seeds.

Keywords

Ficus religiosa seeds, Phyto-constituents, Antioxidant activity, FRAP, DPPH

Abbreviations

TPC: Total Phenolic Contents; **TTC**: Total Tannins Contents; **TFC:** Total Flavonoid Contents; **FRAP**: Ferric reducing Antioxidant power assay; **DPPH**: 2, 2-Diphenyl-1-picrylhydrazyl; **TAC**: Total Antioxidant Capacity; **BHT:** Butylated Hydroxytoluene; **BHA:** Butylated Hydroxyanisole; **GAEq:** Gallic Acid Equivalents; **TAEq;** Tannic Acid Equivalents; **QEq:** Quercetin Equivalent; **DMSO:** Dimethyl Sulfoxide; **TPTZ:** 2, 4, 6-Tri Pyridyltriazine; **ANOVA:** Analysis of Variance

Introduction

As old is the existence of mankind so old is the use of plants and their products for treating different diseases. Since time immemorial just like leaves, stems, roots, and other plant parts, even seeds have been used as a natural medicament for the management of several ailments as advocated by conventional physicians. The non-nutrient bioactive compounds found in fruits, vegetables, cereals, legumes,

and most of the medicinal plants are known as phytochemicals. These phytochemicals protect the plant from biotic and abiotic stress. This protective effect of the phytochemicals in plants is exploited in recent times for treating several ailments in accordance with the usage of plant parts in traditional medicinal practices. All seeds contain reserve foods, viz. carbohydrates, and oils. Apart from these, the seeds contain a spectrum of specific secondary metabolites, most of which are very limited in distribution, and some of which have complex chemical structures. Seeds show diverse medicinal properties chiefly, because of the presence of different secondary plant metabolites which are pharmacologically active chemical constituents, demonstrating therapeutic activities against a multitude of diseases [1].

Ficus religiosa (L.) is a large perennial or deciduous tree belonging to the Moraceae family, largely planted as a roadside tree. Bestowing to the multitudinous benefits it provides, where it has been used to cure the disorders of the central nervous system (epilepsy, migraine, etc.), endocrine system (diabetes, etc.), gastrointestinal tract (vomiting, ulcers, stomatitis, constipation, liver diseases, etc.), reproductive system (menstrual irregularities, etc.), respiratory system (asthma, cough, etc.) and infectious diseases (chickenpox, elephantiasis, leprosy, tuberculosis, gonorrhea, scabies, etc.), *F. religiosa* is considered as an herb [2]. The leaves are pink when young and dark green upon maturity, glossy, alternate, coriaceous (leathery), broadly ovate and spirally arranged, stipulate, and with unusual tail-like tips. Flowers are axillary sessile, and unisexual. Figs are in pairs, rounded, flat-topped, smooth, sessile, axillary, basal bracts, and green when young and purple with red dots when ripen. Petioles are slender and 7.5-10 cm long. In Southeast Asia, it is commonly planted in temple areas. This tree has various nicknames such as the Tree of Life, Bodhi Tree, Wisdom Tree, Sacred Tree, etc. The variety of traditional medical uses of *F. religiosa* has prompted researchers to rationalize its use, looking for the biological activity of extracts or isolated compounds from different parts of this plant. Six parts of the trees (i.e., bark, leaves, seeds, fruits, roots, and latex) are assessed for their therapeutic qualities [3].

According to previous studies, the fruit of *F. religiosa* contains good amounts of β-caryophyllene, α-terpinene tyrosine, undecane, dendrolasine, tridecane, tetradecane, α-trans bergamotene, (e)-β-ocimene, α-pinene, limonene, asparagine, α-ylangene, α-thujene, α-copaene, β-bourbonene, aromadendrene, δ-cadinene, α-humulene, β-pinene, alloaromadendrene, germacrene, γ-cadinene and bicyclogermacrene [4]. As per data provided by various studies, figs of *F. religiosa* are reported as an excellent source of flavonoids, phenols, fiber, antioxidants, and other compounds like vitamins, proteins, minerals, carbohydrates, serotonin, etc. The high amount of serotonin content showed anticonvulsant activities [5], and anti-amnesic activity was observed using a methanol extract of dried *F. religiosa* figs [6]. The *F. religiosa* seeds reportedly contain fatty matter, albuminoids, colouring matter, phytosterolin, carbohydrate, glycoside, and β-sitosterol [7]. Therefore, it is of great interest to carry out a phytochemical screening of the *F. religiosa* seeds in order to validate their use in traditional folk medicine and to reveal the active principle by isolation and characterization of various constituents.

Free radicals attack several macromolecules of the cell, like lipids, proteins, and nucleic acids leading to cell damage and disruption of cellular homeostasis. A lot of free radicals are generated during various physical and metabolic activities of the body, which may harm the body in many ways, resulting in the loss of function and sometimes even death [8]. Spontaneous oxidation may even cause food rancidity and spoilage of medicines [9]. Although there are several enzymes within the body that scavenge free radicals, the principal micronutrient antioxidants (vitamins) are vitamin E (α-tocopherol), vitamin C (ascorbic acid), and β-carotene. The body cannot manufacture these micronutrients in sufficient quantities in all conditions, so they must be supplied in the diet. Synthetic antioxidants especially synthetic phenolic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are used routinely in the food industry, cosmetics, and therapeutic industry [10]. Because of their high volatility and instability at elevated temperatures, and their carcinogenic nature, synthetic antioxidants are not advisable for consumption. In view of increasing risk factors for human survival due to various deadly diseases, there has been a global trend toward the use of natural substances present in medicinal plants and taking a plant-based diet as therapeutic antioxidants. Several parts of *F. religiosa* are previously studied for their phytochemical composition and antioxidant activities however, not many studies are reported on the activity of *F. religiosa* seeds. Hence, the present study is carried out to assess the phytochemical composition and antioxidant activity of *F. religiosa* seed extracts.

Materials and Methods

Plant material

Figs of *F. religiosa* were collected from the region of Andhra University [17° 43' 47.3880" N and 83° 19' 17.3820" E]. Seeds were separated from fresh figs manually and cleaned thoroughly. The cleaned seeds were then shade dried and ground into fine powder by using a laboratory-grade mechanical blender. This seed powder was then sieved through a fine mesh to obtain uniform-sized particles. The finely ground seed powder was then shade dried. The dried seed powder was then stored in airtight containers at room temperature in aseptic conditions for the further extraction procedure.

