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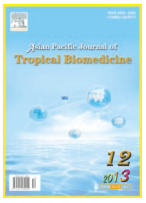

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Phytochemical, Anti-oxidant and Anthelmintic activities of various leaf extracts of *Flacourtia sepiaria* Roxb

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PEER REVIEW

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Comments

This is an appreciable research work in which authors have established the antioxidant and anthelmintic activities of *F. sepiaria*.
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ABSTRACT

Objective: The present study was carried out to investigate the phytochemical constituents, *in vitro* antioxidant potential and anthelmintic activities of *Flacourtia sepiaria* Roxb leaves.
Methods: The dried powdered leaves of *Flacourtia sepiaria* were extracted using petroleum ether, chloroform, ethyl acetate and methanol by a soxhlet extractor and preliminary phytochemical screening was performed using standard protocols. All the extract was evaluated for their potential antioxidant activities using test such as DPPH, superoxide anion radical, hydroxyl radical, nitric oxide radical scavenging abilities, ferrous chelating ability and total phenolic and flavanoid content. Anthelmintic activity of extract was screened in adult Indian earthworm model.
Results: Preliminary screening revealed the presence of bioactive compounds especially phenolics, tannins and terpenoids in all extracts. The phenolic and flavanoid content was highest in methanolic extract and lowest in petroleum ether extract. The paralytic (9.46±0.212) and death time (31.43±0.148) of methanolic extract was found to be significant ($P<0.05$) when compared with paralytic (7.33±0.206) and death time (18.60±0.229) of standard piperazine citrate at 100 mg/mL concentration.
Conclusions: The results of the present study indicate that the leaf extracts of *Flacourtia sepiaria* exhibited strong antioxidant activity and possess significant anthelmintic activity and thus it is a good source of antioxidant and anthelmintic constituents.

KEYWORDS
Antioxidant, Anthelmintic, *Flacourtia sepiaria*, Piperazine citrate.

1. Introduction

Natural antioxidant have a wide range of biochemical activities including inhibition of reactive oxygen species generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential[1]. Free radicals and other reactive oxygen species are generated continuously via normal physiological process, more so in pathological conditions. These free radicals are associated directly or indirectly with most of the pathologies known to date[2]. The use of natural antioxidants has gained much attention from consumers because they are considered safer than synthetic antioxidants. Recently there has been a worldwide trend towards the use and ingestion of natural antioxidants present in different parts of plants due to their phytochemical constituents[3,4].

Helminthic infestations are now being recognized as a cause of chronic ill health and sluggishness amongst the

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children. More than half of the world population suffers from worm infestations of one or other. Helminthes also affects domestic animals and live stocks, causing considerable economic loss. Various alternative and traditional systems of treatments report the efficacy of several natural products eliminating helminthes[5]. As per WHO only synthetic drugs are frequently used in the treatment of human beings but these synthetic drugs are out of reach of millions of people and have a lot of side effects[6].

Flacourtia sepiaria (F. sepiaria) belonging to the family Flacourtiaceae is a medium sized tree widely distributed in the dry jungles of Bengal, Bihar, Orissa and all districts of the Madras presidency. Various parts are widely used in folk medicine; an infusion of the leaves is given in case of snake bites and its bark triturated with sesame oil is used as a liniment in rheumatism and gout. The ashes of root are also given in the kidney diseases[7] and have also been proved to possess anti microbial activity[8]. Xanthine oxidase inhibitory activity has been reported for the aerial parts[9]. However no such report is available in the literature regarding antioxidant and anthelmintic activities of the *F. sepiaria* leaves.

Thus in the light of knowledge that *F. sepiaria* is having wide folklore uses, we intend to evaluate the antioxidant and anthelmintic activities of the various extracts of *F. sepiaria* Roxb leaves using *in vitro* models.

