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In-vitro DPPH Free Radical Scavenging Activity of the Plant *Murraya koenigii* Linn (Curry Leaf) in Rajshahi, Bangladesh

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Authors' contributions

This work was carried out in collaboration between all authors. Author IAD performed the experimental analysis. Authors MBI and SZ managed the analyses of the study as well as final submission of the manuscript. Authors MAI and MAJ managed the literature searches. Author NUA performed the statistical analysis. Author MAR designed the study. Authors MMHM and AAM wrote the first draft of the manuscript. Author MHS checked the draft of the manuscript and finalized for submission as per as JOCAMR authors guidelines. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The use of herbal medicine is becoming popular day by day due to toxicity and side effects of allopathic medicines and hence the present study was carried out to assess the in-vitro DPPH free radical scavenging potential of the methanolic extract and their different fractions and aqueous extracts of *Murraya koenigii* Linn leaves.

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Materials and Methods: Considering the medicinal importance of the plant *Murraya koenigii* Linn (Curry leaf), 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.
Results: Among the fractions, DIA-ion resin adsorbed fraction showed the highest total antioxidant activity with absorbance 2.320±06 and petroleum ether fraction showed the lowest total antioxidant activity with absorbance 1.944 at 100 mg/ml concentration. The TPC were found to be ranges from 13.285 to 17.52 mg GAE/g while highest amount of TFC (16.65 mg CatE/g) was found in different extracts of leaves. DPPH free radical scavenging activity of different extracts of leaves was also measured and their activity was observed in the following order: DAF>PE>EE>CE, with IC_{50} values from 15.53 µg/ml to 24.62 µg/ml.

Keywords: Murraya koenigii Linn; antioxidant; crude extract; free radical; scavenging.

1. INTRODUCTION

Plants which form the backbone of traditional medicines are being used for thousands of years as a source of invaluable bioactive compounds [1]. In most Asian countries, herbal products play an important role to treat wounds, burns, intestinal problems, coughs, and general torpor [2]. 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 healing [5]. Several indigenous plants and formulations for managing cuts, bruises, burns and wounds described in folkloric as well as the Ayurvedic system of medicine [6,7]. In Bangladesh 5,000 species of angiosperm reported to occur [8]. 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 Bangladesh [9]. Dhaka, Rajshahi, Shylet and Chittagong division is rich in medicinal plants[8]. 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 resources [10].

Murraya koenigii, commonly known as curry leaf or Kari Patta, basically known for its aroma and medicinal property, belonging to Family Rutaceae [11]. However, *M. Koenigii* is a semi-deciduous aromatic shrub or tree-let with culinary and medicinal properties [12]. Leaves are aromatic which contain proteins, carbohydrates, fiber, minerals, carotene, nicotinic acid, and vitamin C, and are also rich in vitamin A and calcium. The leaves contain high amount of oxalic acid, crystalline glycosides, carbazole alkaloids, koenigin and resin. Fresh leaves contain 2.5% yellow color, volatile oil [13]. 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 industry [14]. Recent studies found some compound include the alkaloids, mahanimbine [15], girinimbine [16], numerous carbazole alkaloids [17], and cinnamaldehyde [18] in curry tree leaves, stems, and seeds. It was also reported that the fresh leaves of *M. koenigii* possesses anti-microbial, mosquitocidal, topo-isomerase inhibition, and antioxidant properties [19]. However, the aims of this study is to determine total phenols, flavonoids and the antioxidant potential of *M. koenigii* leaves extracts with different solvents.

2. MATERIALS AND METHODS

2.1 Plant Material and Sample Preparation

The *M. koenigii* (plant) leaves were collected from the adjacent areas of BCSIR (Bangladesh Council of Scientific and Industrial Research) 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 passing 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.