Preparation of plant extracts

F. religiosa seed extracts were prepared by soxhlet extraction, a type of continuous extraction using a series of organic solvents with increasing polarity order in terms of their polarity index (hexane: 0.1, followed by chloroform: 4.1, then ethyl acetate: 4.4, then methanol: 5.1, and finally water: 10.2). *F. religiosa* seed powder 450 grams was first extracted in hexane at 70 °C, the residue obtained after extraction in hexane was further extracted consecutively with chloroform at 61.2 °C, then in ethyl acetate at 77 °C, then in methanol at 64.7 °C for 14 hours using soxhlet apparatus. The obtained seed extracts were concentrated further by evaporating the solvent using distillation and then the concentrated extracts were further dried and stored in a desiccator at room temperature for further study. For the preparation of aqueous extract, 10 grams

of *F. religiosa* seed powder was dissolved in 100 ml of distilled water and was stirred using a magnetic stirrer for 10 mins then the mixture was boiled at 80 ºC for 30 minutes. This aqueous extract was then filtered through whattmann filter paper no.1 and then stored at -4 ºC for further use. The dried extracts were weighed and calculated the percent yield.

Qualitative phytochemical analysis

Preliminary qualitative Phytochemical examinations were carried out for all the extracts for the presence of alkaloids, saponins, phytosterols, phenols, tannins, flavonoids, diterpenes, anthocyanins, cardiac glycosides, quinones, and coumarins as per the standard methods. Extracts were evaluated for the presence of alkaloids through Dragendroff's test and Wagner's test. The presence of Saponins was analyzed by doing a frothing and foaming test with distilled water. Phytosterols like terpenoids and triterpenoids were assayed by salkowski's test and Hirshonn's reaction. Phenols were screened in the extracts using 10% aqueous ferric chloride. Tannins were screened in the extracts by the addition of basic lead acetate solution. Flavones were screened by the addition of 10% NaOH to the extract. Flavonones in the extract were screened by the addition of concentrated sulphuric acid. Anthocyanins were screened by the addition of concentrated sulphuric acid to extract in equal volume. Cardiac glycosides were screened by the addition of a few drops of glacial acetic acid, 5% $FeCl_3$, and concentrated sulphuric acid to the extract. Quinones presence in the extract is screened by adding sulphuric acid in equal volumes to extract. Extracts were screened for the presence of coumarins by the addition of 10% NaOH to extract in equal volumes.

Quantitative phytochemical analysis

Total phenolic content (TPC) was determined by the previously described Folin-Ciocalteu (FC) method [11]. To 50 µl of the plant extract, 2.5 ml of 10% FC reagent, and 2.0 ml of 7.5% (w/v) sodium carbonate was added and incubated at 45 ˚C for 15 minutes. The absorbance values of all samples were measured at 765 nm in a spectrophotometer. Using the regression equation (y = 0.009x + 0.116) obtained from the standard curve of gallic acid with R^2 = 0.964, the results were calculated and expressed as milligrams (mg) of gallic acid equivalents (GAEq) per gram (g) dry weight of the extract (DWE).

Total tannin content (TTC) was determined by using the method of FC (1927) with some modifications [12], To 50 µl of sample 950 µl of distilled water, 1.5 ml of FC reagent, and 0.5 ml of 20% Na_2CO_3 were added and incubated at room temperature for 45 minutes. After incubation, the reaction mixture was read spectrophotometrically at 725 nm. TTC was calculated as milligrams of tannic acid equivalents (TAEq) per gram of DWE using the regression equation (y = 1.13x + 0.007) obtained from the standard curve of tannic acid with R^2 = 0.999.

Total flavonoid content (TFC) was determined by a previously reported method using Aluminium chloride colorimetric method with slight modification [13]. To 1 ml of extract, 4 ml of distilled water, and 0.3 ml of 5% $NaNO_2$ were added and incubated at room temperature for 5 minutes. After incubation 0.3 ml of 10% of aluminum chloride, and 2 ml of 1M NaOH were added and the total volume was made up to 10 ml with distilled water. The reaction mixture was read spectrophotometrically at 510 nm. TFC was calculated as milligrams of quercetin equivalent (QEq) per gram DWE using the regression equation (y = 0.229x + 0.244) obtained from the standard curve of quercetin with R^2 = 0.998.

In vitro antioxidant analysis

Estimation of total antioxidant capacity

The determination of total antioxidant capacity (TAC) was carried out by the phosphomolybdate method given by Prieto, P et al. with slight changes [14]. Different concentrations of the plant sample, i.e., 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml, were dissolved in dimethyl sulfoxide (DMSO) in different reaction tubes. To 0.2 ml of each sample extract, 1.8 ml of distilled water was added to make up the total volume to 2 ml. To this reaction mixture, 2 ml of phosphomolybdate reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid) was added. The entire reaction contents were incubated at 90 ºC for 90 minutes. After cooling the reaction tubes, the reaction was assessed spectrophotometrically at 695 nm against DMSO as a control. BHT and ascorbic acid were used as standards. All the reactions were performed in triplicates. The total antioxidant capacity was expressed as µg/ml of ascorbic acid equivalents (AsEq) calculated by using the standard ascorbic acid graph. The extract concentration corresponding to 50 percent activity (IC_{50}) was calculated from the curve of ascorbic acid equivalents against extract concentration. The lower the IC_{50} value the higher the total antioxidant capacity of the sample.

Ferric reducing antioxidant power assay (FRAP)

The antioxidant power of the sample was assayed by the method of Benzie and Strain which was developed to measure the ferric reducing power of human plasma [15]. The ferric reducing antioxidant power (FRAP) assay is a typical method that measures the reduction of ferric ion (Fe^{3+})-ligand complex to the intensely blue-colored ferrous (Fe^{2+}) complex by antioxidants in an acidic medium. To 3 ml of FRAP reagent (a mixture of 300 mM acetate buffer at pH 3.6,10 mM 2,4,6-tri pyridyltriazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1), 100 µl of extracts dissolved in DMSO in different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml) were added and read spectrophotometrically at 593 nm against DMSO as blank. BHT and ascorbic acid were used as standards. All the tests were done in triplicates. Ascorbic acid equivalents were determined using the regression equation (y = 0.0118x + 0.0394) obtained from the calibration curve of ascorbic acid (100µM -1000 µM). The results are expressed as µ moles of Ascorbic acid equivalents per ml which were converted as FRAP units by multiplying with the conversion factor i.e., 2. The extract concentration corresponding to 50 percent activity (IC_{50}) was calculated from the curve of FRAP units against extract concentration. The lower the IC_{50} value the higher the free radical scavenging activity of the sample.

DPPH radical scavenging assay:

DPPH assay is one of the most widely used tests in food

biochemistry for the evaluation of the free radical scavenging capacity of plant extracts. The DPPH assay was carried out as described by Cuendet et al. [16]. To 0.5 ml of each extract dissolved in DMSO in different concentrations 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml, 0.5 ml of 0.004% DPPH was added and incubated for 30 minutes at room temperature in dark. After incubation, the colour change was measured using a spectrophotometer at 517 nm. For control, instead of the extract, DMSO was used. BHT and ascorbic acid were used as standards. Free radical scavenging capacity is expressed in terms of the percentage of inhibition of oxidation which is calculated using the formula:

$$\text{Inhibition}\,\% = \left(\frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}}\right)\times 100$$

The extract concentration corresponding to 50 percent inhibition (IC_{50}) was calculated from the curve of inhibition percentage against extract concentration. The lower the IC_{50} value the higher the free radical scavenging activity of the sample.

