Original Article

Phytochemical Analysis and *In vitro* Antioxidant Properties of *Murraya koenigii* (L.) Fruits

Atish N. Waghmare*, Sachin V. Tembhurne and Dinesh M. Sakarkar

Sudhakarrao Naik Institute of Pharmacy, Nagpur road, Pusad Dist. Yavatmal 445204 (M.S.) India

Address for Correspondence

Sudhakarrao Naik Institute of Pharmacy, Nagpur road, Pusad Dist. Yavatmal 445204 (M.S) India.
E-mail: atish.w31@rediffmail.com

ABSTRACT

Objective: The present study was undertaken to evaluate the phytochemical and *In vitro* antioxidant property of fruits extract of *Murraya koenigii* (FEMK). The present study also evaluated the presence and quantification of flavonoids contents in crude extract.
Methods: The *in vitro* antioxidant activity was performed using inhibition of superoxide anions; free radicals scavenging activity by DPPH, H_2O_2, NO, reducing power assay and lipid peroxidation method. The investigation was also carried out to evaluate the presence and quantification of flavonoids contents in crude extracts. HPTLC fingerprint profile of FEMK was developed which serves as reference standard for quality control of the extracts.
Results: The results of antioxidant activity of Fruit extracts of *Murraya koenigii* (FEMK) demonstrate to scavenge the free radicals in all in-vitro models indicates antioxidant property. The observed effects of extract could be due to the presence of flavonoids. The flavonoids content was found to be 0.117 %, 0.206 %, and 0.348% w/w in 5mg/ml, 10 mg/ml and 15 mg/ml concentrations of FEMK respectively.
Conclusion: The study concludes that the Fruit extract of *Murraya koenigii* shows moderate to strong antioxidant activities. Thus fruit extract of *Murraya koenigii* could be useful for the application of disorders related to free radicals.

Keywords: *Murraya koenigii*, Fruits, Antioxidant, HPTLC fingerprint.

INTRODUCTION

The plant *Murraya koenigii* (L.) belongs to family of rutaceae, which is widely distributed in almost part of the India. The leaves of this plant, called curry leaves, are commonly used for flavoring the Indian dishes. Plants from *Murraya* species are also been used as traditional medicine in southern Asia. The leaves of this plant increase digestive secretions and relieve nausea, indigestion, and vomiting[1].

Phytochemical screening of *M. koenigii* leaves shows the presence of some vitamins phenolic compounds, terpenoids, carbazole alkaloid[2] and mineral contents such as zinc, calcium, iron and vanadium etc[3]. Carbazole alkaloid of *M. koenigii* has been reported to show hypoglycemic[4,5], antioxidant[6,7] activities. The leaves of this plant also found to posses antimicrobial activity[8,9]. However very little study has been investigated on fruits of *Murraya koenigii*, except the work done on fruit juice of *Murraya koenigii*[10]. So the present work was investigated to study phytochemical analysis and antioxidant property of FEMK by *in vitro* methods.

MATERIALS AND METHODS

Collection of plant material

The fruits of *Murraya koenigii* were collected from the region of Yavatmal district, Maharashtra, India during the month of June to September 2012. The fruits were authenticated by Dr. N.M. Dongarwar, Head of the Department; Botany Department, RTM Nagpur University, Nagpur. A voucher specimen of herbarium (No. 9916) was deposited at, Botany department of RTM Nagpur University Nagpur, India.

Extraction and isolation

One (1) kg of *Murraya koenigii* fruits were dried, powdered and defatted with petroleum ether by solvent extraction method. The defatted crude extract was further extracted with ethanol by Soxhlet extraction. The extract was freed from solvent under reduced pressure to give a black brown, highly viscous syrup. The percent yield was observed around 4.2%. The extract was stored in sterile bottle and kept under refrigerated condition for further analysis.

Chemicals

Sodium nitroprusside, 2, 2-diphenyl-1-picryl hydrazyl (DPPH), Thiobarbituric acid, Hydrogen peroxide, Potassium ferrocynate were used of analytical grade.

Phytochemical analysis

The extract was subjected to various phytochemical tests to determine the nature of constituents of the extract and HPTLC fingerprinting[11].

Flavonoids content determination

Stock solution of extract was diluted with methanol to give 5mg/ml, 10mg/ml and 15mg/ml of test solutions. To the 0.5 ml of test solution, 1.5 ml of methanol, 0.1 ml of 1M potassium acetate, 0.1ml of 10% Aluminium chloride, and 2.8 ml of distilled water was added. Within 30min the absorbance of the test solution was checked at 415 nm against blank prepared in same manner except test solution. The flavonoids content present in test solution was determined by extrapolating absorbance from quercetin standard curve (10ppm-100ppm)[12].

HPTLC fingerprint profiles

Stock solution (1mg/ml) of each extracts was prepared in methanol. 2µg/ml, 5µg/ml, 10µg/ml and 15µg/ml of sample extracts were spotted on pre-coated Silica gel G60 F254 TLC plates using CAMAG Linomat V automatic sample spotter and the plates were developed in solvent systems to resolve polar and non-polar components of the fractions. The plates were scanned using TLC Scanner 3 (CAMAG) at 254nm.

Antioxidant assay

DPPH free radical scavenging activity

Free radical scavenging activity of different concentration in range of 100 µg to 5 mg/ml of ethanolic extract of FEMK were carried out by DPPH (2, 2-diphenyl-1-picryl

hydrazyl) assays. To 1 ml of different concentrations of extracts (0.1-5 mg/ml), 0.3 ml of 100 uM DPPH was added and final volume was made to 3.0 ml with distilled ethanol and incubated for 20 minutes at room temperature. The absorbance was measured at 517 nm. The assay was carried out in triplicate and the final absorbances were noted for calculating % of free radicals scavenging activity[13,14].

