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**Investigation on Phytochemical and Antioxidant Activity of the Plant *Murraya Koenigii* Linn
(Curry leaf) in Rajshahi, Bangladesh**

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Abstract

The use of herbal medicine is becoming popular day by day due to toxicity and side effects of allopathic medicines. Considering the medicinal importance of the plant *Murraya koenigii* Linn (Curry leaf), the antioxidant activity (AC), total phenolic content (TPC), total flavonoid content (TFC), of different fractions of methanolic extract (DIA-ion resin adsorbed fraction, chloroform, Ethyl acetate and petroleum ether) of *M. koenigii* were investigated. Among the fractions, DIA-ion resin-adsorbed fraction showed the highest total antioxidant activity with absorbance 2.320 ± 0.06 and petroleum ether fraction showed the lowest total antioxidant activity with absorbance 1.944 at 100 mg/ml concentration. The TPC were found range between 13.285 to 17.52 mg GAE/g while the highest amount of TFC recorded among the extracts was 16.65 mg CatE/g. DPPH free radical scavenging activity of different extracts of leaves was also measured where DIA-ion resin-adsorbed fraction had the highest free radical scavenging activity with IC_{50} value 15.53 μ g/ml. In the present study phenolic compound were found to be the predominant components in the leaves of *M. koenigii* indicating that they are potent antioxidant.

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Introduction

Plants which, form the backbone of traditional medicines, have been used for thousands of years as a source of invaluable bioactive compounds¹. In most Asian countries, herbal products play an important role to treat wounds, burns, intestinal problems, coughs, and general torpor². The use of plants as traditional remedies, and to treat burns and wounds is an important aspect of health management and is also an efficient way to promote cheaper health care options^{3,4}. Many researchers have reported *in vitro* and *in vivo* evidence to support various plant materials as topical anti-microbial agents to enhance wound healing⁵. Several indigenous plants and formulations for managing cuts, bruises, burns and wounds have been described in folkloric as well as the Ayurvedic system of medicine^{6,7}. In Bangladesh 5,000 species of angiosperm are reported to occur⁸. The numbers of medicinal plants included in the 'materiamedica' of traditional medicine in this subcontinent now stand at about 2000. More than 500 of such medicinal plants have so far been enlisted as growing in Bangladesh⁹. Dhaka, Rajshahi, Shylet and Chittagong division is rich in medicinal plants⁸. Bangladesh is situated at the complex interface of the Indian, Himalayan and Southeast Asian biographic regions, and historically it is well-endowed with very diverse complements of terrestrial and aquatic flora and fauna that include a considerable number of medicinal plant resources¹⁰.

Murraya koenigii, commonly known as curry leaf or Kari Patta in Indian dialects, belongs to the family Rutaceae, which represents more than 150 genera and 1600 species. *Murraya* comprises of about 11 species of shrubs, and small trees. Its distribution ranges

encompass the tropical and subtropical regions of Sri Lanka, India, South China to South-East Asia, Malaysia, New Guinea, North-East Australia, and New Caledonia¹¹. Two of such plants namely *M. Koenigii* (L.) Spreng & *M. Paniculata* (L.) Jack, were reported from India. The latter commonly called 'orange jasmine' is grown mainly as an ornamental in Jammu and Kashmir. However, *M. Koenigii* is a semi-deciduous aromatic shrub or tree-let with culinary and medicinal properties¹². The leaves are aromatic which contain proteins, carbohydrates, fiber, minerals, carotene, nicotinic acid, and vitamin C, and are also rich in vitamin A and calcium. They also contain high amount of oxalic acid, crystalline glycosides, carbazole alkaloids, koenigin and resin. Fresh leaves contain 2.5% yellow color, volatile oil¹³. Fresh leaves, dried leaf powder and essential oil are widely used for flavorings soups, curries, fish and meat dishes, eggs dishes, traditional curry powder blends, seasoning and other food preparations have a versatile role to play in traditional medicine and also in soap & cosmetic aromatherapy industry¹⁴. Recent studies found some compound include the alkaloids, mahanimbine¹⁵, girinimbine¹⁶, numerous carbazole alkaloids¹⁷, and cinnamaldehyde¹⁸ in curry tree leaves, stems, and seeds. It was also reported that the fresh leaves of *M. koenigii* possesses anti-microbial, mosquitocidal, topoisomerase inhibition, and antioxidant properties¹⁹. However, the aims of this study is to determine total phenols, flavonoids and the antioxidant potential of *M. koenigii* leaves extracts with different solvents.