Statistical analysis

All the procedures for extraction, phytochemical analysis, and antioxidant studies were repeated in triplicate and statistical analysis was done separately for each test. The results were expressed as means ± standard error means (SEM) of three parallel replicates. For all the reports, one-way analysis of variance (ANOVA) with Tukey's test of multiple comparisons was performed by using Microsoft Excel 2022. IC_{50} values for each test were calculated by using regression analysis and the Pearson correlation coefficient (scatter plots) between antioxidant activities and the sample's TPC, TTC, and TFC were analyzed using = CORREL function. Statistical significance was defined at $p \leq 0.05$.

Results and Discussion

Dried *F. religiosa* seeds were extracted via soxhlet extraction, a solid-liquid extraction technique for 14 hours. The percentage yield of *F. religiosa* seed in hexane, chloroform, ethyl acetate, and methanol is 5.69 ± 0.035%, 0.62 ± 0.015%, 1.43 ± 0.024%, and 0.54 ± 0.023% respectively as indicated in figure 1.

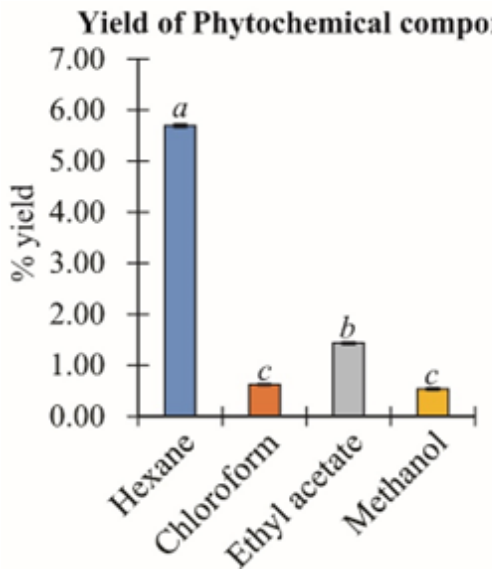

Figure 1: The percentage yield of *F. religiosa* seed extract in different solvents.

The results of the qualitative phytochemical analysis of *F. religiosa* seed extracts are summarised in table 1. Flavonoids, cardiac glycosides, quinones, and coumarins were found in all extracts, i.e., hexane, ethyl acetate, chloroform, methanol, and aqueous extracts. Tannins were found in hexane, ethyl acetate, methanol, and aqueous extracts. Phenols were found in hexane, ethyl acetate, and chloroform extracts. Triterpenoids were found in all extracts except methanol extract. However, alkaloids and terpenoids were not found in any of the above-mentioned extracts. These results were similar to the results reported in methanol extracts of *F. religiosa* fruits by Yaso et al. [17].

Quantitative phytochemical analysis has revealed that the seed extracts of *F. religiosa* contain appreciable levels of TPC. The F-statistic is [$F(4,10) = 241350.69, p<0.05$], and the Tukey test analysis of TPC has revealed that the mean concentration of all extracts has shown a significant difference at $p<0.05$. Of all the *F. religiosa* seed extracts, the highest amount of TPC (total phenolic content) was found in ethyl acetate extract (6.00 ± 0.003), followed by chloroform extract (3.30 ± 0.002),

Table 1: Phytochemical constituents present in different extracts of *F. religiosa* seed.

S.no	Phytochemical constituents	Solvent extracts of *Ficusreligiosa*				
		Hexane	Ethyl Acetate	Chloroform	Methanol	Aqueous
1	Alkaloids	-	-	-	-	-
2	Tannins	+	+	-	+	+
3	Flavonoids	+	+	+	+	+
4	Phenols	+	+	+	-	-
5	Terpenoids	-	-	-	-	-
6	Cardiac Glycosides	+	+	+	+	+
7	Saponins	-	-	-	-	-
9	Quinones	+	+	+	+	+
10	Coumarins	+	+	+	+	+
11	Triterpenoids	+	+	+	-	+

+ indicates the presence of the constituent and - indicates the absence of the constituent.

hexane extract (1.85 ± 0.005), methanol extract (1.66 ± 0.005) and least in aqueous extract (1.12 ± 0.004), as shown in figure 2A. The results are in agreement with the previous study of TPC content in aqueous extract of *F. religiosa* fruit reported by Dharmender et al. [18].

The highest amount of TTC (total tannins content) was present in ethyl acetate extract (14.44 ± 1.78), followed by chloroform extract (10.82 ± 1.38), methanol extract (6.19 ± 0.91), hexane extract (5.95 ± 0.85) and least in aqueous extract (2.74 ± 0.52), as shown in figure 2B. The F-statistic is [$F(4,10) = 15.35$, $p<0.05$], and the Tukey test analysis of TTC has revealed that there is a significant difference between the mean pairs of concentrations of hexane - ethyl acetate extract, chloroform - aqueous extract, ethyl acetate - methanol extract, and ethyl acetate - aqueous extract only.

The highest amount of TFC (total flavonoid contents) was present in ethyl acetate extract (89.00 ± 0.089), followed by chloroform extract (34.85 ± 0.222), methanol extract (12.21 ± 0.196), hexane extract (11.64 ± 0.056) and least in aqueous extracts (3.63 ± 0.551), as shown in figure 2C. The F-statistic is [$F(4,10) = 15095.01$, $p<0.05$], and the Tukey test analysis of TFC has revealed that there is a significant difference between all the mean pairs of concentrations except hexane and methanol extracts.

F. religiosa seed extract yielded differently with different solvents used based on solvent properties. Quantitatively hexane yielded the highest amount of extract followed by ethyl acetate then chloroform and finally methanol. Though methanol and water have a greater polarity, ethyl acetate has high solubility to extract the phytoconstituents from the seeds via the soxhlet extraction procedure. Hence, ethyl acetate can be considered the best of all solvents used in this study to recover bioactive phytoconstituents from *F. religiosa* seeds. Based on the results of the quantitative phytochemical analysis in the present study, the most abundant phytochemical constituents of *F. religiosa* seed are flavonoids followed by tannins and phenols which is contrary to that reported in the *F. religiosa* fruits by Anand et al. [19].

