International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No.04, March 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

Analysis of antioxidant activity, total phenolic and total flavonoid contents of *Allium sativum, Mentha arvensis and Murraya koenigii*

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ABSTRACT

Antioxidants can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and can thus prevent or repair damage to body's cells by reactive oxygen species. In our study we have selected Allium sativum bulbs, Mentha arvensis leaves and Murraya koenigii leaves for quantitative analysis of antioxidants activity. Three solvent systems, 70% ethanol, 70% methanol and distilled water were used in extraction process. The extracts were tested for their total phenolic content, total flavonoid content and antioxidant activities using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and reducing power ability. We found methanolic extract of Allium sativum and Murraya koenigii have higher total phenolic content and total flavonoid content, while Mentha arvensis have higher total phenolic content and total flavonoid content in ethanolic extract. We also found that Allium sativum and Mentha arvensis extracts show better DPPH scavenging action and reducing power.

Key Words : Allium Sativum, Antioxidant, DPPH, Mentha Arvensis, Murraya Koenigii.

I INTRODUCTION

Metabolic pathways as well as external sources such as exposure to particulate matter (PM), x-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals leads to generation of free radicals [1]. Several in-vivo and in-vitro studies have shown that inhaled PM gets deposited in lungs and can trigger pro-inflammatory responses contributing to oxidative stress [1,2]. Oxidative stress is the imbalance between free radical production and antioxidant defence [3]. It is caused by tissue injury, infection or heat, toxins, and excessive exercise. These injured tissues produce enzymes like xanthine oxidase, lipo-oxygenase and cyclo-oxygenase that are regarded as radical generating enzymes. These enzymes activate phagocytes, release free iron and copper ions, or disrupt the electron transport chain of oxidative phosphorylation and therefore producing excess reactive oxygen species (ROS) and reactive nitrogen species (RNS). Cancer, diabetes mellitus, age-related diseases, and neurodegenerative diseases such as Parkinson's disease are also linked with imbalance between ROS and antioxidant defence system [4,5]. To reduce the rise of free radicals consumption of fruits and vegetables or certain dietary supplements should be increased to boost protection against excessive production of ROS. Since

early times people have been using products derived from plants as well as animals to treat various diseases. Whole plant or their parts likevegetables, fruits, leaves, oilseeds, cereal crops, barks and roots are the potential sources of various types of antioxidants [6,7]. In our study we have selected three plant materials-*Allium sativum* bulbs, leaves of *Murraya koenigii* and *Mentha arvensis* from local market to check antioxidant potential of plants materials usually used in south Delhi.

Allium sativum commonly called garlic is a source of various biologically active phyto-molecules including organosulfur compounds, phenolic acid, allyl-thiosulfinates, flavonoids and vitamins. Health benefits of *Allium sativum* owe to its bioactive compounds, especially phenolic compounds [8]. *Allium sativum* bulbs have been widely used in the prevention and treatment of cardiovascular diseases [9]. In an *in vivo* study, intravenous administration of *Allium sativum* extract has shown to produced slight reductions in both systolic and diastolic pressures [10]. Further, oral ingestion of *Allium sativum* extract in hypertensive animals brought the blood pressure back to the normal level [11].

Mentha arvensis (common name, mint) is a traditional item of culinary and herbal medicine, and it grows wild in different parts of India [12]. The plant has been reported in traditional system of medicine against various ailments like jaundice, peptic ulcer, diarrhoea, bronchitis inflammation of liver, and skin diseases. The plant also has anti–inflammatory and sedative–hypnotic, hepatoprotective and antioxidant activities besides antibacterial, anti-fungal and also some radio protective activity [13–19].

Murraya koenigii is widely used in Indian cooking for centuries, has a versatile role to play in traditional medicine. Roots and bark are used externally to cure eruptions and bites of poisonous animals like snakes, viper. Green leaves are eaten raw to cure dysentery, diarrhoea and vomiting. Leaves and roots are also used traditionally as analgesics, in curing piles, blood disorders, itching, inflammation and leukoderma [20–23].

For a plant used in medicine, it is mandatory to know its phytochemical blueprint besides its radical scavenging potential. The present study was designed with an attempt to screen these plants to evaluate their phenolic and flavonoid content in different solvents besides their radical scavenging activity.

2. MATERIAL AND METHODS

Allium sativum bulbs, *Mentha arvensis* leaves and *Murraya koenigii* leaves were obtained from the local market area of Delhi, India. Plants were subjected to extraction methods using methanol, ethanol and water as the solvents of extraction.