Materials and Methods

Plant Material and Sample Preparation

M. koenigii (plant) leaves were collected from the adjacent areas of BCSIR Laboratories Rajshahi, and

Botanical Garden, University of Rajshahi. The collected materials were washed thoroughly in water, chopped, air dried for a week at 35-40°C and pulverized in electric grinder. Dried ground leaves of *Murraya koenigii* were exhaustively extracted with methanol (MeOH, Analytical Grade, BDH Laboratory Supplies) in soxhlet apparatus. The resulting juicy extract was filtered through Whatman paper No.1 and concentrated under reduced pressure at 45°C using rotary evaporator to obtain a crude residue (23.5%). Then water triturate part was collected from crude extract. The water triturate fraction was passed through a previously well packed dia-ion resin column which has selectivity to collect only the phenolic group containing compounds. Then the materials, which were bound in resin column, were collected by passing methanol solvent. Then Petroleum ether, Ethyl acetate and Chloroform solvents were passed through the residue respectively. Finally petroleum ether, Ethyl acetate and Chloroform triturate were collected. The extracts thus obtained were kept in refrigerator until further analysis.

Chemicals and Reagents

All chemicals used were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, catechin, BHT, gallic acid, anhydrous sodium carbonate (Na_2CO_3), aluminum trichloride, potassium acetate, sodium acetate, ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), Folin-Ciocalteu reagent were purchased from Sigma-Aldrich. All chemicals and reagents were used without further purification.

Determination of Total Phenolic Content

Total phenolic content of different extractives of *Murraya koenigii* were determined by adopting the method²⁰ involving Folin-Ciocalteu reagent as oxidizing

agent and gallic acid as standard. An aliquot from each of the plant extract (0.5 ml) and standard gallic acid solution (100, 200, 300, 400, and 500 $\mu\text{g/ml}$) was added with 2.5 ml of Folin-Ciocalteu (Diluted 10 times with water) reagent and 2.5 ml of Sodium carbonate (7.5%) solution. The test tube was incubated for 20 minutes at 25°C to complete the reaction. A typical blank solution containing all reagents except plant extract or standard was also prepared. Then the absorbance of the solution was measured at 760 nm using a spectrophotometer against blank. Total phenolic contents of extracts were expressed as mg gallic acid equivalent (GAE)/g dried extract. All samples were analyzed in triplicate.

Determination of Total Flavonoids Content

Total flavonoid content was determined using the aluminum colorimetric method^{21,22} with some modifications using catechin as the standard. A calibration curve of catechin was prepared in the range of 0–200 $\mu\text{g/ml}$. Briefly, extract (0.5 mL) and standard (0.5 mL) were placed in different test tubes and to each 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 80% methanol (1.5 mL) and distilled water (2.8 mL) were added and mixed. A blank was prepared in the same manner where 0.5 mL of distilled water was used instead of the sample or standard, and the amount of aluminum chloride was also replaced by distilled water. All tubes were incubated at room temperature for 30 min. The absorbance was taken at 415 nm. The concentration of flavonoid was expressed as mg catechin equivalent (CatE) per gram of extract.

Determination of Reducing Power Capacity

Solutions of various concentrations (5, 10, 20, 40 and 80 $\mu\text{g/ml}$) were prepared in corresponding solvents with each type of extractive of *M. koenigii*. This mixture

was kept at 50°C in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). Control was prepared in similar manner excluding samples. The absorbance was measured at 700 nm. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicated the increase in reducing power.

Determination of Total Antioxidant Activity

Total antioxidant activity of different extracts of *M. koenigii* was determined with some modifications²³. Solutions of various concentrations (5, 10, 20, 40 and 80 µg/ml) were prepared in corresponding solvents with each type of extractive of *M. koenigii*, and standard substance (Ascorbic acid) and mixed with 3 ml of reaction mixture containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate. The mixtures were incubated at 95°C for 10 minutes to complete the reaction. Then the absorbance was measured at 695 nm.

Determination of DPPH Radical Scavenging Activity

The antioxidant activity of *M. koenigii* extracts were measured in comparison to standard antioxidant ascorbic acid (BDH, England) depending on the scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The whole procedure was performed according to procedure described by Bracae *et al.* (2001)²⁴. Briefly, different concentrations (10, 20, 40, 80, and 100 µg/ml in methanol) of ascorbic acid solution (1 ml) as well as *M. koenigii* extracts solution (1 ml) were mixed with 3 ml of 0.4 mM DPPH solution.