The total antioxidant capacity (TAC) of all the evaluated *F. religiosa* seed extracts and standards (ascorbic acid and BHT) at different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml) are summarised in table 2. The cumulative total antioxidant activity of all concentrations of different *F. religiosa* seed extracts and standards was analysed, the highest activity was observed in the hexane extract (93.21±0.79 µg/ml) which is lower than that of standards ascorbic acid (295.63±1.14 µg/ml) and BHT (330.00±1.13 µg/ml). These cumulative results were analysed with an F-statistic, [$F(6,14) = 12359.1$, $p<0.05$] which is represented in figure 3 (A). IC_{50} of total antioxidant activity of hexane, chloroform, ethyl acetate, methanol, and aqueous extracts, respectively, were 104.27±2.21 µg/ml, 28.62±8.31 µg/ml, 90.07±10.09 µg/ml, 228.28±5.99 µg/ml, and 672.57±13.35 µg/ml, with an F-statistic of [$F(6,14) = 752.18$, $p<0.05$], is represented in figure 3B.

The phosphomolybdate method is a quantitative method

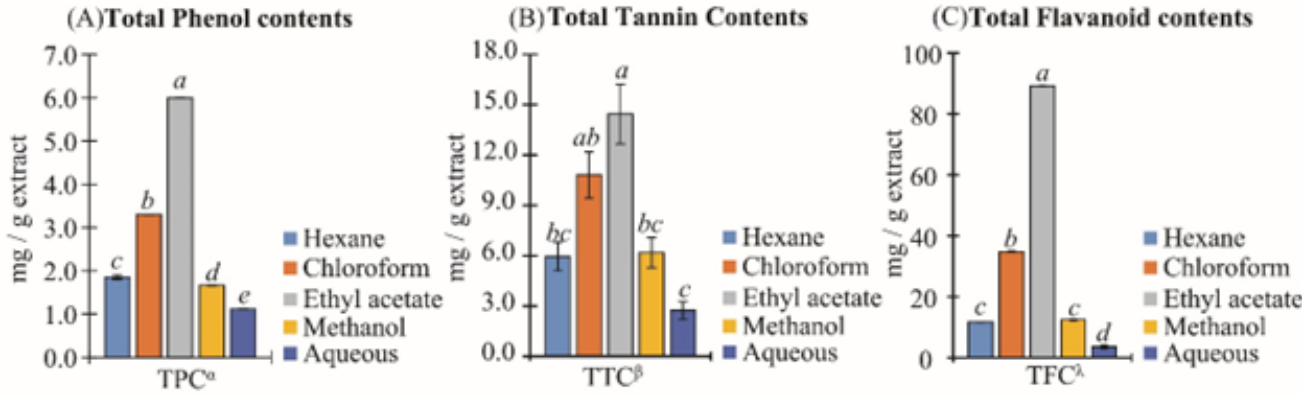

Figure 2: Quantitative Phytochemical yield of *F. religiosa* seed extract in different solvents (A) Total phenolic content in mg GAEq/g, (B) Total tannin content in mg TAEq/g, and (C) Total flavonoid content in mg QEq/g. Values followed by different letters are significantly different [ANOVA (n=3) with Tukey's test, $p<0.05$].

Table 2: Total antioxidant activity of various *F. religiosa* seed extracts in Ascorbic acid equivalents (µg/ml) at different concentrations. Values followed by different letters are significantly different [ANOVA (n=3) with Tukey's test, $p<0.05$].

Concentration µg/ml	Ascorbic acid equivalents (µg/ml)						
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous	Ascorbic acid	BHT
100	42 ±0.92^{utu}	45.11±0.73^{s}	42.61± 1.38st	31.11± 1.44^{w}	24.11± 1.28^{x}	53.5± 1.71^{qr}	52.28± 1.6^{qr}
200	73 ± 0.19^{p}	78.78± 1.15^{nop}	74.11± 1.28^{p}	47.61± 1.38^{rs}	31.17± 1.48^{vvw}	198.33± 0.96^{g}	142.28± 1.22^{b}
300	104.67± 1.25^{k}	81 ± 1.13^{no}	81.44± 1.22^{no}	56.67± 0.96^{q}	35.83± 1.29^{uvvw}	338.67± 1.04^{e}	282.44± 1.26^{f}
400	115± 0.96^{j}	84.22±1.33^{mn}	89.06± 1.24l^{m}	77.11± 1.06^{op}	37.83± 1.46^{tuv}	379 ± 1.2^{d}	548.94± 1.19^{b}
500	131.39± 1.18^{i}	89.94 ± 1.28^{lm}	101.5± 1.23^{k}	91.11± 1.44^{l}	44.22± 0.45st	508.67± 1.04^{c}	624.06± 0.39^{a}

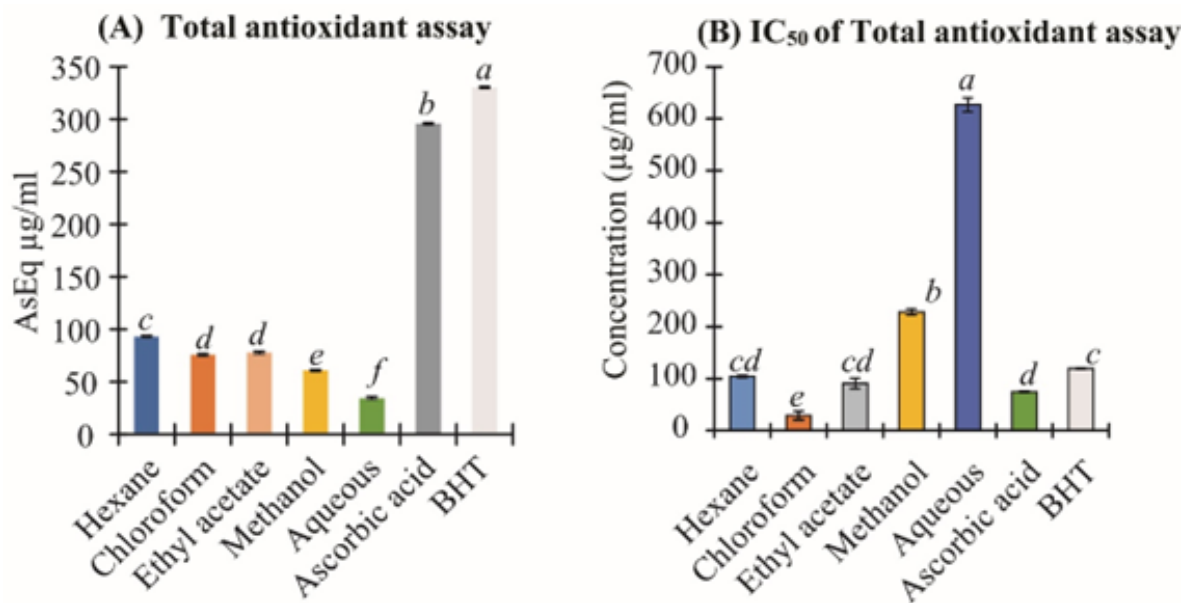

Figure 3: Total antioxidant capacity of *F. religiosa* seed extract in different solvents (A) mean cumulative values of all concentrations, (B) IC50 in µg/ml. Values followed by different letters are significantly different [ANOVA (n=3) with Tukey's test, $p \leq 0.05$].

of analysing antioxidant activity since the total antioxidant capacity is expressed as AsEq. The antioxidant capacity of the extracts was measured using a spectrophotometer by phosphomolybdenem method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) complex with maximum absorption at 695 nm. This assay is highly useful for the detection of both water as well as fat soluble antioxidant compounds [20]. Based on the obtained results, it is observed that all the extracts show significant total antioxidant activity in a concentration-dependent manner. Although at the highest evaluated concentration (500 µg/ml), ethyl acetate extract has shown maximum activity, the cumulative total antioxidant capacity of all concentrations revealed the highest in the hexane extract.