Nitric oxide scavenging activity

The procedure involves the principle that, nitric oxide (NO) generated from sodium nitroprusside (SNP) is responsible to generate nitrite ions that can be found using Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylenediamine dichloride and 3% phosphoric acid). SNP in aqueous solution at physiological pH generates NO, which reacts with oxygen to produce nitrite ions that can be determined by the use of Griess Reagent. Scavengers of NO compete with oxygen which leads to reduced production of NO. SNP (10 mM) in phosphate buffer saline was mixed with different concentration of fruit extract (0.1-5 mg/ml) and incubated at 25°C for 180 minutes. The above samples were reacted with Griess reagent. The absorbance of the chromophores produced during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dichloride was measured at 546 nm and compared to the absorbance of ascorbic acid, used as a positive control treated in the same manner with Griess reagent[15].

In vitro lipid peroxidation

It was estimated by quantifying thiobarbituric acid reactive substances (TBARS) which are present in homogenates of liver as an index of end point oxidative damage. The present study used ferrous sulphate and hydrogen peroxide to bring about the lipid peroxidation in tissue homogenate. The experiment was performed in liver homogenate of mice. The 0.5 gm of liver was homogenate in 1.15% ice cold KCl solution and centrifuge. The supernatant was used as source of polyunsaturated fatty acid (PUFA) for calculation of extent of thiobarbituric acid reactive substances. Supernatant from different concentration of each fruit extract (0.1-5 mg/ml) were incubated with hydrogen peroxide (100uM) and ferrous sulphate (20uM) at 37^0c for 60 min. After incubation the content of TBARS as an index of lipid peroxidation was measured by UV spectrophotometer at 530 nm[16].

Reducing power assay

The reducing power of the fruit extract was estimated according to the method prescribed[17]. The mixture containing 2.5ml of 0.2M phosphate buffer (pH 6.8) and 2.5 ml of K_3FeCN_6 (1%w/v) was added to 1.0 ml of each fruit extract (0.1-5 mg/ml). The mixture was incubated at 50^0 C for 20 min, to it 2.5 ml (10%w/v) of trichloroacetic acid was added. The mixture was centrifuged at 3000 rpm for 10 min to obtain the supernatant solution (2.5ml), mixed with 0.5ml of $FeCl_3$ (0.1%w/v) and distilled water (2.5 ml). The absorbance was then read at 700 nm against blank sample[18].

Hydroxyl radical scavenging method

Solution of hydrogen peroxide (40mM) was prepared in phosphate buffer pH 7.4 and its concentration was determined by using spectrophotometer at 230 nm. Hydrogen peroxide solution (0.6 ml) was added to fruit extract of *Murraya koenigii* (0.1-5 mg/ml). After 10 minutes the absorbance of hydrogen peroxide was determined at 230 nm against the blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by fruit

extract and the standard compound vitamin C were calculated[19].

Statistics

The data are expressed as mean ± S.D. Inhibition percentages are calculated from the differences between test and control. One-way analysis of variance (ANOVA) followed by Student's T test for multiple comparisons of unpaired data were used for statistical evaluation.

RESULTS

Preliminary phytochemical analysis

Preliminary phytochemical studies of fruit extract of *Murraya koenigii* indicated the presence of alkaloids, flavonoids and phenolic substances. (Table 1).

HPTLC fingerprint profile

HPTLC fingerprint profile of extract of *Murraya koenigii* was recorded. (Table 2) and Fig. 1A-1F.

Total flavonoids content

The total amount of flavonoids content was calculated from equation of standard curve of Quercetin in each fruit extract of *Murraya koenigii*. The flavonoids content was found to be 0.158, 0.226, and 0.328% w/w, respectively in 0.5, 1.0 and 1.5% of extract.

Antioxidant activity

Inhibition of nitric oxide radical

Concentration of fruit extract required for 50% inhibition was found to be 2.90 mg/ml for fruit extract. Vitamin C was used a reference standard and showed 70.62% inhibition at 100 µg/ml which was comparatively higher than fruit extract of FEMK (63.85) at highest concentration (5 mg/ml) used (Fig. 2A).

Scavenging of DPPH radical

The different concentration of fruit extract showed a concentration dependent radical scavenging activity with EC50 value of 2.69 mg/ml for fruit extract (Fig 2B). The free radical scavenging activities for maximum concentration (5 mg/ml) fruit extract is less (66.36) compared to standard vitamin C (88.41 % at 100 µg/ml).

Inhibition of TBARS activity

Fruit extract of *Murraya koenigii* showed a concentration dependent inhibition of TBARS with EC50 value of 3.12 mg/ml (Fig 2C). The TBARS inhibition for maximum concentration (5 mg/ml) fruit extract is less (61.13%) compared to standard vitamin C (78.00% at 100 ug/ml).

Reducing power assay

Various concentrations of fruit extract were found to have significant reducing power. All the concentration of fruit extract exhibited a concentration dependent increase in reducing power. Compared with ascorbic acid the reducing powers of the fruit extract was found to be less (Fig. 2D). The EC50 for the reducing power of fruit extract was found to be 2.75 mg/ml respectively while the reducing power of vitamin C at 100 µg/ml was found to be 67.57% which was comparatively more than highest concentration (5 mg/ml) of FEMK extract used (60%).