2.2 Chemicals and Reagents

All chemicals used were of analytical grade. 1,1-Diphenyl-2- picrylhydrazyl (DPPH), Ascorbic acid, catechin, gallic acid, anhydrous sodium carbonate (Na_2CO_3), aluminum tri chloride, potassium acetate, sodium acetate, ferric chloride hexahydrate ($FeCl_3.6H_2O$), Folin–Ciocalteu reagent, purchased from Sigma–Aldrich. All chemicals and reagents were used without further purification.

2.3 Determination of Total Phenolic Content

Total phenolic content of different extractives of *Murraya koenigii* were determined by adopting the method [20] 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 µg/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.

2.4 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 lg/ 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.

2.5 Determination of Reducing Power Capacity

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.* This mixture was kept at 50ºC 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.

2.6 Determination of Total Antioxidant Activity

Total antioxidant activity of different extractives of *M. koenigii* were determined by the method of Prieto et al. [23] with some modifications. 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 (AA) 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.

2.7 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 Bracaet et al. [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^{TM} 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).

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content (TPC)

Phenolic compound can be from a simple phenol to highly polymarized giant molecule lignin. These 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 Fig. 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).

3.2 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 diseases [25]. Total flavonoid content of different fractions of *M. koenigii* are shown in Fig. 2. Among the fractions, the highest total flavonoid content was found in Dia-ion resin adsorbed fraction (16.65 mg CatE/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.

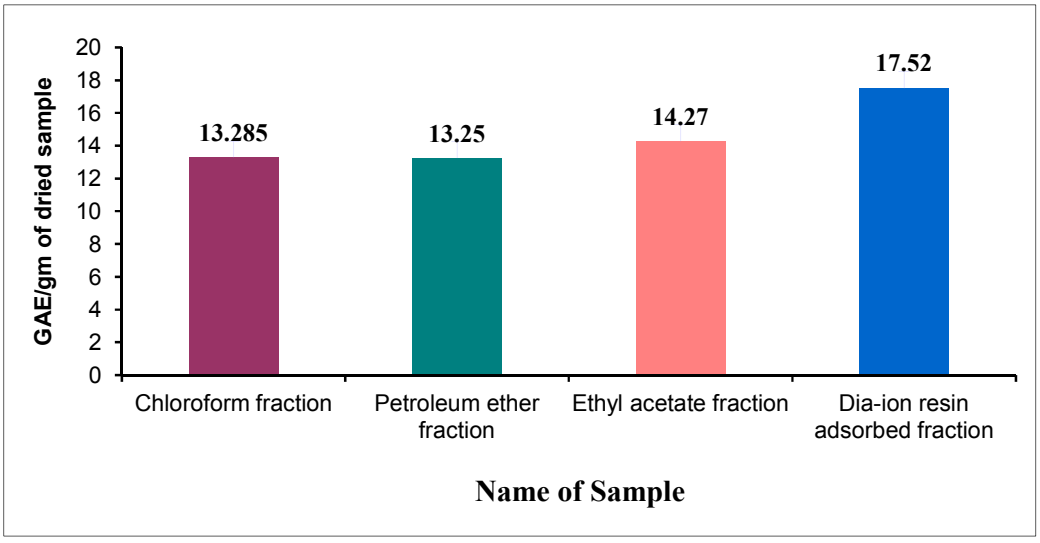

Fig. 1. Total phenolic content (mg GAE/g extract) of different extract of *M. koenigii*

3.3 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, chloroform fraction ethyl acetate and Dia-ion resin adsorbed fraction have been investigated. Among the four different extractives Dia-ion resin adsorbed fraction showed the highest iron reducing capacity with absorbance of 2.504 at 80 μg/ml concentration, followed by Chloroform fraction with absorbance 2.263±0.003 at 80 μg/ml while Ethyl acetate fraction showed iron reducing capacity with absorbance of 1.675 at 80 μg/ml and Petroleum ether fraction showed the iron reducing capacity with absorbance 1.421 at 100 μg/ml Fig. 3. Different extractives, and standard exhibited the reducing power activity as follows: AA>DAF > CE> EE > PE.