FRAP activity of all the evaluated *F. religiosa* seed extracts and standards (ascorbic acid and BHT) at different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml) are summarised in table 3. Cumulative percent inhibitions of all concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, and 500 µg/ml) of different *F. religiosa* seed extracts and standards were analysed, and the highest activity in terms of FRAP units was observed in the ethyl acetate extract (47.42 ± 0.10) which is lower than the standards, ascorbic acid (61.56 ± 0.05) and BHT (114.47 ± 0.02). The cumulative results were analysed with an F-statistic, [$F(6,14) = 184910.98$, $p<0.05$] which is represented in figure 4 (A). IC_{50} of FRAP activity of hexane, chloroform, ethyl acetate, methanol, and aqueous extracts, respectively, were 662.69 ± 1.57 µg/ml, 402.9±0.21 µg/ml, 325.41±1.09 µg/ml, 621.55 ± 3.55 µg/ml, and 785.59±5.46 µg/ml with an F-statistic, [$F(6,14) = 11322.43$, $p<0.05$] represented in figure 4B.

The FRAP assay measures the antioxidant effect of any substance as its reducing ability in the reaction medium. The antioxidant potential of the *F. religiosa* seed extracts was estimated for their ability to reduce TPTZ-Fe(III) complex to TPTZ-Fe(II) complex. The results of the present study revealed that ethyl acetate extract has shown the highest activity among all extracts both cumulatively and at the highest concentration.

DPPH activity of all the evaluated *F. religiosa* seed extracts and standards (ascorbic acid and BHT) at different concentrations (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml,

Table 3: FRAP activity of various *F. religiosa* seed extracts in FRAP units at different concentrations. Values followed by different letters are significantly different [ANOVA (n=3) with Tukey's test, $p < 0.05$].

Concentration µg/ml	FRAP units						
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous	Ascorbic acid	BHT
100	14.17 ± 0.1^{x}	21.34 ± 0.2^{u}	28.8 ± 0.15^{r}	16.2 ± 0.2^{w}	16.43 ± 0.3^{w}	36.15 ± 0.15^{o}	63.55 ± 0.15^{b}
200	$15.36 \pm 0.1w^{x}$	26.49 ± 0.62^{s}	35.41 ± 0.11^{qp}	17.9 ± 0.34^{v}	18.07 ± 0.1^{v}	53.38 ± 0.06^{j}	93.15 ± 0.1^{d}
300	24.37 ± 0.12^{t}	39.93 ± 0.2^{m}	46.46 ± 0.18^{l}	27.28 ± 0.15^{s}	24.28 ± 0.15^{t}	64.68 ± 0.2^{h}	113.94 ± 0.06^{c}
400	35.64 ± 0.11^{qp}	50.44 ± 0.26^{k}	59.14 ± 0.34^{i}	37.84 ± 0.5^{n}	32.76 ± 0.11^{q}	71.8 ± 0.1^{f}	138.41 ± 0.29^{b}
500	37.81 ± 0.22^{n}	59.88 ± 0.06^{i}	67.31 ± 0.21^{g}	40.55 ± 0.11^{m}	34.56 ± 0.06^{p}	81.8 ± 0.1^{e}	163.32 ± 0.2^{a}

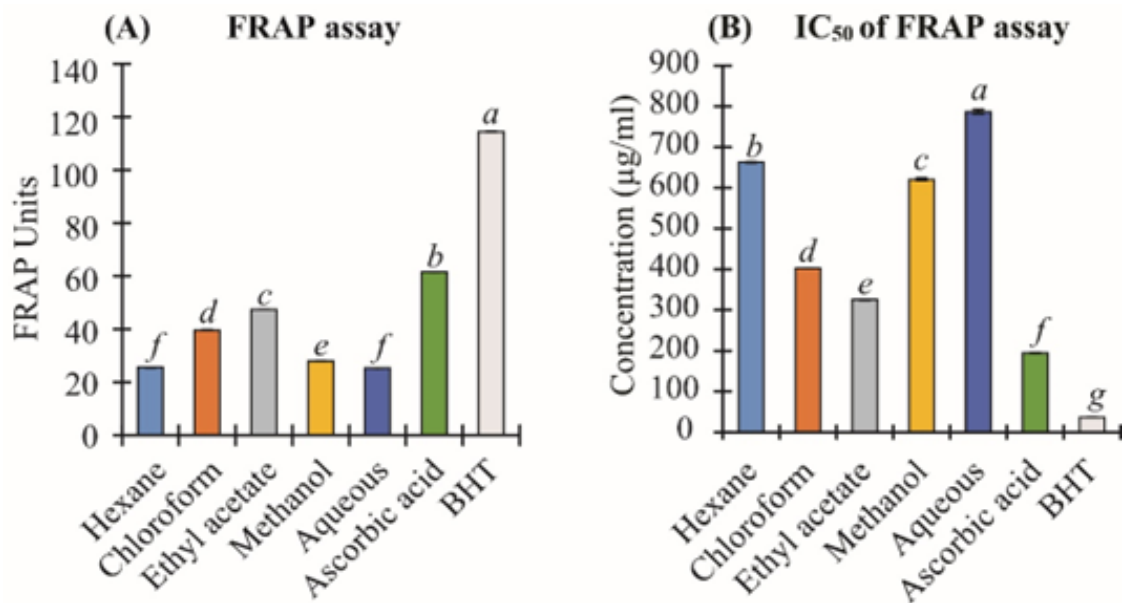

Figure 4: FRAP activity of *F. religiosa* seed extract in different solvents (A) mean cumulative values of all concentrations expressed as FRAP units, (B) IC50 in µg/ml. Values followed by different letters are significantly different [ANOVA (n=3) with Tukey's test, $p \leq 0.05$].