2. Materials and methods

2.1. Plant material collection and extraction

The leaves of *F. sepiaria* Roxb (Family : Flacourtiaceae) were collected from Tirunelveli district, Tamilnadu, India during the month of March 2011. The plant was identified and authenticated by Mr. chelladurai, Research officer-Botany, Central council for research in Ayurveda and Sidha, Government of India, Ref No: NCP/CH/PS02.

The *F. sepiaria* leaves were collected, shade, dried, powdered mechanically and sieved through No.20 mesh sieve. About 100 g of the powdered leaves is first extracted with petroleum ether (PEL), 60-80 °C and then consecutively with chloroform (CEL), ethylacetate (EEL) and methanol (MEL) by soxhlet extraction. The extract collected was filtered and evaporated using rotary evaporator and stored in vacuum desiccators. The percentage yield of the extract is listed in Table 1.

Table 1
Percentage yield of various extracts.

Extracts	% Yield (w/w)
PEL	9.2
CEL	1.6
EEL	4.9
MEL	7.2

PEL: pet ether extract of *F. Sepiaria* Roxb leaves; CEL: chloroform extract of *F. Sepiaria* Roxb leaves; EEL: ethyl acetate extract of *F. Sepiaria* Roxb leaves; MEL: methanolic exract of *F. Sepiaria* Roxb leaves.

2.2. Chemicals and instruments

DPPH were purchased from Sigma-Aldrich, USA. 2-deoxy-2-ribose, ascorbic acid, curcumin, gallic acid and quercetin were purchased from Himedia Labs, PVT LTD Mumbai, India. Piperazine citrate was purchased from ENZAL chemicals India LTD. All other chemicals used for the work were purchased commercially and were of analytical grade. U-V spectrophotometer Schimadzu was used to measure the absorbance.

2.3. Experimental model

Adult Indian earthworm used for the study was obtained from the College of Agriculture, Trivandrum and washed with normal saline to remove all the fecal matter and waste surrounding their body.

2.4. Phytochemical screening of the extracts

Chemical tests were carried out for all the extracts of *F. sepiaria* for the presence of phytochemical constituents like phenols, tannins, saponins, flavonoids, terpenoids, alkaloids, glycosides and steroids[10,11].

2.5. DPPH radical scavenging activity

DPPH assay gives an account on the free radical scavenging ability[12]. Briefly about 1 mL of DPPH solution (0.1 mmol/L) prepared in methanol was added to 3 mL of test or standard (gallic acid) solution at different concentration (1-64 μg/mL). The mixture was incubated in dark at 30 °C for 30 min and the absorbance was measured at 517 nm and percentage inhibition was calculated. A control reaction was carried out without the test sample.

2.6. Superoxide radical scavenging activity

The superoxide radicals are generated in a phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT)[13]. In this experiment, the superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mmol/L, pH 8.0) containing 78 mmol/L NADH, 50 mmol/L NBT, 10 mmol/L PMS and extracts to be tested at different concentrations (10-160 μg/mL). The color reaction between superoxide radicals and NBT was detected at 560 nm and the percentage inhibition was calculated. Ascorbic acid (10-160 μg/mL) was used as positive control.

2.7. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extract was determined by its ability to scavenge the hydroxyl radicals produced by the EDTA-Fe^{3+}-H_2O_2-ascorbic acid system by a reaction known as Fenton reaction[14]. The reaction mixture amounts to a final volume of 1 mL which contains 100 μL of 2-deoxy2-ribose (28 mmol/L) in phosphate buffer solution (20 mmol/L, pH 7.4), 500 μL of the extracts at various

concentrations (10-160 μg/mL) in buffer solution, 200 μL of 1.04 mmol/L EDTA and 200 μmol/L $FeCl_3$ (1:1v/v), 100 μL of H_2O_2 (1 mmol/L) and 100 μL of ascorbic acid (1 mmol/L). Test samples were incubated at 37 °C for 1 h. The free radical damage inflicted on the substrate, deoxyribose was assessed with the thiobarbituric acid test. The positive control used for this assay was quercetin (10-160 μg/mL). The percentage inhibition of the extracts and standard was calculated.