Inhibition of hydroxyl radical

Effect of different concentration of fruit extracts for the inhibition of hydroxyl radical induced by H_2O_2 is shown in Fig. 2E. The extract shows concentration dependant inhibition with EC50 at 3.30 mg/ml, respectively. Inhibition for maximum concentration (5 mg/ml) fruit extract is less (59.39) compared to standard vitamin C (75.04 % at 100 ug/ml).

DISCUSSION

Free radicals are highly reactive molecules or chemical species containing unpaired electrons that cause oxidative stress[20]. The phytochemical analysis of *Murraya koenigii* fruit extract revealed the presence of alkaloids, flavonoids and phenolic contents. Anion radicals present in flavonoids serve as health promoting compound[21].

The presence of the phenolic compounds in this plant contributed to their antioxidative activity and thus the utility of these plants in herbal medicament. DPPH scavenging assay indicates that the fruit extracts was potently active. The ability to inhibit the formation of ABTS+ due to the reflection of the ability of this plant extract to scavenge DPPH; this means that the extract may be used to treat radical related pathological damage especially at higher concentration[22]. One of the strongest reactive oxygen species among the free radicals is Superoxide anion radical[23]. The plant is also a potent scavenger of superoxide radical is due to scavenging activity of this radical by the fruit extract which is compared favorably with the standard reagents such as ascorbic acid. Thus hydrogen peroxide is an important reactive oxygen species because of its ability to penetrate biological membranes.

The phenolic contents of fruit extracts which donate electron to H_2O_2, thus reducing it to water also scavenges H_2O_2. Sodium Nirtoprusside (SNP) in aqueous solution at physiological pH generates Nitric oxide (NO) which is a reactive free radical and interacts with oxygen to produce nitrite ions which can be determined by the use of Griess Reagent. The level of nitric oxide was significantly ($p< 0.001$) reduced in this study by the crude extract.

From the results it was observed that the fruit extracts of *Murraya koenigii* were found to act as radical scavengers against different free radicals under the various conditions of oxidative stress. The reducing power determined in the present study depends on the redox potentials of the compounds present in fruit extract of *Murraya koenigii*. The highest reducing power ($p<0.001$) was observed at 5 mg/ml of fruit extract. Thus, it can be expected that the extract of *Murraya koenigii* may have scavenging activity against other oxidizing agents. Fruit extract of *Murraya koenigii* showed a concentration dependent radical scavenging activity by reducing DPPH radical as indicated by different significant level such as 1mg/ml ($p<0.01$), 3 mg/ml ($p<0.001$).

Fruit extract of *Murraya koenigii*, scavenges the NO formed from the sodium nitroprusside by inhibiting the chromophores formation and hence the absorbance decreases as the concentration of the fractions increases. In present study the HPTLC fingerprinting profile of fruit extract of *Murraya koenigii* was generated in solvent system in order to ascertain the total number of chemical moieties which will also help in designing the method of isolation and characterization of the bioactive components.

CONCLUSION

From the results it indicates that *Murraya koenigii* fruit extract has strong antioxidant potential when compared with the results of the standard compounds such as Ascorbic acid. The antioxidant potential of plan extract could be due to the presence of flavonoids. *In vivo* potential of this plant needs to clarify the management of human diseases resulting from oxidative stress. Thus fruit extract of *Murraya koenigii* could be useful for the application of disorders related to free radicals.

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Conflict of interest statement

Authors declare that the present work is done by the authors and there is no any financial support from any agency and there are no conflicts of interest.

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Table 1. Phytochemical evaluation of fruit extracts of *Murraya koenigii*

S. No.	Compound	Test	Inference
1	Flavonoids	Shinoda test	+
2	Alkaloid	Lead acetate	+
		Wagner	-
3	Phenolic content	Dragendroffs	+
		Ferric chloride test	+
		Libermann's test	+

+ indicates the positive test; - indicates negative test.

Table 2. HPTLC fingerprinting profile of *Murraya koenigii* fruit extract

S. No.	Screening for Alkoloid (compared with Brucine as a standard)								Screening for Flavonoid (compared with Quercetin as a standard)							
	Fruit Extract 5 µg/ml		Fruit Extract (10 µg/ml)		Fruit Extract (15 µg/ml)		Brucine 5 µg/ml		Fruit Extract (5 µg/ml)		Fruit Extract (10 µg/ml)		Fruit Extract (15 µg/ml)		Quercetin 5 µg/ml	
	Rf values	% area	Rf Values	% area	Rf values	% area	Rf values	% area	Rf values	% area	Rf Values	% area	Rf values	% area	Rf values	% area
1	0.13	2.70	0.13	1.98	0.13	3.26	0.25	93.20	0.07	1.00	0.11	1.44	0.11	1.07	0.79	99.40
2	0.24	6.51	0.24	7.19	0.24	8.28			0.16	8.18	0.16	6.54	0.17	6.95		
3	0.28	2.88	0.28	3.30	0.28	4.35			0.21	6.24	0.21	6.38	0.21	6.38		
4	0.35	9.27	0.35	11.0	0.35	12.5			0.27	11.2	0.28	11.53	0.28	11.83		
5	0.40	9.19	0.40	10.3	0.40	11.8			0.36	7.79	0.37	10.6	0.38	10.77		
6	0.47	2.77	0.47	3.79	0.47	4.66			0.67	38.8	0.67	39.0	0.68	39.89		
7	0.55	31.69	0.55	31.0	0.55	29.2			0.73	21.1	0.74	21.1	0.74	21.21		
8	0.64	34.99	0.64	31.3	0.64	25.8			0.78	2.85	0.78	3.17	0.78	1.90		