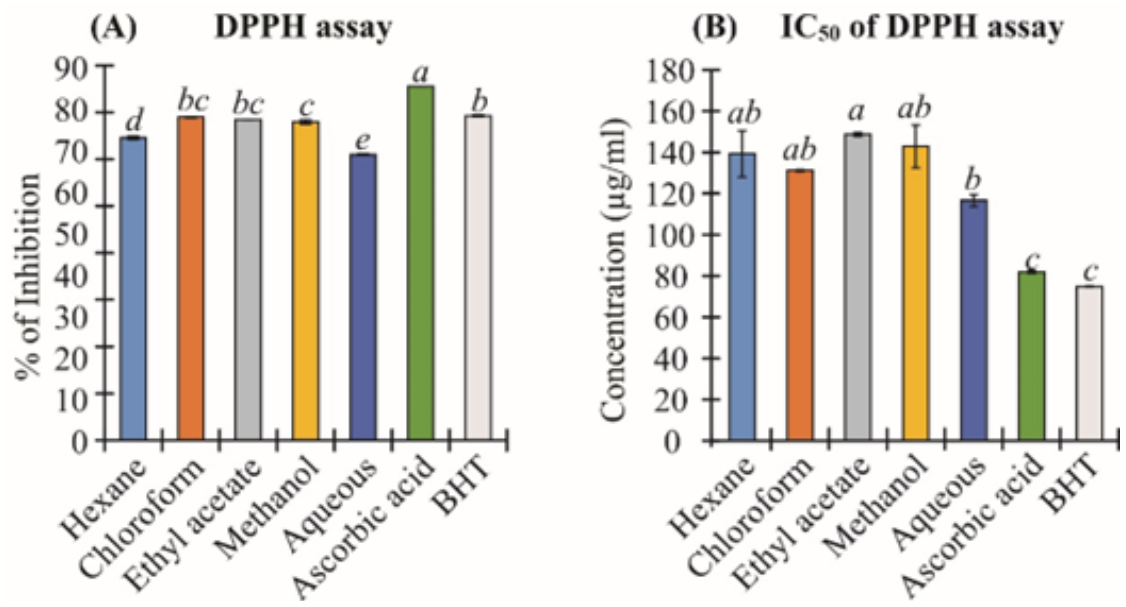

Figure 5: DPPH activity of *F. religiosa* seed extract in different solvents (A) mean cumulative values of all concentrations expressed as % inhibition, (B) IC50 in µg/ml. Values followed by different letters are significantly different [ANOVA (n=3) with Tukey's test, $p \leq 0.05$].

and 500 µg/ml) are summarised in table 4. Cumulative percent inhibitions of all concentrations of different *F. religiosa* seed extracts were analysed, and the highest percentage of inhibition amongst the seed extracts was observed in the chloroform extract (68.97 ± 0.08) which is close to that of standards ascorbic acid (75.60 ± 0.03), and BHT (69.30 ± 0.15) with an F-statistic, [$F(6, 14) = 339.79$, $p<0.05$] which is represented in figure 5A. IC_{50} of DPPH activity of hexane, chloroform, ethyl acetate, methanol, and aqueous extracts, respectively, were 140.25 ± 11.22 µg/ml, 131.17±0.41 µg/ml, 148.78±0.92 µg/ml, 143.87±10.37 µg/ml, and 116.52±2.74 µg/ml with an F-statistic of [$F(6,14) = 25.776$, $p<0.05$] represented in figure 5B.

The present investigation has shown that all tested extracts, exhibited DPPH radical-scavenging activity, and results were close to that of the standard compounds indicating their potent abilities to act as radical scavengers. The results revealed that the percent inhibition of oxidation in each extract increased with increasing concentration. At the highest evaluated concentration (500 µg/ml), of all extracts, ethyl acetate extract has shown the highest activity which might be due to the presence of high phenolic (phenols and flavonoids) compounds. As the concentration of phenolic compounds or degree of hydroxylation of the phenolic

Table 4: DPPH activity of various *F. religiosa* seed extracts in terms of % inhibition at different concentrations. Values followed by different letters are significantly different [ANOVA (n=3) with Tukey's test, $p < 0.05$].

Concentration (µg/ml)	% of Inhibition						
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous	Ascorbic acid	BHT
100	45.93 ± 1.91^{rst}	45.32 ± 0.11st	43.47 ± 0.09^{t}	47.6 ± 2.44^{rs}	48.73 ± 0.16^{qr}	48.52 ± 0.05^{qr}	48.4 ± 0.11^{qr}
200	52.2 ± 0.09^{o}	56.43 ± 0.2^{n}	56.72 ± 0.06^{lm}	51.62 ± 0.06o^{p}	50.95 ± 0.15^{opq}	64.45 ± 0.04^{m}	66.55 ± 0.17^{kl}
300	68.68 ± 0.19^{ijkl}	71.56 ± 0.21^{i}	66.46 ± 0.08^{m}	67.71 ± 0.06^{ikl}	66.76 ± 0.15^{kl}	81.27 ± 0.01^{f}	69.58 ± 0.21^{ijk}
400	76.87 ± 0.06^{gh}	83.62 ± 0.1^{ef}	85.6 ± 0.12^{be}	83.87 ± 0.06^{ef}	68.78 ± 0.09^{ijkl}	88.65 ± 0.05^{bc}	74.85 ± 0.24^{h}
500	79.21 ± 0.09^{g}	87.91 ± 0.19^{bcd}	90.16 ± 0.21^{b}	88.94 ± 0.02^{bc}	69.84 ± 0.08^{ig}	95.1 ± 0.21^{a}	87.1 ± 0.23^{cd}