The mixtures were kept in dark for 30 minutes to measure the absorbance at 517 nm using GENESYS™ 20 Spectrophotometer (Thermo Spectronic, USA). The scavenging activity against DPPH was calculated using the equation:

$$\text{Scavenging activity (\%)} = [(A - B) / A] \times 100$$

Where A is absorbance of control (DPPH solution without the sample), B is the absorbance of DPPH solution in the presence of the sample (extract/ ascorbic acid).

Results and Discussion

Total Phenolic Content (TPC)

Phenolic compound are able to absorb free radicals and can chelate metal ions that could catalyze formation of ROS which promotes lipid peroxidation. Phenolic compounds have redox properties, acting as free radical scavenging or primary antioxidants; therefore, it is justifiable to determine phenolic content in plant extract. Total phenolic content of different fractions of *M. koenigii* are shown in Figure (1). Among the fractions, the highest phenolic content was found in Dia-ion resin adsorbed fraction (17.52 mg GAE/gm of extract), followed by Ethyl acetate fraction (14.27 mg gallic acid/gm of extract), Chloroform fraction (13.285 mg gallic acid/gm of extract) and petroleum ether fraction (13.25 mg gallic acid/gm of extract).

Total Flavonoid Content (TFC)

Plant flavonoids are the secondary metabolites with both in vitro and in vivo antioxidant activity and they help human body to defend against oxidative stress related diseases²⁵. Total flavonoid content of different fractions of *M. koenigii* are shown in Figure (2). Among the fractions, the highest total flavonoid content was found in Dia-ion resin adsorbed fraction (16.65 mg CatE/

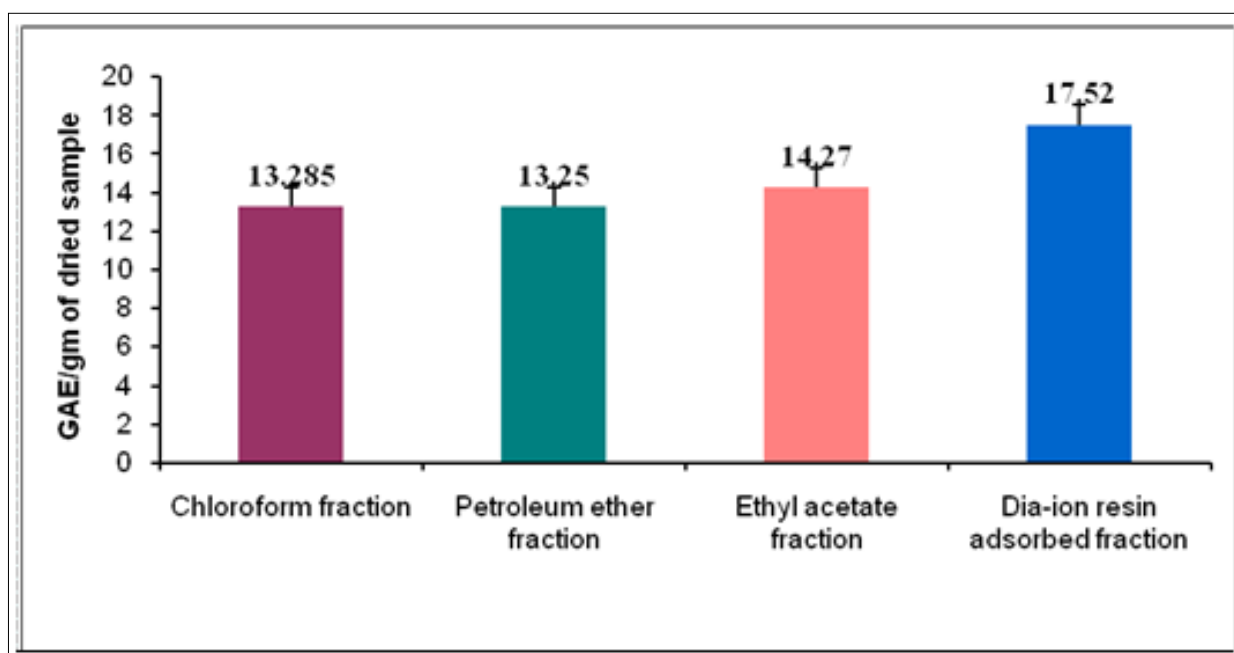


Figure 1. Total phenolic content (mg GAE/g extract) of different extract of *M. koenigii*.