2.1. Chemicals

Methanol, Ethanol, Gallic acid powder, Folin-ciocalteau reagent, Sodium carbonate anhydrous, Hydrochloric acid, Aluminium trichloride, Rutin, Phosphate buffer saline, Potassium hexa cyanate ferrate, Trichloroacetic acid, Ascorbic acid, DPPH were used in the study and all were procured from Himedia.

2.2. Sample Preparation

2.2.1. Preparation Aqueous extract

10 g *Allium sativum* bulbs and leaves of *Murraya koenigii* were weighed, sliced into fine pieces and crushed in water using pestle and mortar to form a paste. 200 ml of distilled water was added to it and was boiled for 20 minutes in the water bath at 100 °C. Then the extract was filtered using Whatman filter paper. The filtrate was then evaporated to dryness under vacuum in a rotary evaporator at 40°C[24].

For *Mentha arvensis* leaves extraction was done as designed by *Marcocci et al* [25]. 15g of *Mentha arvensis* leaves were washed and dried at room temperature. Leaves were cut and ground into a paste using pestle and mortar. The paste was transferred into a conical flask and dissolved in 100ml distilled water. The crude preparation was left overnight in the shaker at 35^{o}C and the next day centrifuged at 2500 rpm for 10 minutes. The supernatant containing the plant extract was then filtered using Whatman filter paper and transferred to a beaker. Finally the filtrate was evaporated to dryness under vacuum in a rotary evaporator at 40°C.

2.2.2 Preparation of ethanolic extract

For ethanolic extract preparation 10 g of dried, peeled *Allium sativum* bulbs, 20 g of *Murraya koenigii* leaves, and 20 g of *Mentha arvensis* leaves respectively were ground using 70% ethanol. Then finely ground paste was soaked in 100 ml of 70% ethanol and was covered to prevent evaporation of ethanol and kept in a rotary shaker for 2 days at 250 rpm. Resultant crude extract was centrifuged at 8000 rpm for 10 minutes. Supernatant was collected and concentrated using Rotary Evaporator [26].

2.2.2. Preparation of methanolic extract

For methanolic extract preparation, 20 g of peeled *Allium sativum* bulbs, or 20 g of *Murraya koenigii* leaves, or 20 g of *Mentha arvensis* leaves were ground using 70% methanol. Finely ground paste was soaked in 200 ml of 70% methanol and was covered to prevent evaporation of methanol and kept in a rotary shaker for 2 days at 250 rpm. Resultant crude extract was centrifuged at 8000 rpm for 10 minutes. Supernatant was collected and concentrated using Rotary evaporator [26].

2.3. Determination of polyphenolic content

2.3.1. Estimation of total phenolic content

The concentration of total phenolic in aqueous, methanolic and ethanolic extracts of all three plants were determined by using Folin-Ciocalteu reagent by the method of Singleton et al[27]. A 1mg/ml standard solution of gallic acid was made in 70% methanol and total phenolic content were expressed as mg/g gallic acid equivalent (GAE).The reaction mixture was prepared by mixing 0.5 ml (1mg/ml) of each sample with 2.5ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2 ml of 7.5% sodium carbonate (Na_2CO_3). The same procedure was repeated for standard solution of gallic acid with all the reagents. Blank was concomitantly prepared, containing 0.5ml of 70% methanol, 2.5ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2 ml of 7.5% Na_2CO_3.The tubes were covered and allowed to stand for 45 minutes at room temperature. Absorbance was measured at 760 nm by UV-visible spectrophotometer. Standard curve was plotted using gallic acid (0.01- 0.06 µg/ml). The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained [28,29].

2.3.2. Total flavonoid content

Flavonoid content determination was done in the extracts by aluminium chloride method[30,31].1 ml of 2% aluminium chloride ($AlCl_3$) was dissolved in80% methanol was mixed with the same volume of the extract solution and incubated for 1hour at room temperature. Absorbance was assessed at 415nm against blank using spectrophotometer. Total flavonoid content was determined using a standard plot of rutin (10-50µg/ml), then expressed as mg of rutin equivalents (RE)/g of extract. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained.

2.4. Antioxidant activity

2.4.1. Reducing power activity assay

Reducing power assay measures ability of extract to reduce Fe^{3+} to Fe^{2+}.The reducing power of methanolic, ethanolic and aqueous extract of *Allium sativum* and *Mentha arvensis* extracts were determined by the method of Oyaizu [32]. 2.5ml concentrations of the plant extracts (10-100µg/ml) in deionized water were mixed with equal volumes of phosphate buffer 0.2 M, pH 6.6 (2.5 ml) and potassium ferricyanide (2.5 ml) and incubated at 50^0C for 20 min. Aliquots of 10% trichloroacetic acid (TCA) (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5ml of freshly prepared ferric chloride 0.1% solution. Absorbance was measured at 700 nm using spectrophotometer. A blank was prepared without adding extract. Ascorbic acid at various concentrations (10-100µg/ml) were used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power [33].