2.8. Nitric oxide radical scavenging activity

Nitric oxide generated from sodium nitroprusside at physiological pH results in the formation of nitrite ions (NO^{2-}) which reacts with Griess' reagent to form a pink colour complex which can be measured spectrophotometrically[15]. Sodium nitroprusside (10 mmol/L, 2 mL) in phosphate buffer saline (0.025 mol/L, pH 7.4) and test solutions at different concentrations (10-160 μg/mL) in a total volume of 3 mL was incubated at room temperature for a period of 150 min. After which, 0.5 mL of the incubated solution and 1 mL Griess' reagent were added togather and allowed to react for 30 min. Control samples without the test compounds but with equal volume of buffer was prepared in a similar manner as done for the test. The absorbance of the reaction mixture was measured at 546 nm. The experiment was carried out using curcumin (10-160 μg/mL) as positive control. The percentage inhibition of the extract and standard was calculated.

2.9. Ferrous chelating ability

In the ferrous chelating assay, Fe^{2+} level in the assay mixture was determined by measuring the formation of the ferrous ion-ferrozine complex[16]. Briefly, different concentrations (10-160 μg/mL) of the extracts were added to 2 mmol/L ferric chloride (0.1 mL) and the reaction initiated by adding 5 mmol/L ferrozine (0.2 mL) solution and the mixture shaken and left to stand for 10 min at 25 °C. The absorbance of the assay solution was measured at 562 nm. The experiment was carried out using ascorbic acid (10-160 μg/mL) as positive control. The percentage chelating effect of ferrozine-ferrous ion complex formation was calculated.

2.10. Estimation of total phenolic content

Total phenolics present in the extract were estimated using Folin-Ciocalteu reagent and gallic acid as the standard[17]. An aliquot of 0.5 mL of extract solution, 1 mL of saturated sodium carbonate and 0.5 mL of Folin-Ciocalteu reagent in a test tube was mixed, and allowed to stand at ambient temperature for 45 min. The blank was prepared in the same manner, and it was centrifuged if any precipitate was formed. The absorbance of supernatant solution was measured against blank at 725 nm. The total phenolic compounds present in the extracts were determined as μg gallic acid equivalent (GAE) with the use of the standard gallic acid graph.

2.11. Estimation of total flavonoid content

For estimation of total flavonoid content, 1 mg/mL of extract was prepared in methanol. From this 1 mL was pipetted out into test tube and made upto 5 mL using distilled water and 0.3 mL of 5% sodium nitrite added. Then 2 mL of 1 mol/L sodium hydroxide was added and total volume made upto 10 mL with distilled water. The solution were mixed well and the absorbance was measured against a blank at 510 nm. The total flavonoid compounds present in the extracts was expressed as μg quercetin equivalent (QE) with the use of the standard quercetin graph[18].

2.12. Calculation of 50% inhibitory concentration (IC_{50})

The concentration (μg/mL) of the extract required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the extracts. Percentage inhibition (I%) was calculated using the formula:

$$I\% = \frac{A_c - A_t}{A_c} \times 100$$

where A_c is the absorbance of the control and A_t is the absorbance of the test sample.

2.13. Anthelmintic activity

Adult Indian earthworms, *Pheretima posthuma* resemble the intestinal roundworm parasites of human beings both anatomically and physiologically[19,20] and hence were used to study the anthelmintic activity. Indian adult earthworm 5-7 cm in length and 0.1-0.2 cm in width were used for the *in vitro* anthelmintic bioassay of petroleum ether, chloroform, ethylacetate and methanol extracts. The worms were divided into the respective group containing six earthworms in each group. All the prototypes were dissolved in minimum quantity of 2% v/v Tween80 and then the volume was adjusted to 10 mL with normal saline for making the concentration of 25, 50 and 100 μg/mL. All the prototypes and standard drug solution were freshly prepared before commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 10 mL of respective formulation as follows, vehicle (2% v/v Tween 80 in normal saline), standard piperazine citrate (25, 50 and 100 mg/mL) and prototypes (25, 50 and 100 mg/mL), and then the anthelmintic activity was determined.

Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour. They were observed for their spontaneous motility. Observations were made for time taken to paralysis and death of individual worms.

2.14. Statistical analysis

All the experiments were carried out in triplicate and results expressed as mean±SEM. Significant differences among means of samples were evaluated by one-way analysis of variance.

3. Results

3.1. Phytochemical screening of the extract

Phytochemical analysis showed the presence of tannins, phenolics, flavanoids, terpenoids and steroids in the extract(Table 2).

Table 2
Phytochemical screening of various extracts.

Phytochemicals	PEL	CEL	EEL	MEL
Tannins and phenolics	+	+	+	+
Saponins	–	–	–	–
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	–	+	+	+
Glycosides	–	–	+	+
Steroids	+	+	+	+
Proteins	–	+	+	+

PEL: pet ether extract of *F. Sepiaria* Roxb leaves; CEL: chloroform extract of *F. Sepiaria* Roxb leaves; EEL: ethyl acetate extract of *F. Sepiaria* Roxb leaves; MEL: methanolic exract of *F. Sepiaria* Roxb leaves.

3.2. DPPH radical scavenging activity

DPPH radical scavenging of various extracts of the leaves of *F. sepiaria* was investigated and results were shown(Table 3). All the extracts showed a dose dependent scavenging activity, of which the methanolic extract showed the highest activity. However the scavenging activity of gallic acid used as standard was greater than all the extracts. The highest activity was shown by MEL [IC_{50}=(2.190±0.120) μg/mL] and the order of decreasing scavenging ability is MEL>EEL [(19.370 ±0.268) μg/mL]>CEL [(52.560±0.232) μg/mL]. All extracts showed significant (P<0.05) scavenging ability when compare with standard gallic acid [(IC_{50} =(1.820±0.432) μg/mL].

Table 3
DPPH radical scavenging activity of *F. Sepiaria* Roxb leaves.

CONC	Percentage inhibition (%)				
(μg/mL)	PEL	CEL	EEL	MEL	Gallic acid
1	–	–	–	27.990±0.261	32.210±0.478
2	–	–	06.280±0.233	49.420±0.364	54.140±0.360
4	05.490±0.245	08.480±0.220	15.370±0.222	64.580±0.367	72.170±0.442
8	11.420±0.118	17.500±0.252	34.090±0.417	83.100±0.192	89.100±0.610
16	16.700±0.145	32.000±0.430	46.390±0.271	89.840±0.110	94.370±0.260
32	20.410±0.182	42.360±0.162	63.520±0.245	94.350±0.210	98.300±0.357
64	24.410±0.262	54.260±0.200	77.350±0.240	96.300±0.040	98.940±0.452
IC_{50}	#	52.560±0.232*	19.370±0.268*	2.190±0.120*	1.820±0.432

PEL: pet ether extract of *F. Sepiaria* Roxb leaves; CEL: chloroform extract of *F. Sepiaria* Roxb leaves; EEL: ethyl acetate extract of *F. Sepiaria* Roxb leaves; MEL: methanolic exract of *F. Sepiaria* Roxb leaves. All values determined were mean±SEM; n=3. *P<0.05 when compared with standard.

3.3. Superoxide radical scavenging activity

The superoxide radical scavenging ability was found to increase with increase in concentration of the extract. The MEL [IC_{50}=(45.06±0.106) μg/mL] was found to be an efficient scavenger of superoxide anion radical generated from PMS-NADH system *in vitro* and the activity was significant (P<0.05) when compared to that of standard ascorbic acid [IC_{50}=(30.100±0.432) μg/mL]. The scavenging effects of extracts on the superoxide anion radical decreased in order MEL>EEL [IC_{50}=(83.360±0.614) μg/mL]>CEL [IC_{50}=(151.270± 0.218) μg/mL] (Table 4).