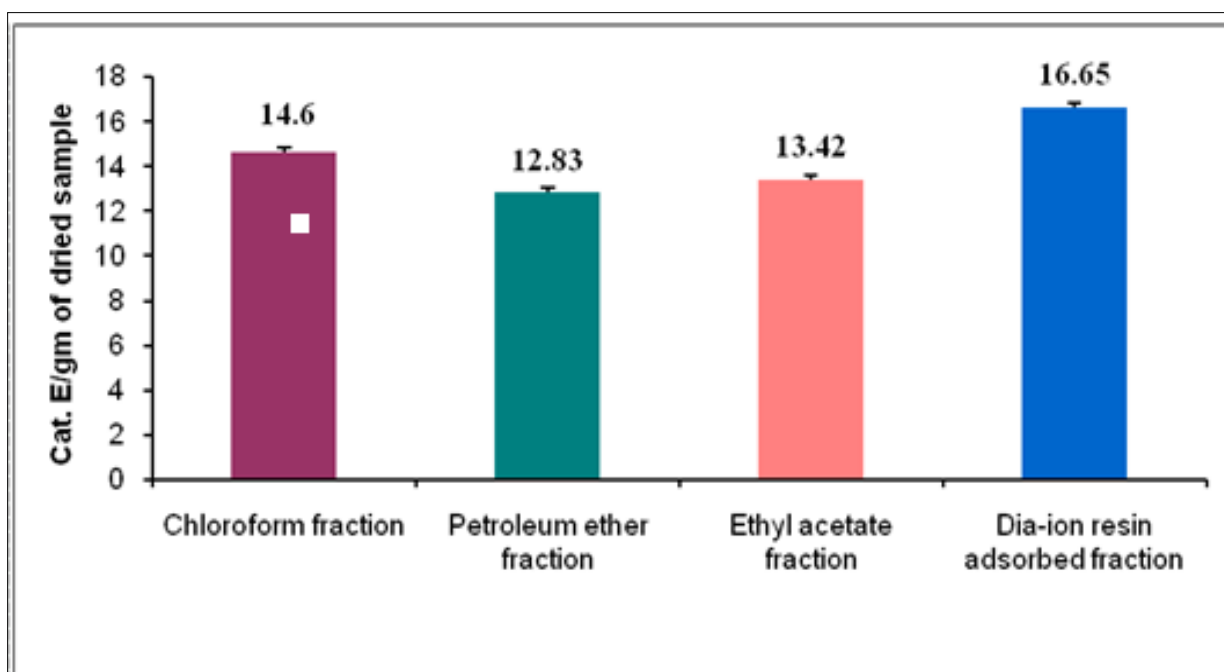


Figure 2. Total flavonoid content (mg CatE/g extract) of different fractions of *M.koenigii*.

g of extract), followed by Chloroform fraction (14.60 mg CatE/g of extract), Ethyl acetate fraction (13.42 mg CatE/g of extract) and petroleum ether fraction (12.83 mg CatE/g of extract). Therefore, it can be said that polyphenolic, and flavonoid may work together with other phytochemicals present in *M. koenigii* and make it medicinally important.

Reducing Power Capacity Content

The reducing potential of plant is mostly associated with the presence of reductones, which exert their mechanism of action by breaking the free radical chain by donating a hydrogen atom. The iron reducing capacity of the four different fractions of *Murraya koenigii* extract such as petroleum ether fraction (PE), chloroform fraction (CE), ethyl acetate (EE) and Dia-ion resin- adsorbed fraction (DAF) have been investigated. Among the four different extracts Dia-ion resin-adsorbed fraction showed the highest iron reducing capacity with absorbance of 2.504 at 80 mg/ml concentration, followed by Chloroform fraction with absorbance 2.263 ± 0.003 at 80 mg/ml while Ethyl acetate fraction showed iron reducing capacity with absorbance of 1.675 at 80 mg/ml and Petroleum ether fraction showed the iron reducing capacity with absorbance 1.421 at 100 mg/ml Figure (3). Different extracts, and standard (vitamin C, AA) exhibited the reducing power activity as follows: AA>DAF > CE> EE > PE.