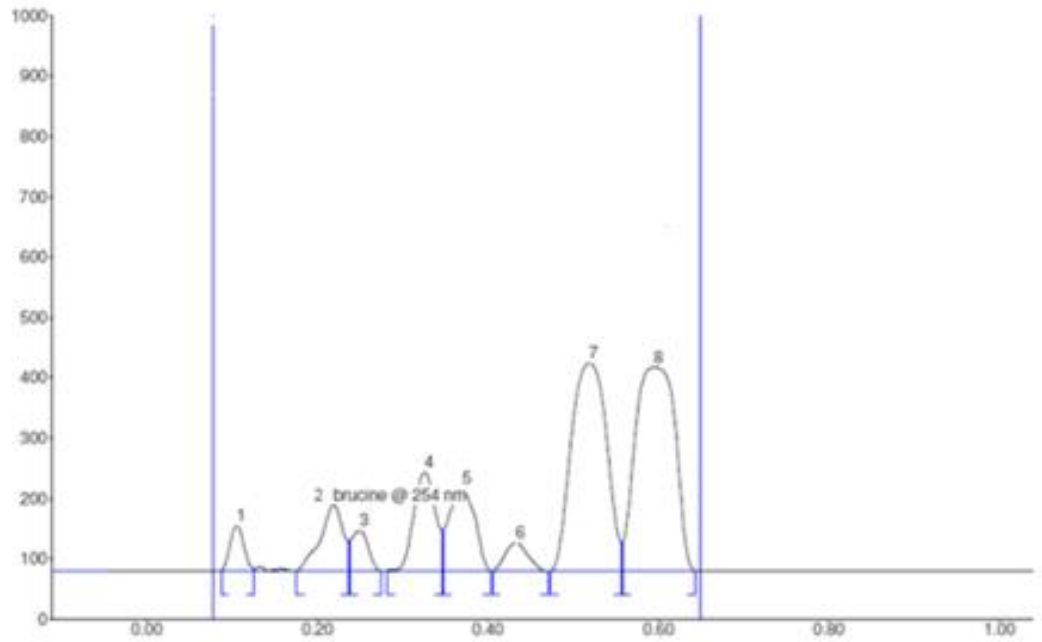

Figure 1A. HPTLC profile of fruit extract 5 µg/ml compared with brucine

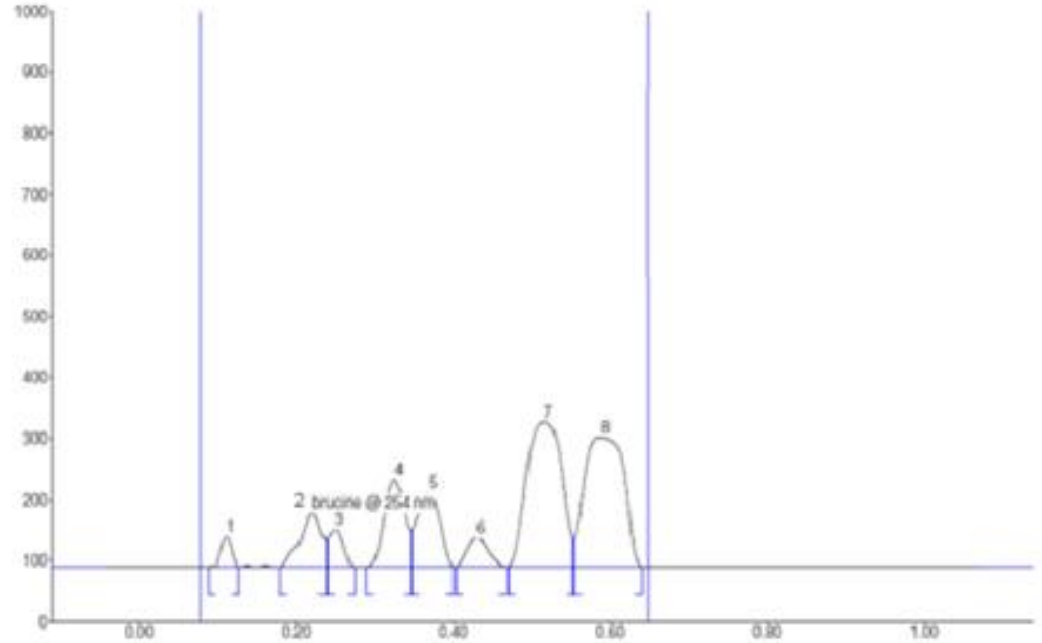

Figure 1B. HPTLC profile of fruit extract 10 µg/ml compared with brucine

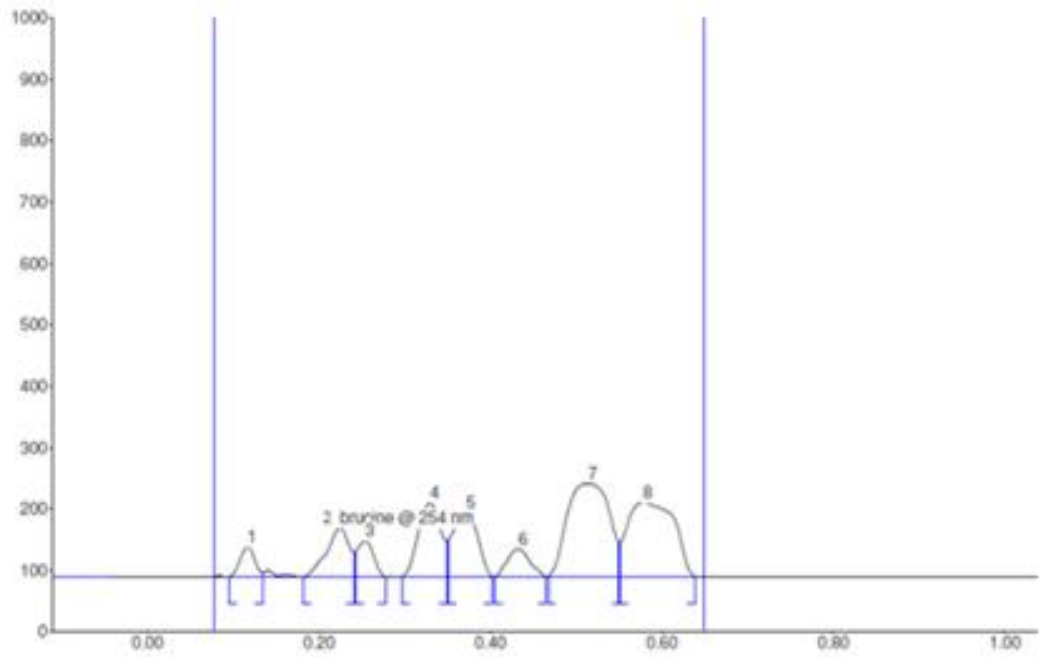

Figure 1C. HPTLC profile of fruit extract 15 µg/ml compared with brucine

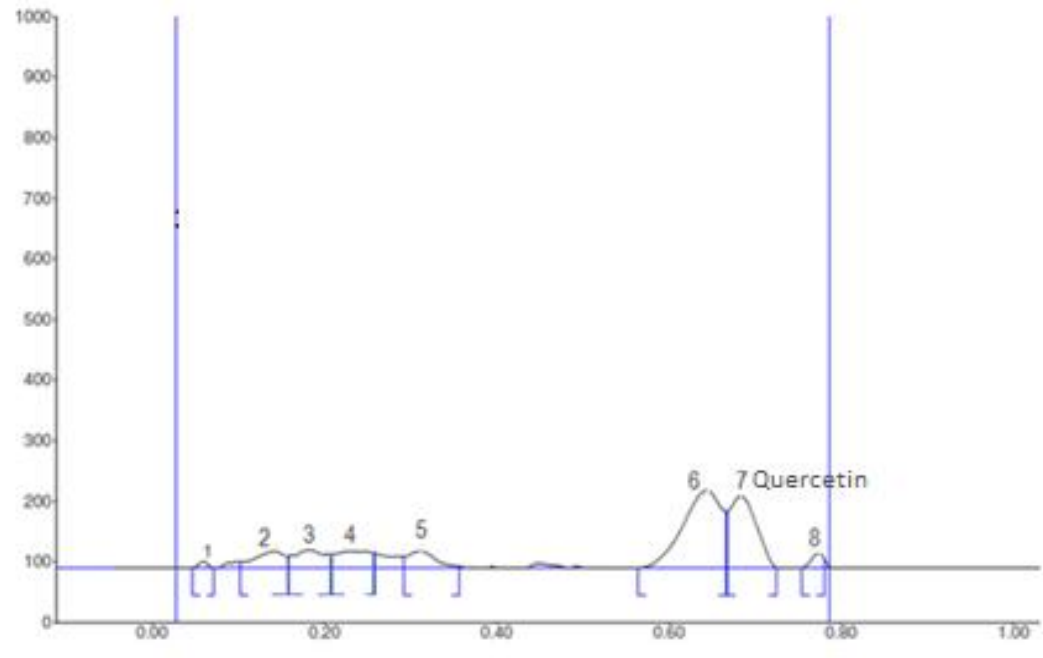

Figure 1D. HPTLC profile of fruit extract 5 µg/ml compared with quercetin

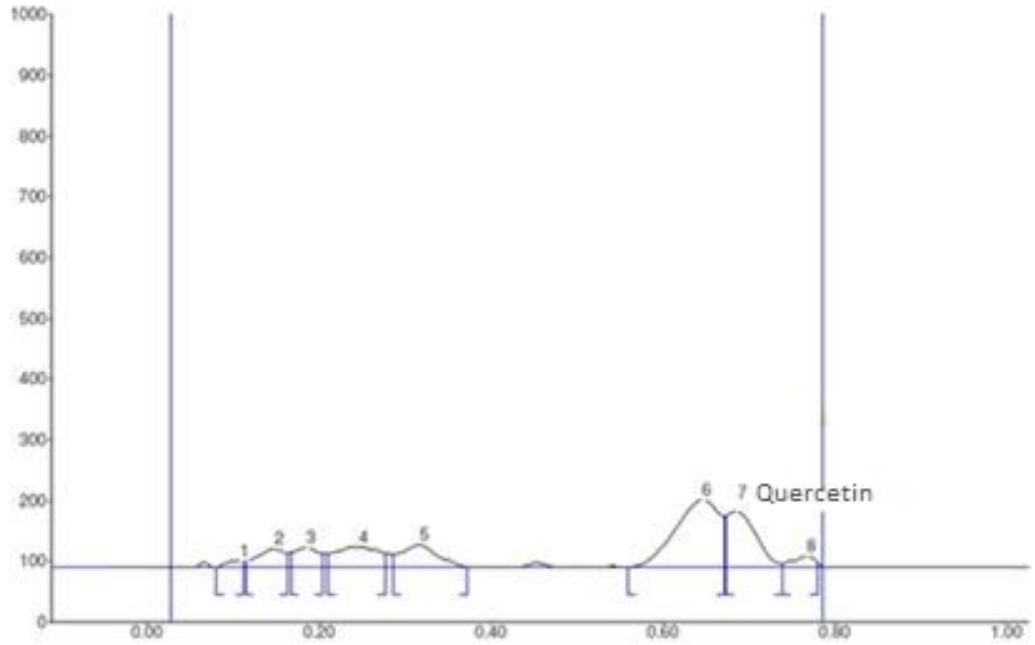