2.4.2. DPPH Radical Scavenging Activity

The free radical scavenging capacity of extracts of *Allium sativum* and *Mentha arvensis* were determined using (2,2-diphenyl-1-picrylhydrazyl) DPPH by following the method of Masoodi et al with some modification[34]. Freshly prepared DPPH solution was taken in test tubes and extracts were added followed by serial dilutions (5µg/ml to 50µg/ml) to every test tube so that the final volume was 3 ml and after keeping it for 30 min in dark, the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid was used as standard. Control sample was prepared containing the same volume without any extract and standard with solvent in which extracts were made served as blank. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula.

$$[\%\ \text{DPPH Radical scavenging} = (Ac - A)/\ Ac \times 100]$$

Where, Ac = Absorbance of the control, A = Absorbance of the sample

International Journal of Advance Research in Science and Engineering
Volume No.07, Special Issue No.04, March 2018
www.ijarse.com
IJARSE
ISSN: 2319-8354

III RESULTS

3.1. Total phenolic and flavonoid content

The amount of total phenolic content (TPC) was determined in the extracts of *Allium sativum*, *Mentha arvensis*, *Murraya koenigii* with Folin–Ciocalteu reagent[27],and expressed in mg/gm. In our study methanolic extract of *Allium sativum* and *Murraya koenigii* shows higher phenolic content than ethanolic and aqueous extracts. In case of *Mentha arvensis* ethanolic extract showed highest phenolic content than methanolic and aqueous extract. Total phenolic content in ethanolic extract exhibited following order *Mentha arvensis* extract> *Allium sativum* extract>*Murraya koenigii* extract. In case of methanolic and Aqueous extract total phenolic content exhibited following order is *Murraya koenigii* extract>*Mentha arvensis* extract >*Allium sativum* extract. Fig.1 shows gallic acid standard curve and table 1 shows results of total phenolic content in terms of gallic acid equivalent.

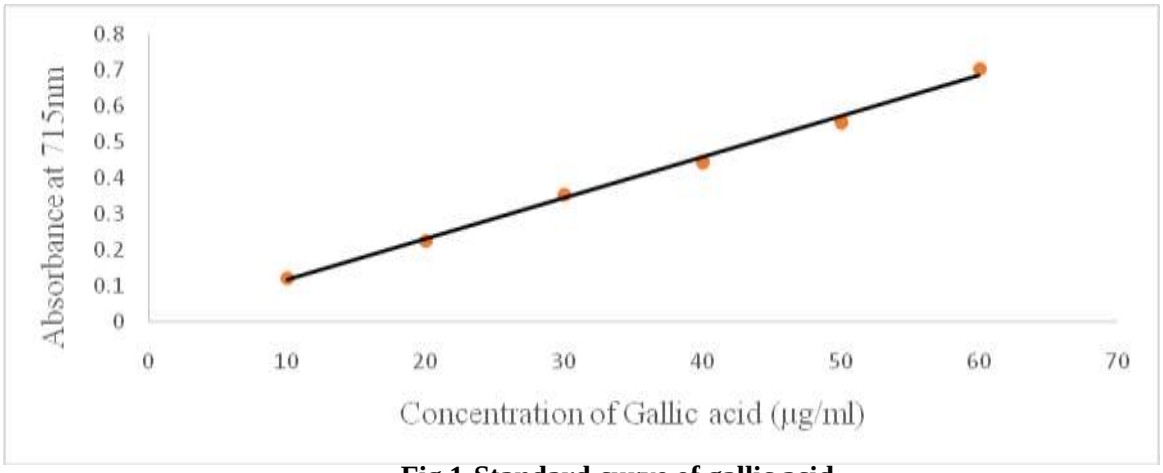

Fig.1-Standard curve of gallic acid

Table. 1- Total Phenolic of *Allium sativum*, *Mentha arvensis* and *Murraya koenigii* extracts that were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent.