Total Antioxidant Activity

The antioxidant ability and radical scavenging properties of plants are associated with its medicinal values. Total antioxidant activity of different fractions of methanolic extract of *Murraya koenigii* leaves such as Dia-ion resin adsorbed fraction, chloroform fraction, Ethyl acetate fraction and petroleum ether fraction were

investigated. Among the fractions, Dia-ion resin adsorbed fraction showed the highest total antioxidant activity with absorbance 2.320 at 100 mg/ml (Figure 4), whereas the Chloroform fraction and Ethyl acetate fraction showed absorbance 2.306 at 100 mg/ml and 2.183 at 100 mg/ml respectively. Petroleum ether fraction showed the lowest total antioxidant activity with absorbance 1.944 at 100mg/ml concentration. Different extractives, and standard exhibited the antioxidant activity as follows: AA> DAF> CE> EE> PE

DPPH free radical scavenging Activity

Plants rich in secondary metabolites, including phenolics and flavonoids, have antioxidant activity due to their redox properties and chemical structures. The DPPH radical is widely used in assessing free radical scavenging activity because of the ease of the reaction. Among the fractions of the extract, highest DPPH radical scavenging activity was found in Dia-ion resin adsorbed fraction having IC₅₀ value 15.53mg/ml. On the other hand, chloroform fraction showed DPPH radical scavenging activity with IC₅₀ value 24.62mg/ml, followed by ethyl acetate fraction with IC₅₀ value 21.97mg/ml and petroleum ether fraction showed DPPH radical scavenging activity with IC₅₀ value 18.56 mg/ml Figure (5) and (6).

Conclusion

The present study investigated the antioxidant activity of different extracts of the plant *M. koenigii*. For this purpose, total phenolic content, total flavonoid content, reducing power capacity, total antioxidant, and DPPH radical scavenging activity tests were performed with four different fractions of the plant. From the above results, we can conclude that, Dia-ion resin-adsorbed fraction showed the highest activity in Total phenolic

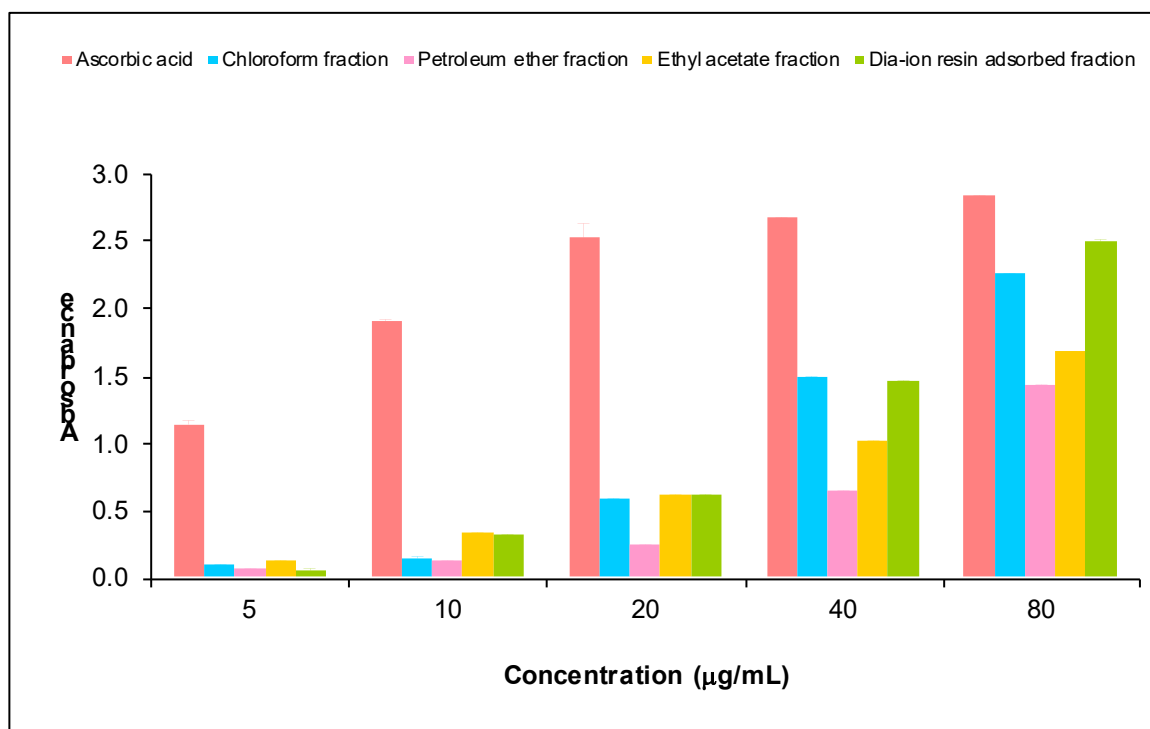


Figure 3. Reducing power capacity of different fractions of methanolic extract of *M.koenigii* and Ascorbic acid (Standard).