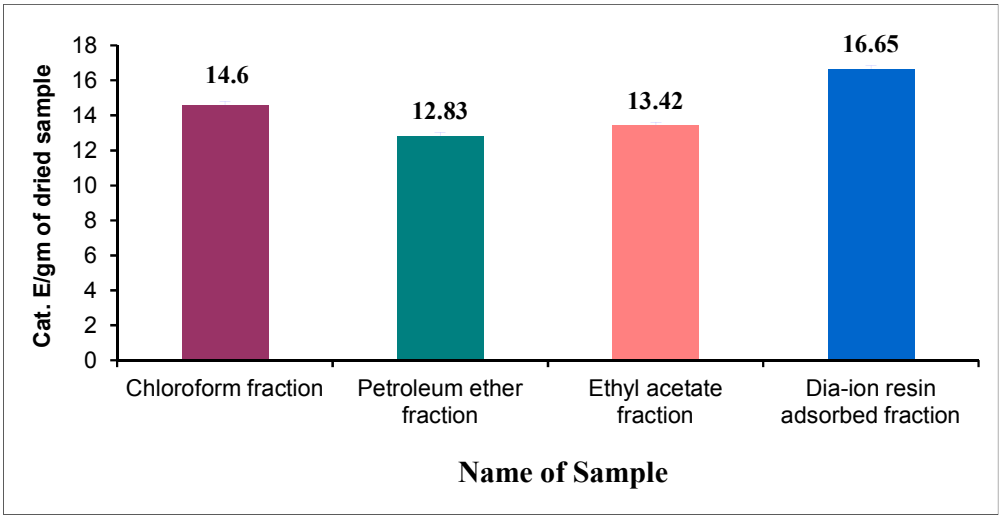

Fig. 2. Total flavonoid content (mg CatE/g extract) of different fractions of *M. koenigii*

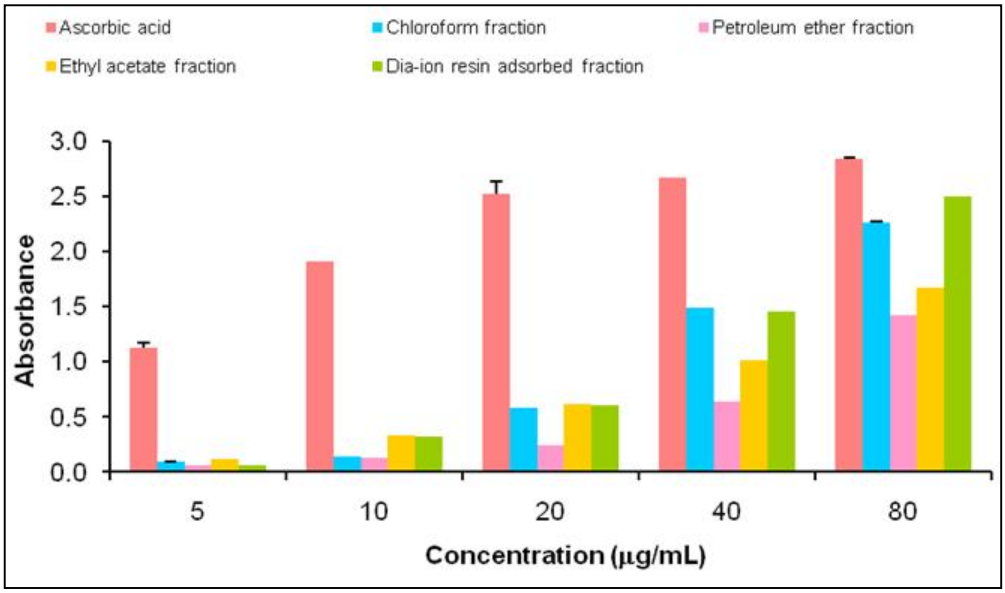

Fig. 3. Reducing power capacity of different fractions of methanolic extract of *M. koenigii* and Ascorbic acid (standard)