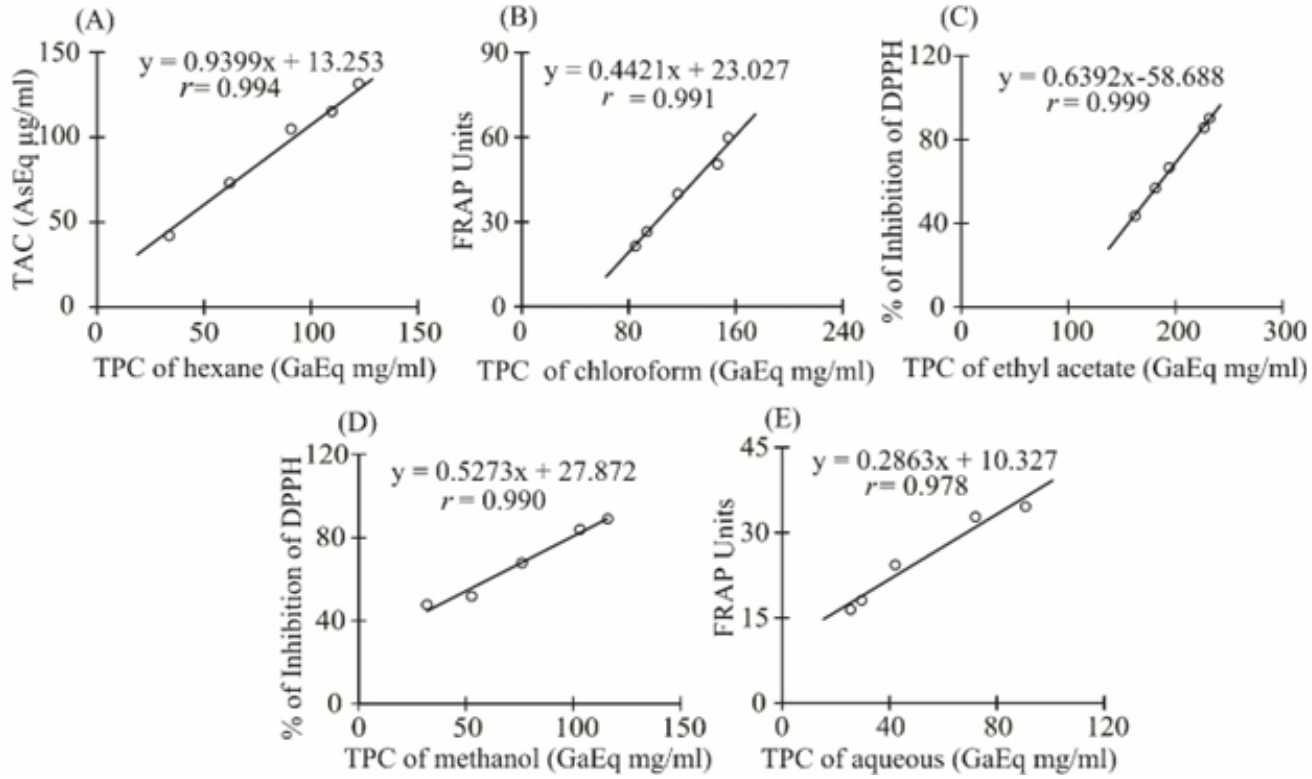

Figure 6: Positive Correlation analysis. (A) correlation between the Total phenolic content of *F. religiosa* seed hexane extract and total antioxidant capacities. (B) correlation between the TPC of *F. religiosa* seed chloroform extract and FRAP activity. (C) correlation between the TPC of *F. religiosa* seed ethyl acetate extract and DPPH activity. (D) correlation between the TPC of *F. religiosa* seed methanol extract and DPPH activity. (E) correlation between the TPC of *F. religiosa* seed aqueous extract and FRAP activity.

compounds increases, DPPH scavenging activity increases, and hence does the antioxidant activity [21]. Cumulative concentrations analysis revealed that chloroform extract has shown the highest activity which is supported by the lowest IC_{50} value of chloroform extract. Despite lower phenols, tannins, and flavonoid content, as well as total antioxidant activity, chloroform extract surprisingly showed the highest DPPH scavenging activity. This is a contradictory result which might be due to the presence of different kinds of antioxidant compounds [22].

Higher values of antioxidant activity might indicate an easily available natural source of antioxidants that could be applied to the pharmaceutical or food industries. A similar study was done on *F. religiosa* leaf extracts which revealed the presence of high amount of phytochemicals with potent antioxidant activity [23]. Therefore, we used Pearson Correlation Coefficient analysis to correlate the phytochemical concentrations with antioxidant activity of the evaluated *F. religiosa* seed extracts. Correlative analyses were performed on the obtained results of the various *F. religiosa* extracts used in the present study. Figure 6A-6E depicts the relationship between the TPC content of hexane, chloroform, ethyl acetate, methanol, and aqueous extracts against the antioxidant activity. DPPH activity showed a high positive correlation with the TPC content of ethyl acetate extract (r = 0.999) and methanol extract (r = 0.990). FRAP activity showed a high positive correlation with the TPC content of chloroform extract (r = 0.991) and aqueous extract (r = 0.978).

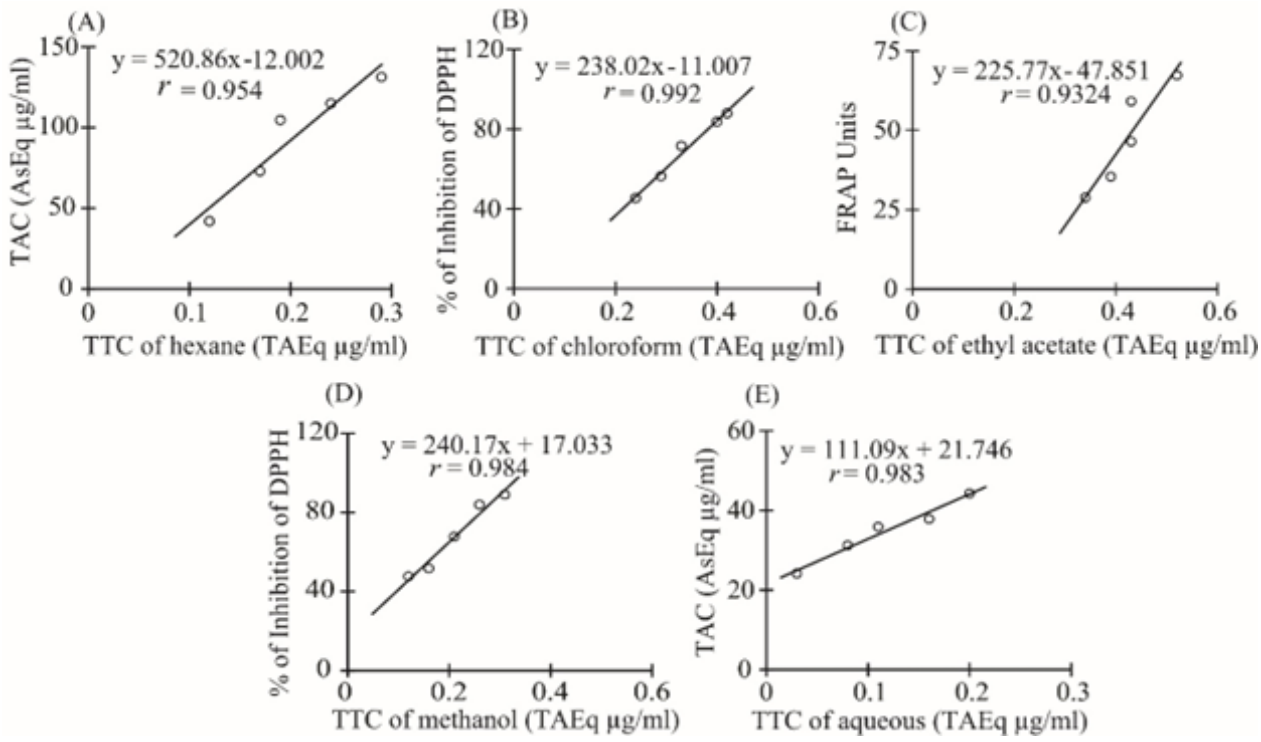

Figure 7: Positive Correlation analysis. (A) correlation between the Total tannin content of *F. religiosa* seed hexane extract and total antioxidant capacities. (B) correlation between the TTC of *F. religiosa* seed chloroform extract and DPPH activity. (C) correlation between the TTC of *F. religiosa* seed ethyl acetate extract and FRAP activity. (D) correlation between the TTC of *F. religiosa* seed methanol extract and DPPH activity. (E) correlation between the TTC of *F. religiosa* seed aqueous extract and total antioxidant activity.