Figure 1E. HPTLC profile of fruit extract 10 µg/ml compared with quercetin

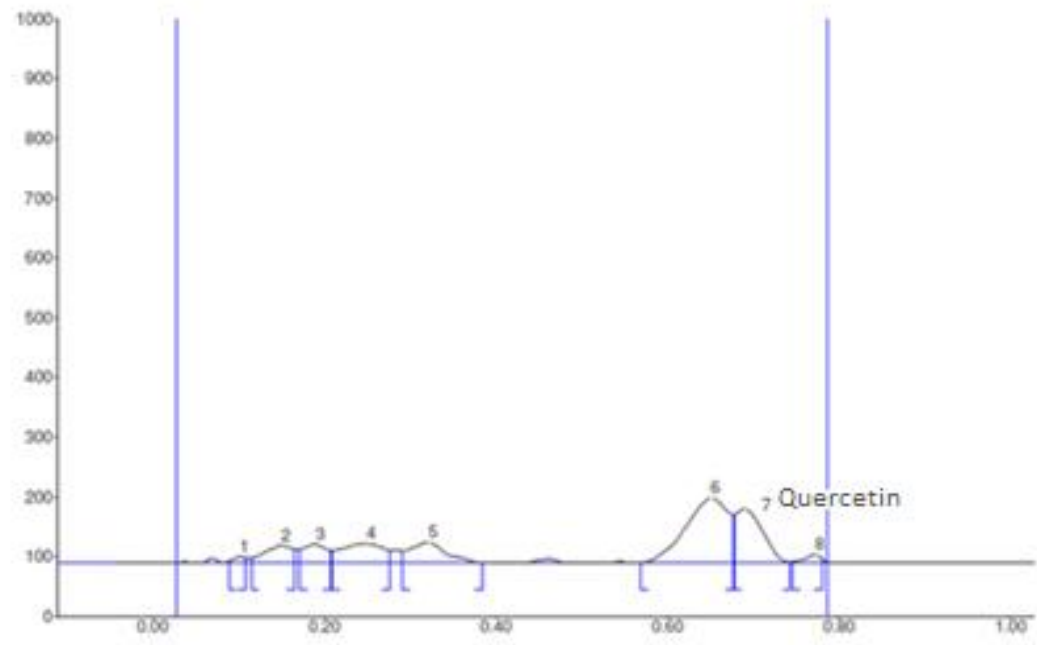

Figure 1F. HPTLC profile of fruit extract 15 µg/ml compared with quercetin

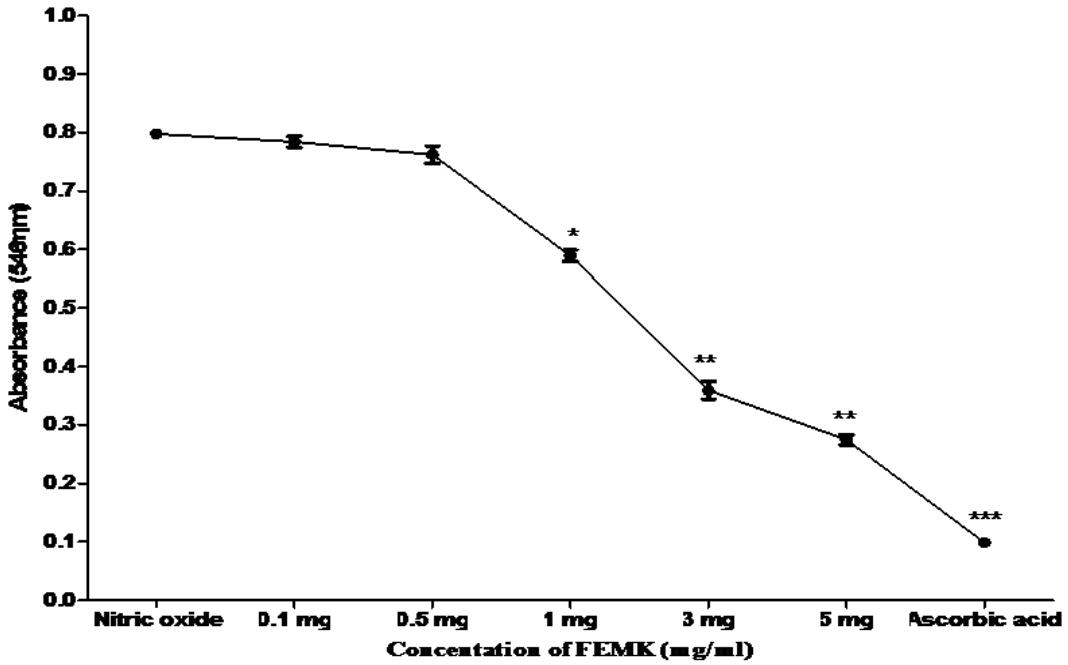

Figure 2A. Inhibition of nitric oxide

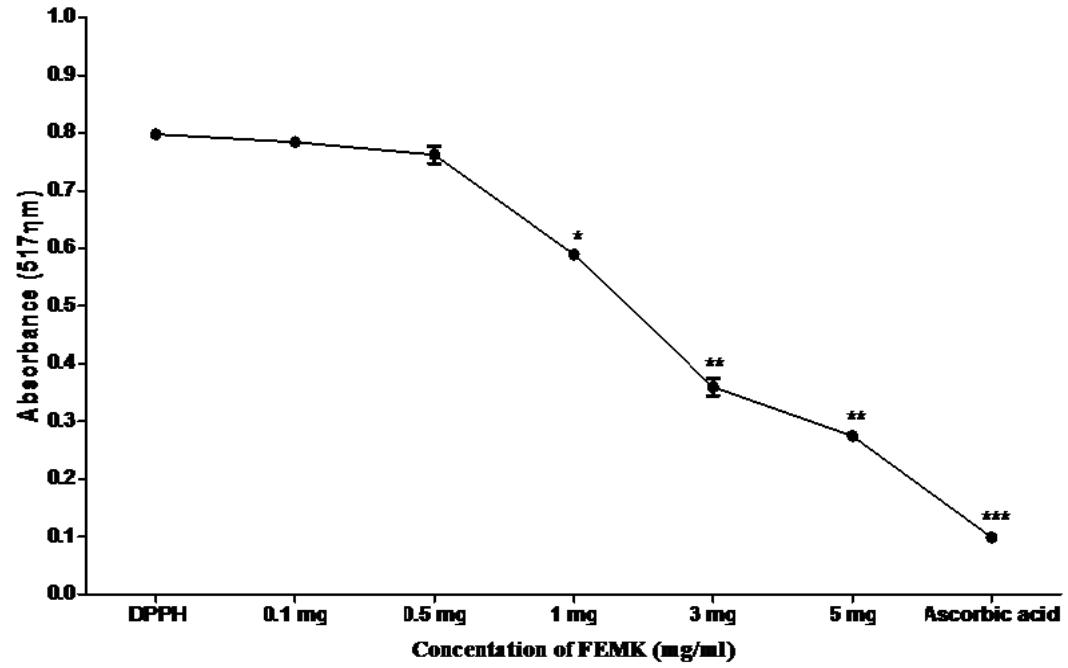

Figure 2B. Scavenging of DPPH radical

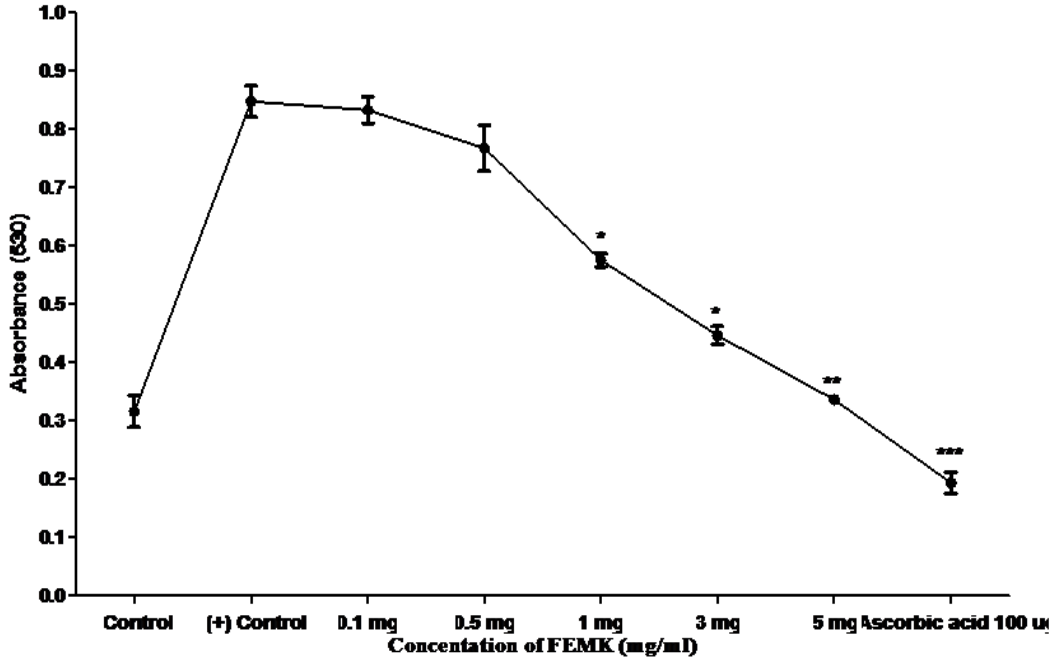

Figure 2C. Inhibition of TBARS in lipid peroxidation

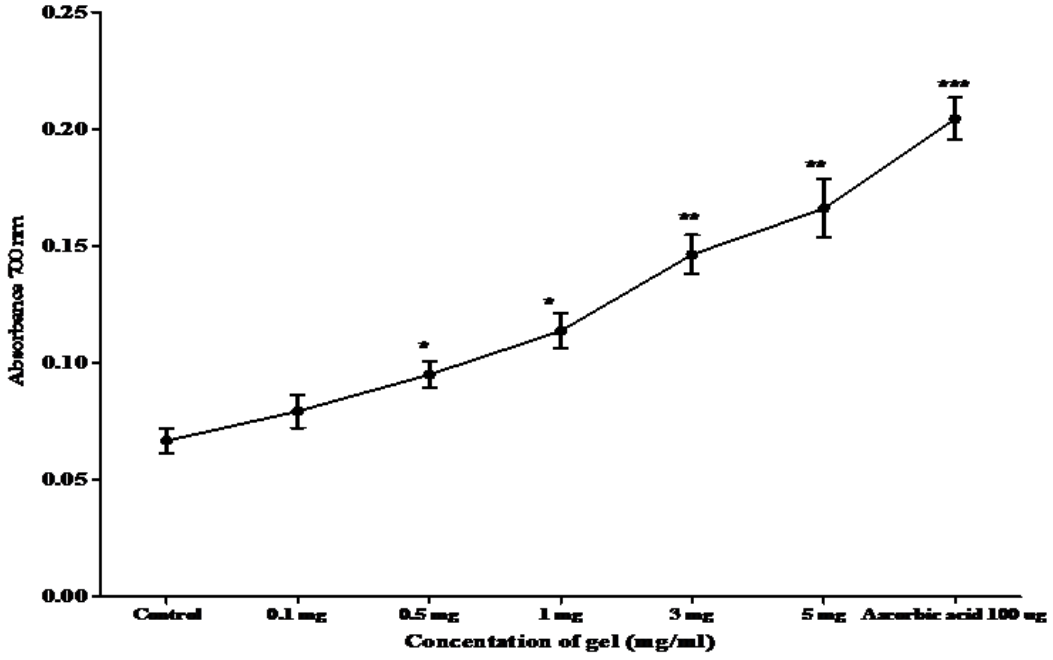

Figure 2D. Reducing power