Table 4
Superoxide radical scavenging activity of *F. Sepiaria* Roxb leaves.

CONC	Percentage inhibition (%)				
(μg/mL)	PEL	CEL	EEL	MEL	Ascorbic acid
10	05.420±0.156	08.470±0.209	10.640±0.135	16.610±0.098	26.300±0.173
20	08.470±0.201	15.590±0.141	21.640±0.069	29.670±0.174	42.510±0.163
40	19.500±0.259	29.670±0.123	40.680±0.257	47.370±0.167	56.330±0.104
80	27.400±0.248	40.200±0.226	49.580±0.087	68.140±0.530	71.580±0.231
160	36.090±0.075	51.210±0.220	59.470±0.170	81.500±0.212	84.550±0.248
IC_{50}	#	151.270±0.218*	83.360±0.614*	45.060±0.106*	30.100±0.432

PEL: pet ether extract of *F. Sepiaria* Roxb leaves; CEL: chloroform extract of *F. Sepiaria* Roxb leaves; EEL: ethyl acetate extract of *F. Sepiaria* Roxb leaves; MEL: methanolic exract of *F. Sepiaria* Roxb leaves. All values determined were mean±SEM; n=3. *P<0.05 when compared with standard.

3.4. Hydroxyl radical scavenging activity

The extracts and the standard (quercetin) inhibited the formation of hydroxyl radical in a dose dependent manner (Table 5). The MEL [IC_{50}=(48.850±0.106) μg/mL] showed the maximum quenching ability followed by EEL [IC_{50}=(77.140± 0.664) μg/mL] and CEL (IC_{50}=(136.600±0.758) μg/mL]. The *in vitro* radical scavenging ability of the extracts were found to be significant (P<0.05) when compared with the standard quercetin [IC_{50}=(24.870±0.752) μg/mL].

Table 5
Hydroxyl radical scavenging activity of *F. Sepiaria* Roxb leaves.

CONC	Percentage inhibition (%)				
(μg/mL)	PEL	CEL	EEL	MEL	Quercetin
10	5.440± 0.181	8.970±0.117	10.470±0.029	13.550±0.265	20.620±0.106
20	11.760±0.048	17.040±0.759	21.150±0.162	26.240±0.195	47.210±0.202
40	18.400±0.210	35.640±0.262	40.940±0.093	47.230±0.187	58.650±0.089
80	20.510±0.225	44.380±0.241	50.670±0.292	60.340±0.210	72.130±0.138
160	24.350±0.129	52.440±0.187	60.500±0.106	78.430±0.274	88.430±0.309
IC_{50}	#	136.600±0.758*	77.140±0.664*	48.850±0.106*	24.870±0.752

PEL: pet ether extract of *F. Sepiaria* Roxb leaves; CEL: chloroform extract of *F. Sepiaria* Roxb leaves; EEL: ethyl acetate extract of *F. Sepiaria* Roxb leaves; MEL: methanolic exract of *F. Sepiaria* Roxb leaves. All values determined were mean±SEM; n=3. *P<0.05 when compared with standard.

3.5. Nitric oxide radical scavenging activity

The extract exhibited a concentration dependent scavenging effect on the nitric oxide radicals and effectively reduced the generation of nitric oxide radicals. All the plant extracts was found to decrease the quantity of nitrite ions *in vitro* of which the MEL showed the maximum scavenging of 76.44% at 160 μg/mL (Table 6). The order of decreasing nitric oxide scavenging ability is MEL [IC_{50}=(53.740±0.228) μg/mL]>EEL [IC_{50}=(84.470±0.620) μg/mL]>CEL [IC_{50}=(143.180 ±0.170) μg/mL] and these values when compared with standard curcumin [IC_{50}=(22.360±0.210) μg/mL] was found to be significant (P<0.05).