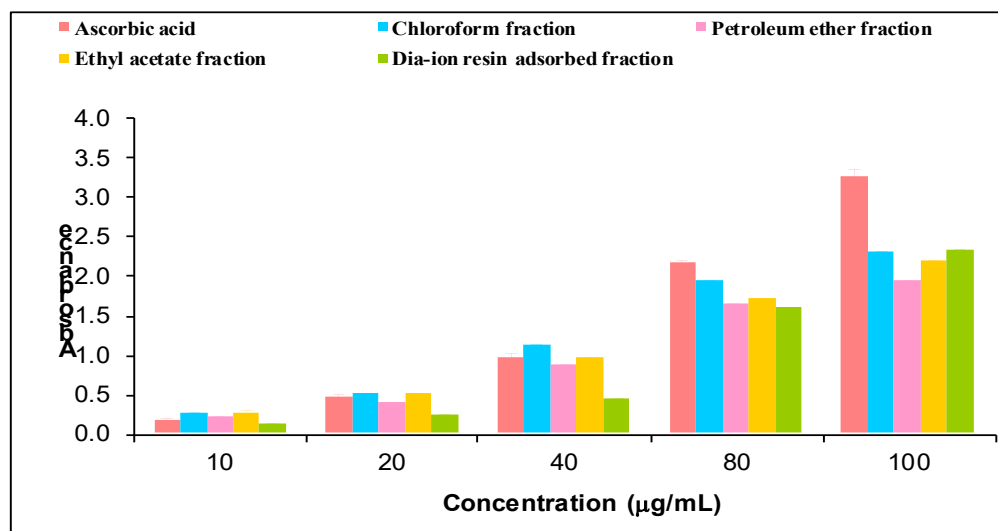


Figure 4. Total antioxidant activity of different fractions of methanolic extract of *M.koenigii* and Ascorbic acid (Standard).