3.4 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* such as Dia-ion resin adsorbed fraction, chloroform frcation, 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 μg/ml Fig. 4. Whereas the Chloroform fraction and Ethyl acetate fraction showed absorbance 2.306 at 100 μg/ml and 2.183 at 100 μg/ml respectively. Petroleum ether fraction showed the lowest total antioxidant activity with absorbance 1.944 at 100 μg/ml concentration. Different extractives, and standard exhibited the antioxidant activity as follows: AA> DAF> CE> EE> PE.

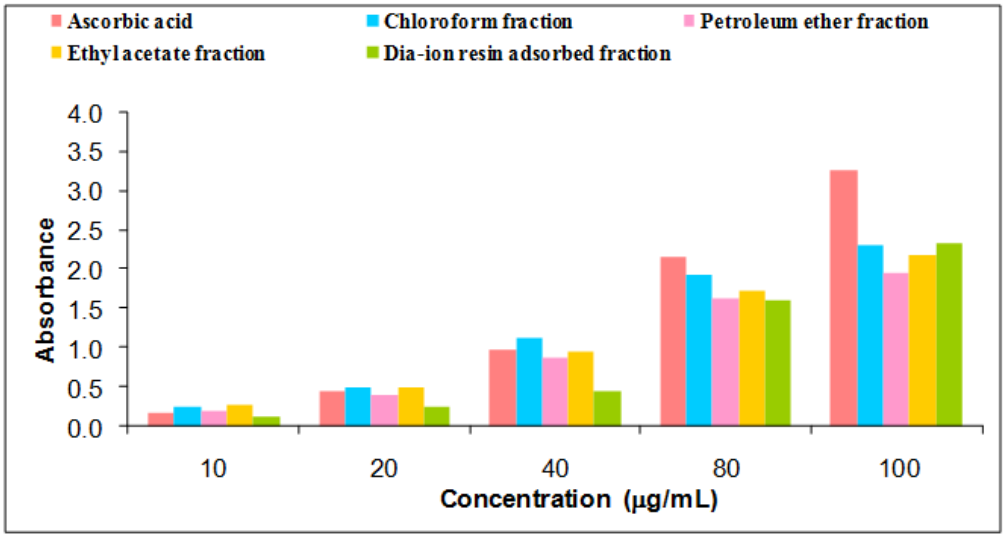

Fig. 4. Total antioxidant activity of different fractions of methanolic extract of *M. koenigii* and Ascorbic acid (standard)