S.no	Extract	Total Phenolic content of *Allium sativum* (mgGAE/gE)	Total Phenolic content of *Mentha arvensis* (mgGAE/gE)	Total Phenolic content of *Murraya koenigii* (mgGAE/gE)
1.	Ethanolic	63.60±0.47	75.90±0.95	54.24± 4.1
2.	Methanolic	67.02±0.28	60.20±1.15	70.93±2.7
3.	Aqueous	25.70±0.50	30.54±0.27	35.75±1.2

Table 1: Total Phenolic content of *Allium sativum, Mentha arvensis* and *Murraya koenigii* extracts. GAE: gallic acid equivalents. All results are presented as mean± standard deviation of three assays.

3.2. Total flavonoid content

The Total Flavonoid Content (TFC) of *Allium sativum* extract, *Mentha arvensis* extract *and Murraya koenigii* extract was expressed as mg/g Rutin Equivalent. Rutin standard curve and results of total flavonoid contents are depicted in Fig. 2 and Table 2 respectively.

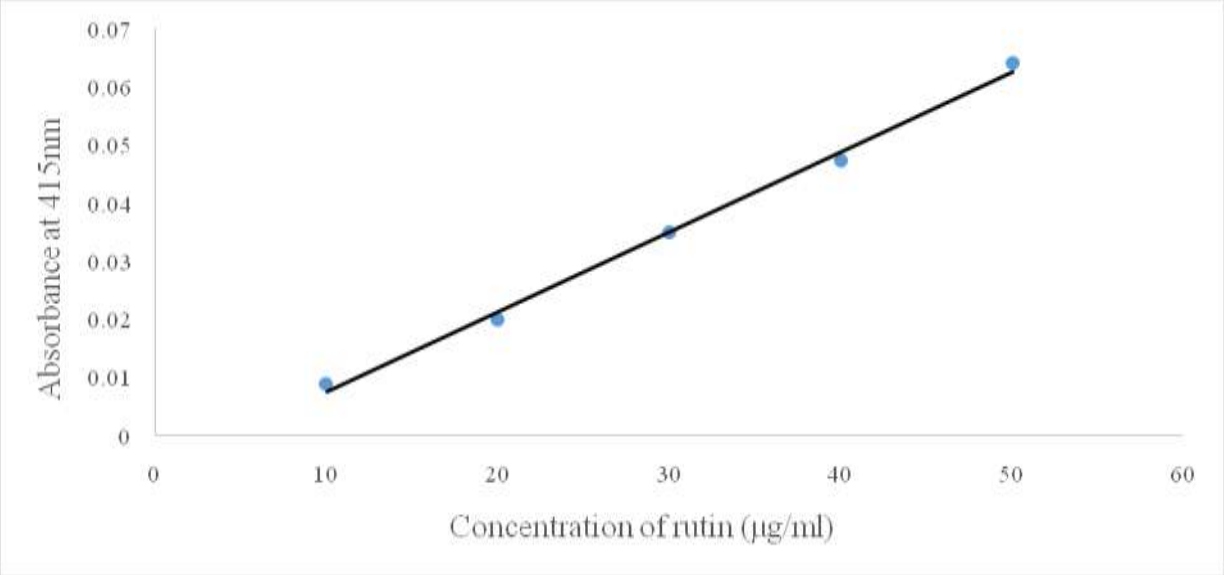

Fig.2- Standard curve for rutin for total flavonoid content.

Table.2-Total Flavonoid content of *Allium sativum*, *Mentha arvensis* and *Murraya koenigii* extracts.

S.No	Extract	Total flavonoid Content of *Allium sativum* (mg RE/gE)	Total flavonoid Content of *Mentha arvensis* (mg RE/gE)	Total flavonoid Content of *Murraya koenigii* (mg RE/gE)
1.	Ethanolic	34.10±2.62	44.14±1.53	43.10±3.509
2.	Methanolic	44.13±3.53	29.83± 1.33	52.13±2.353
3.	Aqueous	12.67±1.50	17.20±1.35	26.67±2.6

RE: rutin equivalents, gE: g of extract. All results are presented as mean ± standard deviation of three assays.

We found total flavonoid content was found higher in methanolic extract of *Allium sativum* and *Murraya koenigii* while in case of *Mentha arvensis* the total flavonoid content was found higher in ethanolic extract. We have also found that total flavonoid content of methanolic extract of *Murraya koenigii* is higher than *Allium sativum* and *Mentha arvensis*. Total flavonoid content in ethanolic extract exhibited following order *Mentha arvensis extract>Murraya koenigii extracts >Allium sativum* extract. In aqueous extract total flavonoid content exhibited following order *Murraya koenigii* extracts> *Mentha arvensis* extract>*Allium sativum* extract. Total

flavonoid content in case of methanolic extract exhibited following order *Murraya koenigii* extract >*Allium sativum* extract> *Mentha arvensis* extract.