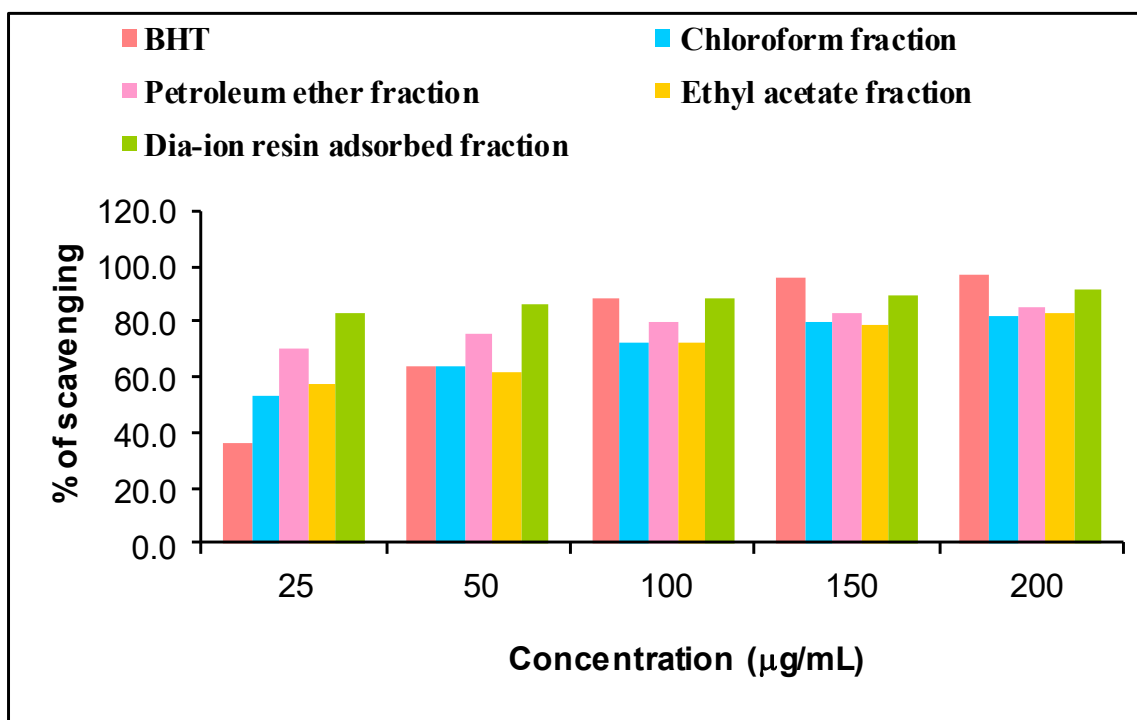


Figure 5. DPPH radical scavenging activity of different fractions of methanolic extract of *M. koenigii* and BHT (standard).

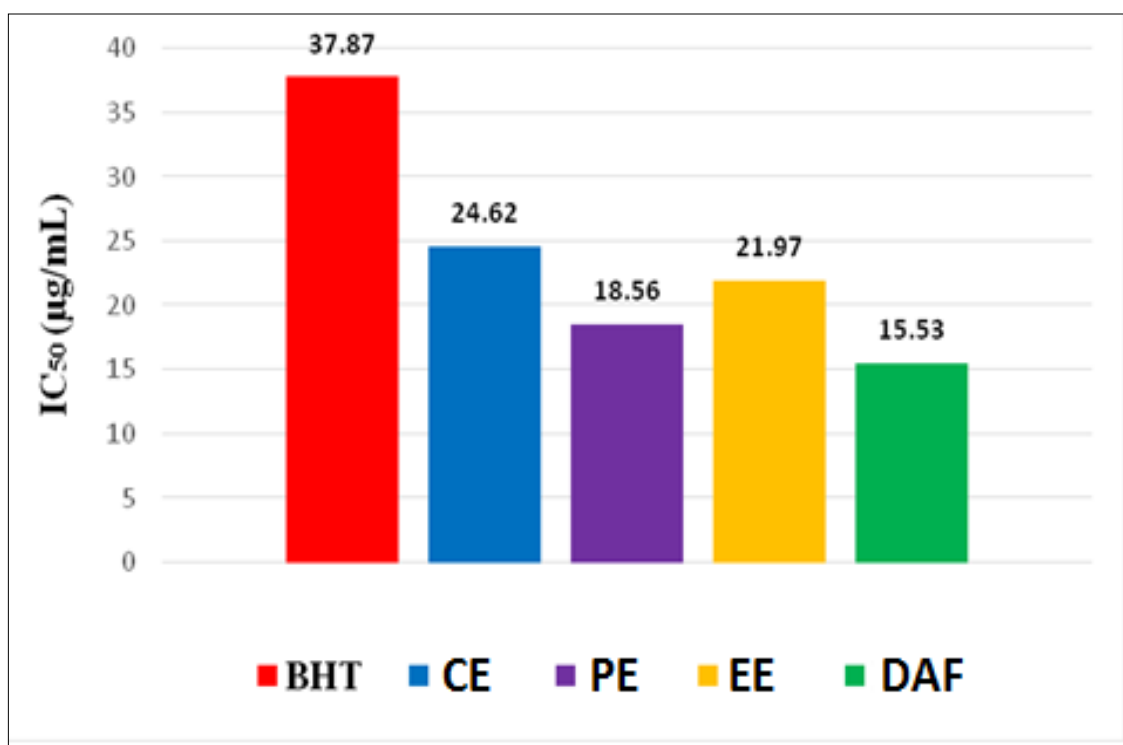


Figure 6. IC₅₀ values of different extractives of *M. koenigii* and standard BHT.

content, total flavonoid content, Reducing power capacity, Total antioxidant and DPPH radical scavenging. Dia-ion resin is good adsorbent for separation of polyphenolic compounds. Plant polyphenols are potent antioxidants widely distributed and accumulated in large amount in various plants consumed by human beings. Plants polyphenols scavenge DPPH free radicals. Total polyphenols were significantly negatively correlated to IC_{50} values of DPPH radicals scavenging. Plant flavonoids are health-promoting, disease-preventing dietary antioxidant compounds that have been shown in numerous in vitro and in vivo experiments. In the present study phenolic compound were found to be the predominant components in the leaves of *M. koenigii* indicating that they are potent antioxidant.

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