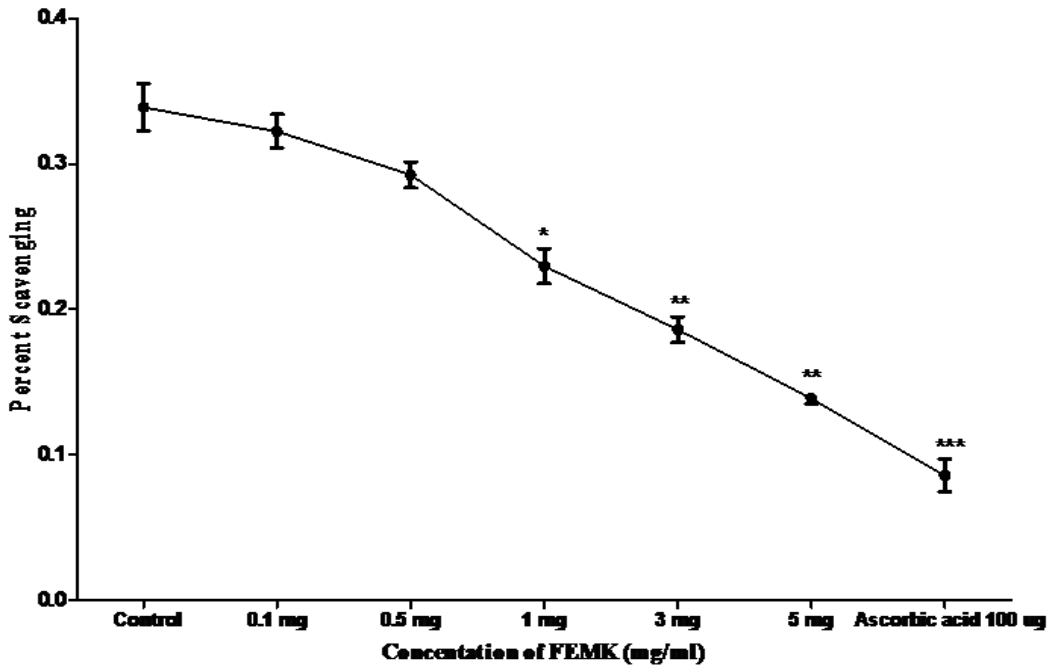

Figure 2E. Inhibition of hydroxyl radical

Figure 2. Free radical scavenging activity of fruit extract of *Murraya koenigii* fruit, [The values are mean of three readings, ± SD].

Figure 2. *In vitro* antioxidant assays: All the values are mean of three reading ± S.D. **(A)** – Inhibition of nitric oxide:- *- statistically significant compared with NO (p< 0.01); **- indicates statistically significant compared with NO (p<0.001); ***- indicates statistically significant compared with NO (p< 0.0001). **(B)** DPPH radical scavenging assay:- *- statistically significant compared with DPPH (p< 0.01); **- indicates statistically significant compared with DPPH (p<0.001); ***- indicates statistically significant compared with DPPH (p< 0.0001). **(C)** TBARS ±assay:- *- statistically significant compared with + control (p< 0.01); **- indicates statistically significant compared with + control (p<0.001); ***- indicates statistically significant compared with + control (p< 0.0001). **(D)** Reducing power assay:- *- statistically significant compared with control (p< 0.01); **- indicates statistically significant compared with control (p<0.001); ***- indicates statistically significant compared with control (p< 0.0001). **(E)** Hydroxyl radical scavenging assay:- *- statistically significant compared with control (p< 0.01); **- indicates statistically significant compared with control (p<0.001); ***- indicates statistically significant compared with control (p< 0.0001). ANNOVA followed by student's T test.