3.3. Reducing power of *Mentha arvensis* and *Allium sativum*

The reducing power was assayed by method of Oyaizu [32]. Through this assay we compared antioxidant property of plants in their ability to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}), with the resulting ferrous ion (Fe^{2+}) formation monitored spectrophotometrically at 700 nm. Reducing power assay may indicate how easily a given antioxidant donates electrons to reactive free radicals species, thus promoting the termination of free radical chain reactions. The ability of the antioxidant to reduce Fe^{3+} to its more active Fe^{2+} form also indicates the ability to act as a pro-oxidant in the system. Our results show that the reducing power capability of extracts increases with the increasing concentration from range of 10 µg/ml to100 µg/ml. We also saw that in case of *Mentha arvensis*, highest capacity of reducing Fe^{3+} to Fe^{2+} was observed in ethanolic extract than methanolic and aqueous extracts with reference to ascorbic acid used as standard, as shown in (fig. 3). In case of *Allium sativum* reducing power capability was found higher in methanolic extract than ethanolic and aqueous extract with reference to standard ascorbic acid, as shown in (fig.4).

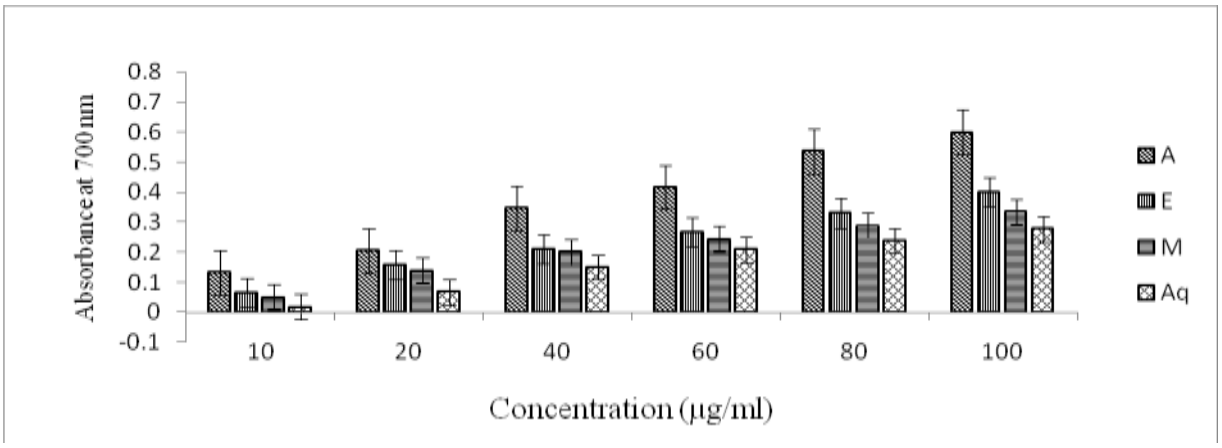

Fig.3- Reducing power of *Mentha arvensis* A: absorbance of Ascorbic acid, Aq: absorbance of aqueous extract, E: absorbance of ethanolic extract, M: absorbance of methanolic extract.

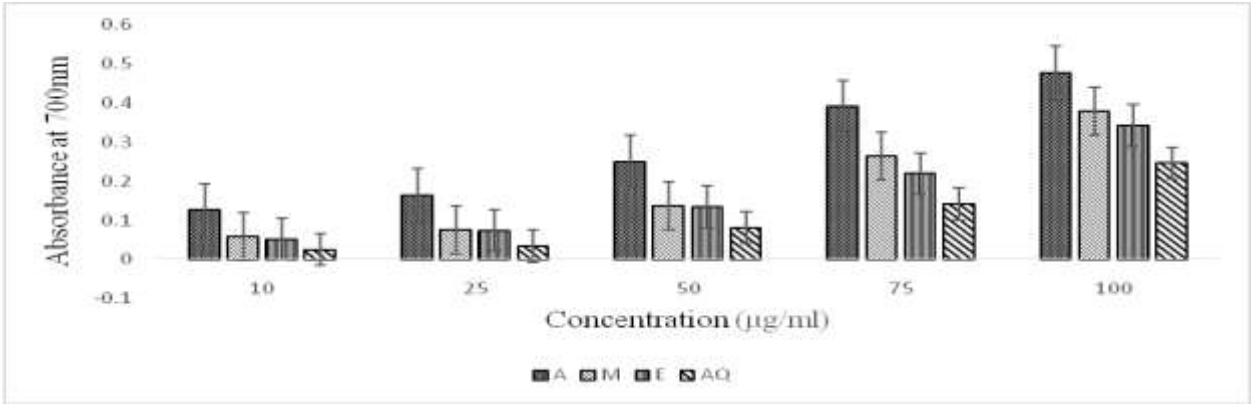

Fig.4- Reducing power of *Allium sativum* extracts A: absorbance of ascorbic acid, Aq: absorbance of aqueous extract, E: absorbance of ethanolic extract, M: absorbance of methanolic extract.