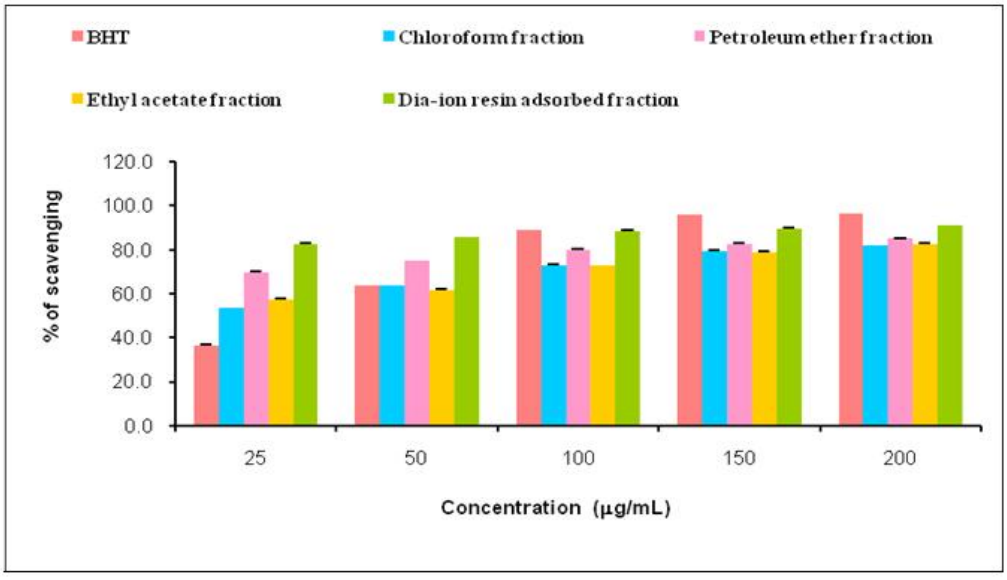

Fig. 5. DPPH radical scavenging activity of different fractions of methanolic extract of *M. koenigii* and BHT (standard)

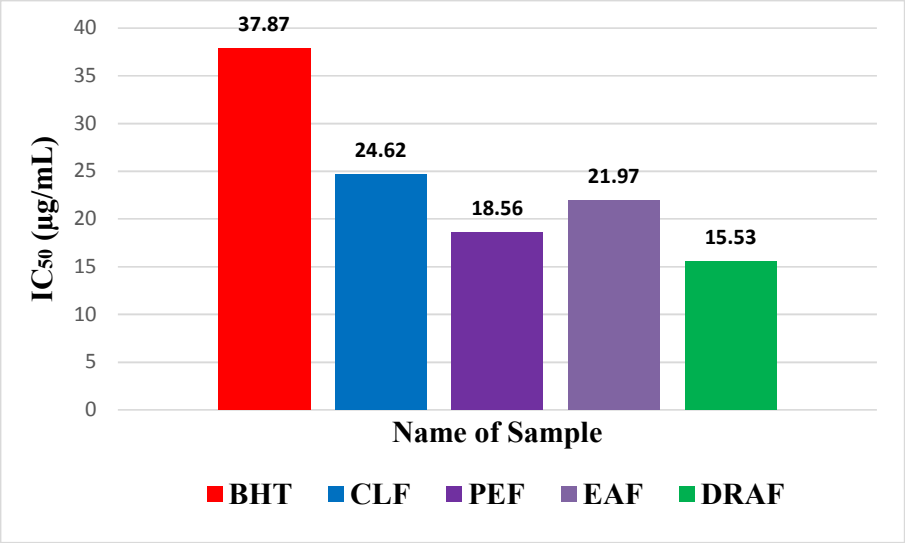

Fig. 6. Comparative graphical presentation of IC_{50} values of different extractives of *M. koenigii* and standard BHT

3.5 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 IC_{50} value 15.53µg/ml. On the other hand, chloroform fraction showed DPPH radical scavenging activity with IC_{50} value 24.62 µg/ml, followed by ethyl acetate fraction with IC_{50} value 21.97 µg/ml and petroleum ether fraction showed DPPH radical scavenging activity with IC_{50} value 18.56 µg/ml Figs. 5 and 6.

4. 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 shows the highest activity in Total phenolic content, total flavonoid content, Reducing power capacity, Total antioxidant and DPPH radical scavenging. Dia-ion resin is good adsorbent for separating polyphenolic compounds [26]. Ogawa et al. [27] also obtain highly purified polyphenols fractions from the seeds of Aesculus turbinate using Dia-ion HP-20. Plant polyphenols are potent antioxidants widely distributed and accumulated in large amount in various plants consumed by human beings. Plants polyphenols inhibit scavenge DPPH free radicals [28]. Total polyphenols were significantly negatively correlated to IC_{50} values of DPPH radicals scavenging [29]. Plant flavonoids are health-promoting, disease-preventing dietary antioxidant compounds that have been shown in numerous *in vitro* and *in vivo* experiments [30]. In this study, similar result was observed for the Dia-ion adsorbed fraction which was rich in phenolic contents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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