Table 6

Nitric oxide radical scavenging ability of *F. Sepiaria* Roxb leaves.

CONC	PERCENTAGE INHIBITION (%)				
(μg/mL)	PEL	CEL	EEL	MEL	Curcumin
10	04.820±0.080	05.400±0.115	10.590±0.242	13.610±0.232	27.290±0.183
20	09.530±0.248	13.620±0.254	20.600±0.220	28.300±0.198	48.480±0.196
40	15.160±0.076	29.490±0.187	36.340±0.140	44.580±0.138	61.360±0.162
80	29.440±0.145	41.400±0.250	49.320±0.223	60.360±0.263	79.640±0.241
160	31.750±0.332	52.290±0.148	61.480±0.184	76.440±0.100	92.400±0.263
IC_{50}	#	143.180±0.170*	84.470±0.620*	53.740±0.228*	22.360±0.210

PEL: pet ether extract of *F. Sepiaria* Roxb leaves; CEL: chloroform extract of *F. Sepiaria* Roxb leaves; EEL: ethyl acetate extract of *F. Sepiaria* Roxb leaves; MEL: methanolic exract of *F. Sepiaria* Roxb leaves. All values determined were mean±SEM; n=3. *P<0.05 when compared with standard.

3.6. Ferrous chelating ability

The formation of the Fe^{2+}–Ferrozine complex was interrupted in the presence of extracts in a dose dependent manner, indicating that the extracts have the ability to chelate the ion. Ferrozine on reaction with ferrous ions developed a red coloured complex and in the presence of the extracts the complex formation was hindered. Among the extracts tested, the methanol extract showed the highest ferrous ion chelating ability [IC_{50}=(53.380±0.416) μg/mL]. the EEL and CEL also showed significant chelating ability but at higher concentration [IC_{50}=(77.530±0.362) μg/mL and (155.830±0.244) μg/mL respectively] when compared with standard ascorbic acid [IC_{50}=(38.02±0.318) μg/mL] (Table 7).

Table 7

Ferrous chelating ability of *Flacourtia Sepiaria* Roxb leaves

CONC	PERCENTAGE INHIBITION (%)				
(μg/mL)	PEL	CEL	EEL	MEL	Ascorbic acid
10	3.790± 0.162	5.650±0.194	7.490±0.363	10.610±0.350	20.420±0.275
20	6.600±0.110	13.480±0.160	16.510±0.475	23.520±0.302	38.580±0.395
40	12.570±0.366	24.430±0.223	29.590±0.265	41.040±0.909	51.260±0.351
80	24.390±0.221	38.150±0.210	51.340±0.376	60.570±0.341	70.340±0.344
160	31.790±0.226	50.650±0.374	59.420±0.384	75.400±0.286	78.360±0.478
IC_{50}	#	155.830±0.244*	77.530±0.362*	58.380±0.416*	38.020±0.318*

PEL: pet ether extract of *F. Sepiaria* Roxb leaves; CEL: chloroform extract of *F. Sepiaria* Roxb leaves; EEL: ethyl acetate extract of *F. Sepiaria* Roxb leaves; MEL: methanolic exract of *F. Sepiaria* Roxb leaves. All values determined were mean±SEM; *n*=3. *P<0.05 when compared with standard.

3.7. Total phenolic content

Total phenolic content assay using Folin–Ciocalteau reagent is an easy, suitable and reproducible method and the total phenolic content of *F. sepiaria* leaves was calculated from the standard gallic acid graph and expressed as μg GAE/g. It is employed regularly in studying phenolic antioxidants. The methanol extract has the highest total phenolic content (122 μg GAE/g). The order of decreasing total phenolic content is MEL>EEL (105 μg GAE/g)>CEL (56.25 μg GAE/g)>PEL (35 μg GAE/g).