3.4 DPPH Radical Scavenging Activity

Radical scavenging ability was checked by DPPH reagent method. DPPH is very stable free radical. Antioxidants present in plant extracts can scavenge radical either by donating hydrogen or by radical scavenging activity. Solution of DPPH is mixed with a substance that can donate a hydrogen atom, which gives rise to the reduced form of 2,2-diphenyl-1-picrylhydrazylwith the loss of its violet colour [35]. Our results show that percent inhibition of DPPH radical by our extracts at a concentration of 50 µg/ml, exhibited following order: ascorbic acid (90%)>ethanolic extract (56%)>methanolic extract (47%) > aqueous extract (33%) in *Mentha arvensis.* At 50µg/ml, *Allium sativum* exhibited following order for percent inhibition of DPPH radical: Ascorbic Acid 89% >methanolic extract 51%> ethanolic 45% >aqueous 24%. We have shown percent DPPH inhibition by *Mentha arvensis* and *Allium sativum* in (fig. 5) and (fig.6) respectively.

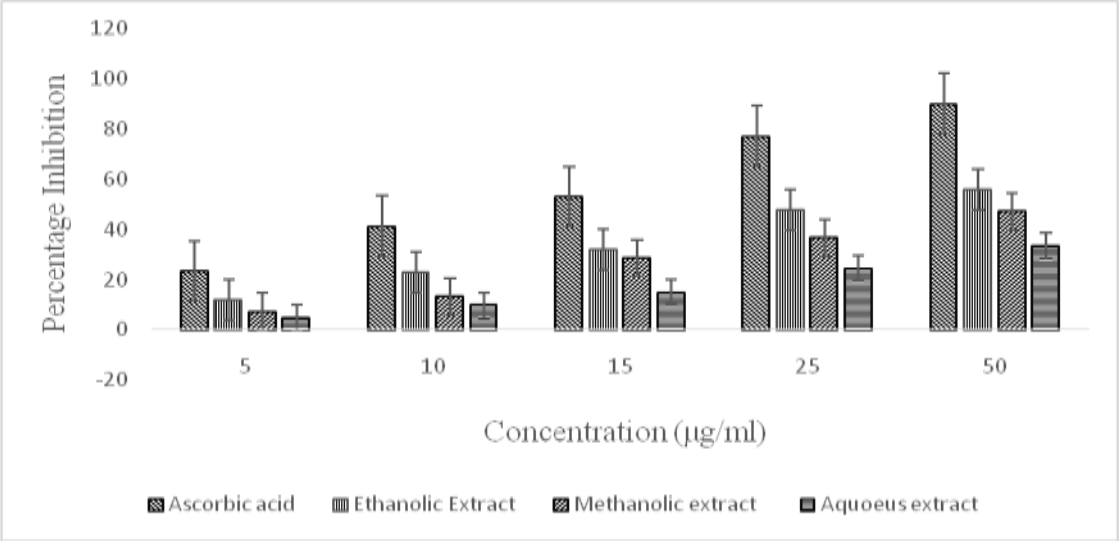

Fig.5- Evaluation of DPPH free-radical scavenging activity of methanolic, ethanolic and aqueous extracts of *Mentha arvensis* and with respect to standard ascorbic acid.