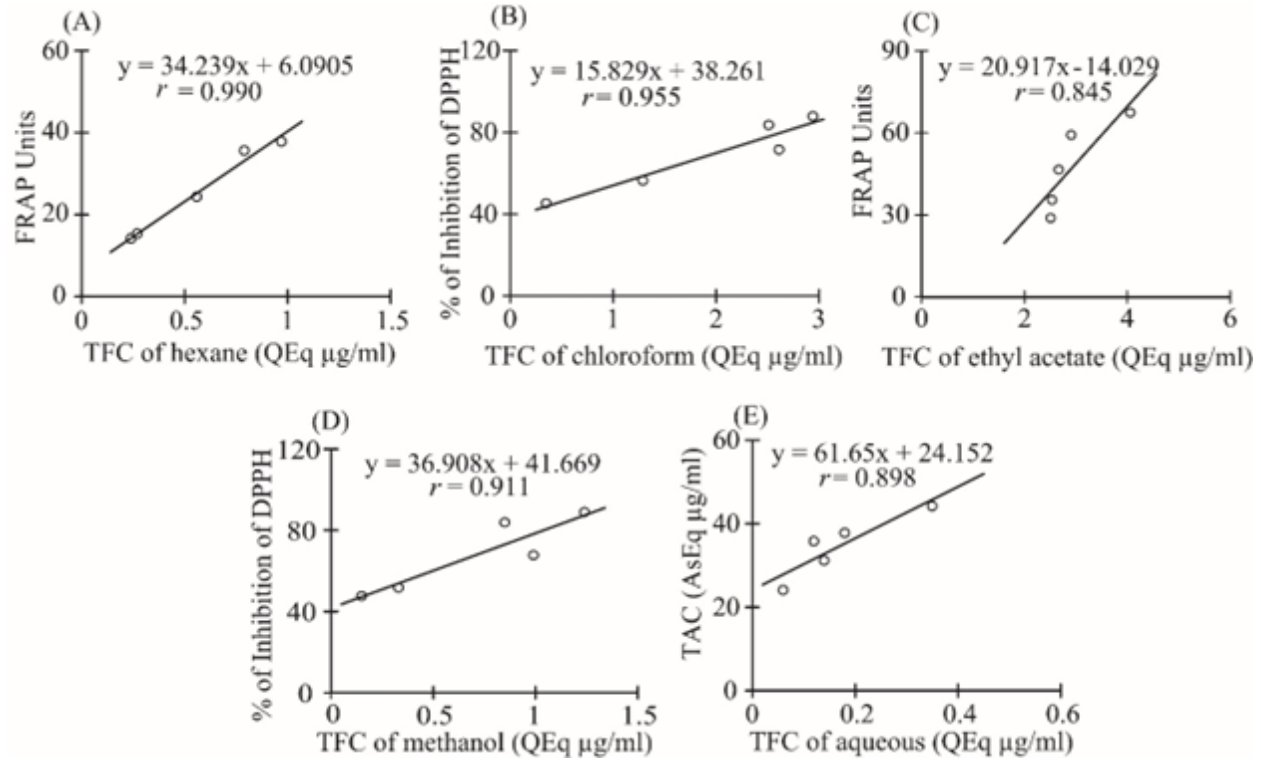

Figure 8: Positive Correlation analysis. (A) correlation between the Total flavonoid content of *F. religiosa* seed hexane extract and FRAP. (B) correlation between the TFC of *F. religiosa* seed chloroform extract and DPPH activity. (C) correlation between the TFC of *F. religiosa* seed ethyl acetate extract and FRAP activity. (D) correlation between the TFC of *F. religiosa* seed methanol extract and DPPH activity. (E) correlation between the TFC of *F. religiosa* seed aqueous extract and total antioxidant activity.

Likewise, Total antioxidant capacity has a high positive correlation with the TPC content of hexane extract (r = 0.994). Figure 7A-7E) depicts the relationship between the TTC content of hexane, chloroform, ethyl acetate, methanol, and aqueous extracts against antioxidant activity assays. DPPH activity showed a high positive correlation with TTC content of chloroform extract (r = 0.992) and methanol extract (r = 0.984). Total antioxidant capacity showed a high positive correlation with TTC content of hexane extract (r = 0.954) and TPC content of aqueous extract (r = 0.983). Likewise, for FRAP activity, a high positive correlation was found with TTC content of ethyl acetate extract (r = 0.932). Figure 8A-8E depicts the relationship between the outcomes of TFC content of hexane, chloroform, ethyl acetate, methanol, and aqueous extracts against antioxidant activity assays. DPPH activity showed a high positive correlation with TFC content of chloroform extract (r = 0.955) and methanol extract (r = 0.911). FRAP activity showed a high positive correlation with TFC content of hexane extract (r = 0.990) and ethyl acetate extract (r = 0.845). Likewise, total antioxidant capacity has a high positive correlation with the TFC content of aqueous extract (r = 0.898).

A linear association between TAC, FRAP, and DPPH values with total phenol, tannin, and flavonoid levels was established, showing that antioxidant activity rose proportionally to the phytochemical concentration. Also, a positive correlation has been recorded between TPC, TTC, and TFC of the hexane, chloroform, ethyl acetate, methanol, and aqueous fractions of *F. religiosa* seed and their total antioxidant and free radical scavenging activities.

Conclusion

The study revealed the presence of potent bioactive compounds in the seed extract which justifies the use of *F. religiosa* seed as a neutraceutical in traditional medicine.

This study showed that *F. religiosa* seed extracts have a high flavonoid/phenolics ratio indicating that the extracts have high flavonoid content. The data obtained from the present study suggests a high correlation between total flavonoid content and antioxidant capacity. Of all the extracts tested, *F. religiosa* seed ethyl acetate extract has the highest flavonoid content, which could be responsible for its high antioxidant activity.

Further extraction of bioactive substances from these potent extracts will help to clarify the rationale behind the obtained results for further investigation.

Conflict of Interest

The authors declare no conflicts of interest.

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