3.8. Total flavanoid content

The total flavanoid content of the *F. sepiaria* leaf extracts was determined as μg QE by means of the standard quercetin graph. The total flavanoid content was found to be highest in methanol extract (312 μg QE/g). The order of decreasing total flavanoid content is MEL>EEL (187 μg QE/g)>CEL (110 μg QE/g)>PEL (68 μg QE/g). The highest amount of flavanoids is extracted in methanol and lowest in petroleum ether.

3.9. Anthelmintic activity

The extracts exhibited more potent activity at a higher concentration (100 mg/mL) against *Pheretima posthuma* (earthworm). When observed the response of worms in case of paralysis and death, there was significant variations among the results produced by the different extracts at different concentrations (25, 50 and 100 mg/mL) (Table 8).

All the extracts exhibited anthelmintic activity in dose dependent manner varying from loss of motility (paralysis) to loss of response to external stimuli, which eventually advanced into death. MEL, EEL and CEL exhibited significant anthelmintic activity in dose dependent manner when compared with reference standard piperazine citrate. The methanolic extract showed less time to cause paralysis (9.460±0.212) min and death (31.430±0.148) min of the earthworms and thus it was found to be more potent than other extracts (MEL>EEL>CEL>PEL) at 100 mg/mL concentration.

Table 8

Anthelmintic activities of *F. Sepiaria* Roxb leaves.

Test Sample	Concentration (mg/mL)	Time taken for paralysis (min)	Time taken for death (min)
Control (0.1% Tween in normal saline)	--	--	--
PEL	25	173.750±0.730*	--
	50	89.070±0.235*	--
	100	42.250±0.622*	197.700±1.120*
CEL	25	65.750±0.816*	180.640±0.904*
	50	44.270±0.509*	104.500±0.712*
	100	32.660±0.204*	82.080±0.668*
EEL	25	36.480±0.164*	91.920±0.414*
	50	21.280±0.268*	66.510±0.735*
	100	12.330±0.235*	39.660±0.512*
MEL	25	29.660±0.144*	74.280±0.548*
	50	17.560±0.204*	52.410±0.164*
	100	9.460±0.212*	31.430±0.148*
Piperazine Citrate	25	26.330±0.208	57.030±0.381
	50	15.300±0.104	36.450±0.217
	100	7.330±0.206	18.600±0.229

PEL: pet ether extract of *F. Sepiaria* Roxb leaves; CEL: chloroform extract of *F. Sepiaria* Roxb leaves; EEL: ethyl acetate extract of *F. Sepiaria* Roxb leaves; MEL: methanolic exract of *F. Sepiaria* Roxb leaves. All values determined were mean±SEM; n=6. *P<0.05 when compared with standard.

4. Discussion

4.1. Phytochemical screening

Various bioactive components such as phenolics, tannins, flavanoids, terpenoids and steroids were prominently revealed during the preliminary phytochemical screening. Phenolics, tannins flavanoids and steroids were present in all the extracts whereas saponins were absent in all the extracts. Alkaloids and proteins were absent in petroleum ether extract. Glycosides were absent in petroleum ether and chloroform

extract.

4.2. Antioxidant assay

Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. Over production of oxidants in certain condition can cause imbalance leading to oxidative damage to large biomolecules such as lipids, DNA and proteins. Many synthetic drugs protect against oxidative damage but they have adverse side effects[21]. Data from both scientific reports and laboratory studies show that the plant contain a large variety of substance called "plant chemicals" or "phytochemicals" that possess antioxidant activity[22,23]. Studies have attributed that antioxidant properties are due to the presence of phenols and flavanoids[24]. Thus the presence of these components would have contributed to significant antioxidant activity of plant extracts. Antioxidant of phenolic compounds is based on their ability to donate hydrogen atom to free radicals[25]. The scavenging activity of a stable radical is considered a valid and easy assay to evaluate scavenging activity of natural compounds[26].