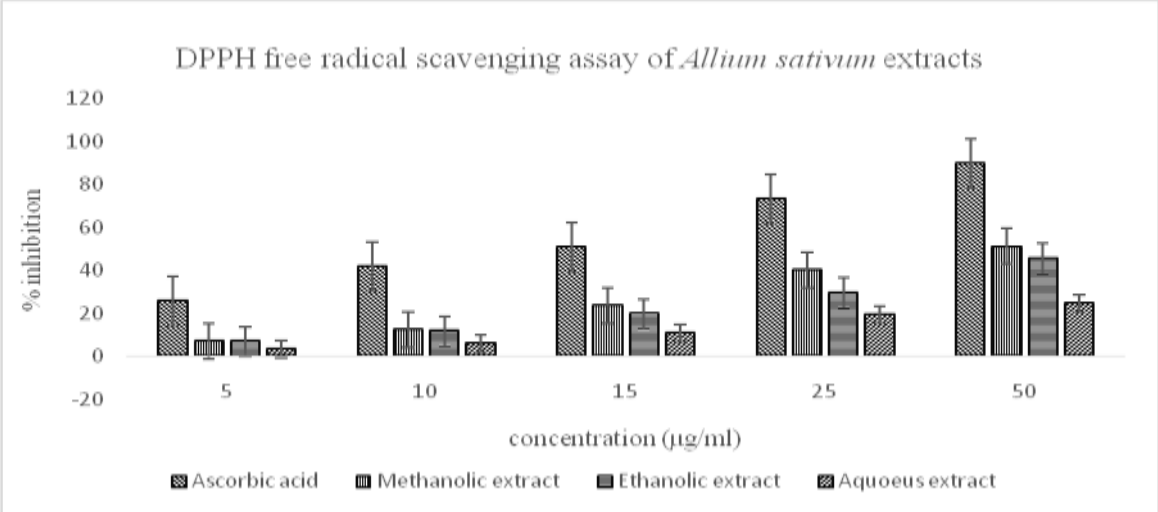

Fig.6- Evaluation of DPPH free-radical scavenging activity of methanolic, ethanolic and aqueous extracts of *Allium sativum* and with respect to standard Ascorbic acid.

4. Discussion

Nature has bestowed plants with antioxidant and radical scavenging properties. Phenols and flavonoids are main constituents in plants that protect cells against oxidative damage caused by free radicals. Sharma et al have reported plants with medicinal importance and chemical composition [36]. Phenolic compounds are secondary metabolites and are highly effective free radical scavengers [37]. Flavonoids are a group of phenolic compounds and their beneficial effects include the ability to scavenge a wide range of nitrogen and chlorine species such as superoxide, hydroxyl radical, reactive oxygen, peroxynitrous acid, peroxylradicals, and hypochlorous acid. Flavonoids also chelate ions, often decreasing the metal ion pro-oxidant activity[38–41]. Antioxidant activity of plants might be due to their phenolic compounds such as flavonoids which are a group of polyphenolic compounds with known properties that include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [42]. Solvent extraction is frequently used for isolation of antioxidant. Extraction yield is dependent on the solvent and method of extraction, due to the different antioxidant potentials of compounds with different polarity [43]. Moreover there is no universal method by which accurate measurement of antioxidant activity can be done [44]. In the present study, we have evaluated total phenolic and flavonoid content of *Allium sativum* bulbs, *Murraya koenigii* and *Mentha arvensis* leaves in three different solvents i.e, methanol, ethanol and water. Our results shows that total phenolic content and total flavonoid content of 70% methanolic extract of *Allium sativum and Murraya koenigii* were higher than 70% ethanolic and aqueous extracts, while in case of *Mentha arvensis* total phenolic content and total flavonoid content was found higher in 70% ethanolic extract than methanolic extract and aqueous extracts. Our result for *Allium sativum* has shown little difference from done by *Raja Zouari Chekki et al* [45].They studied *Allium sativum* from Tunisia and Indian *Allium sativum* in which they found total phenolic content of ethanol extract for Tunisian *Allium sativum* was 43.6 mg GAE/100 g. Total phenolic content of 80% methanol extracts was 64.5 mg GAE/100 g for the Indian extract *Allium sativum* whereas TPC in case of 70% of methanol shows 67.02 mg GAE/100 g [45]. It is reported that garlic extracts have got antioxidant activity and provides protection against free radical damage, they have also reported that these four main compounds from garlic allicin, alliin, allyl cysteine and allyl from garlic are active compounds against free radical damage [46]. In case of *Mentha arvensis*phenolic acids generally exist in a free, esterified or glycosylated form in plants. Ayumi et al have extracted free phenolic acids in rice using 70% ethanol [47]. Antioxidant potential of roots of *Mentha arvensis* of methanolic extract was shown by Dar et al, and they have shown total phenolic content and total flavonoid content in range of 9.12 mg/g and 32.14 mg/g respective [48]. Sulaiman et al had studied 37 raw vegetables with four solvent system like 70% acetone, 70% ethanol, 70% methanol and aqueous solution where they found that 70% acetone extracts of 24 species of vegetables exhibited the highest value of TPC. They also reported in some plants there was more total phenolic content and total flavonoid content in 70% ethanolic solvent while in other plants they found higher total phenolic content and total flavonoid content in 70% methanolic solvent [49]. In our study *Mentha arvensis* also shows higher total phenolic content and total flavonoid content in ethanolic extract than methanolic and aqueous extracts.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. For the estimation of the reducing power ability we investigated the transformation of Fe^{3+} to Fe^{2+} using the method of Oyaizu [32], by measuring the formation of Prussian blue at 700nm. Increase in absorbance of reaction mixture indicates higher reductive ability of sample. Our results show reducing power capability of the extracts increased with increasing concentrations as reported by other studies also [50]. We found methanolic extract of *Allium sativum* exhibiting the highest reducing power at 100 µg/ml among the other extracts with reference to ascorbic acid used as standard. Ethanolic extract of *Mentha arvensis* and ascorbic acid had significant reducing power with increasing concentration in the range of 10-100 µg/ml. These plants have shown iron chelating ability therefore *Mentha arvensis* and *Allium sativum* have potential antioxidant ability and both can prevent lipid peroxidation by chelating Fe^{3+}.

The model of scavenging the stable DPPH radical is a widely used method in the evaluation of the free radical scavenging ability of various compounds [51]. This assay is used as a preliminary test which provides information on the reactivity of test compound with a stable free radical since odd electron of DPPH gives strong absorption band at 517nm, and when it is quenched by the extract, there is a decrease in absorbance. Our studies have shown that the scavenging activities of all the extracts and the standard for DPPH are dose responsive, that is, the higher the concentration, the greater the scavenging activity. Methanolic extract of *Allium sativum* showed a very good anti-radical activity in scavenging DPPH radical (comparable to the standard, Ascorbic acid) with a maximum inhibition of about 51.02±0.52 at a concentration of 50µg/ml. High antioxidant activity of *Allium sativum* has been reported [52], but this activity depends on both phenolic and sulphur compounds of the *Allium sativum*. In our study ethanolic extract of *Mentha arvensis* showed a very good anti-radical activity in scavenging DPPH radical (comparable to the standard ascorbic acid) with a maximum inhibition of about 56.046 at a concentration of 50 µg/ml. At the same concentration, methanolic extract had maximum percentage inhibition of 47.106, followed by aqueous extract with a percentage inhibition of 33.513.Our finding that phenolic and flavonoid content is higher in polar solvents than aqueous solvent is supported by other studies too[53,54].We have not evaluated reducing power capacity and DPPH scavenging capacity of *Murraya koenigii*, but from literature survey it is evident that *Murraya koenigii* shows significant radical scavenging capacity due presence of chemical constituents like Koenimbine, Koenine ,Koenigine, Mahanimbine, Bismurrayafoline E, Euchrestine , Bismahanine, Bispyrafoline, Isomahanine, O-methyl murrayamine A ,O- methyl mahanine, Lutein, Tocopherol Carotene, present in leaves [55].

Polar solvents are able to extract out phenolic compounds in high concentrations [56,57]. Phenols are very important plant constituents; they show high scavenging ability of free radicals due to their hydroxyl group. Therefore, the phenolic content of plants may contribute directly to their antioxidant action [58]. The secondary plant metabolites flavonoids are significant antioxidant and have chelating properties, based on the structure and substitution pattern of hydroxyl groups [59]. Most of the extraction processes have been done with methanol as solvent but it is suggested that pure ethanol and 70% ethanol are safe solvents with lower toxic than methanol. Also, good yield and high concentration of bioactive flavonoid compounds could be isolated with these safe solvents from plant materials[60]. However it is preferred to use solvent with water instead of 100% solvent, since extraction yield increases with increases polarity in solvents used for extraction. Aqueous solvents have

shown least content in all three plant products while 70% methanolic and 70% ethanolic has shown good yield. The reason behind this may be the difference in solubility of chemical compounds in aqueous and organic solvents. And also yield and type of polyphenolics in an extract are influenced by the type and polarity of extracting solvents, time and temperature of extractions as well as physical characteristic of the samples [61,62].

5. Conclusion

It is concluded from our results that 70% methanolic extract, ethanolic extract and aqueous extract of *Allium Sativum*, *Mentha arvensis* and *Murraya koenigii* contain moderate amounts of phenolic and flavonoids; they also exhibit high antioxidant, free radical scavenging activity and reducing power ability. These in vitro assays show that plant extracts are significant sources of antioxidant components so these plants can be used as natural antioxidant sources with consequent health benefits.

Acknowledgments

This work was supported by Department of Bioscience, Jamia Millia Islamia, and New Delhi

Conflict of interest: None declared

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