DPPH is a relatively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH. From the present result it may be postulated that *F. sepiaria* leaves reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles. In the present study, the methanolic extracts exhibited high DPPH radical scavenging activity compared to other extracts. Superoxide anion is oxygen centered radical with selective reactivity. This species is produced by a number of enzyme systems in auto-oxidation reactions and by non enzymatic electron transfers that univalently reduce molecular oxygen. It can also reduce certain iron complexes such as cytochrome[27]. The present study showed potent superoxide radical scavenging activity of *F. sepiaria* extracts. Methanol extract showed potent superoxide radical scavenging activity with IC_{50} value compared to standard ascorbic acid.

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity[28]. Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and cause damage to cell[29]. The present study shows that the extracts had significant scavenging effects on hydroxyl radical, which increased with the increase in concentration from 10-160 μg/mL. Over production of nitric oxide manifest in various pathological conditions mainly by formation of peroxynitrites[30]. The plant extract evaluated were found to decrease the quantity of nitrite ions *in vitro* which can be attributed to the antioxidant constituents present in the extracts. It was reported that the chelating agents that can form sigma bond with a metal, are most efficient as secondary antioxidants because they decrease the redox potential and thus steady the oxidized form of the metal ion[31]. The current study shows that the extract has iron binding ability and thus exhibiting its antioxidant activity.

Phenolics and secondary metabolite in plant kingdom were found in great abundance. It has been reported that the antioxidant activity of phenol is principally due to their redox potentials, hydrogen donors and singlet oxygen quenchers[32]. Flavanoids due to the presence of their phenolic hydroxyl groups are highly capable of scavenging reactive oxygen species and are known to be potent antioxidants[33]. The results of the above study further confirm that the presence of phenolic compounds in the extracts have paved the way for the significant inhibitory values of the extracts.

4.3. Anthelmintic activity

Helminthic infections of the gastrointestinal tract of human beings and animals have been acknowledged to have adverse effects on the health standards with a consequent lowering of resistance to other diseases. Nowadays resistance to the available synthetic drugs is a major problem. Therefore in recent years, a search for plant derived drugs is the primary choice of researchers, as they are believed to have less side effects and more compatible with the physiological flora[34,35]. Phytochemical analysis of the crude extract revealed the presence of tannins, phenolics, flavanoids and alkaloids which are known to exhibit anthelmintic property. Tannins and Phenolics are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation[36] and also bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite, leading to death. Based on these we can assume that tannins, phenolic compounds and flavanoids present in the leaf extract of *F. sepiaria* may be responsible for the anthelmintic activity.

The present study reveals that the leaf extract of *F. sepiaria* has significant antioxidant and anthelmintic activity. But further investigations on the isolation of active compounds present in the extracts and *in vivo* studies are necessary to identify a potential chemical entity for clinical use.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Oxidative stress is one of the main reasons for various biological disorders including cancer and associated ailments. In addition new anthelmintic drugs are in demand especially from natural sources. Hence the research topic in the current manuscript holds significance to the science community.

Research frontiers

The present work described the antioxidant potential and anthelmintic capacity of various extracts of *F. sepiaria* Roxb leaves. *In vitro* antioxidant potential of the plant was thoroughly investigated by authors by various methods.

Related reports

Authors have used highly standardized chemicals. Protocols followed by authors for determining antioxidant potential of *F. sepiaria* are highly appreciable.

Innovations and breakthroughs

Various parts of the plant *F. sepiaria* Roxb are widely used in folk medicine for snake bite, rheumatism, gout, kidney

disorders. Current investigation proven the antioxidant potential and anthelmintic capacity of the folklore medicinal plant.

Applications

Present study confirms the *in vitro* antioxidant potential and anthelmintic activity of *F. sepiaria*.

Peer review

This is an appreciable research work in which authors have established the antioxidant and anthelmintic activities of *F. sepiaria*.

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