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PHYTOCHEMICAL INVESTIGATION AND ANTIOXIDANT ACTIVITY OF EXTRACTS OF SOME MEDICINAL PLANTS

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ABSTRACT

In current study, we accepted out a systematic record of the relative antioxidant activity in selected medicinal plant species extracts. The total phenol varied from 25.3±7 to 278.4±7 mg g^{-1} in the extracts. Flavonoid contents were between 56±4.4 and 80.3±3.7. 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging result of the extracts was determined spectrophotometrically. The maximum radical scavenging consequence was observed in *Metha pulegiam* (Lamiacea) with IC_{50} = 0.018 mg ml^{-1}. The strength of radical scavenging power of *Metha pulegiam* extract was about 4 times better than synthetic antioxidant butylated hydroxy toluene (BHT). The superior amount of phenolic compounds leads to further potent radical scavenging result as shown by *Metha pulegiam* leaves extract.

Keywords: *Metha pulegiam*, phytochemical investigation, Antioxidant activity, *Pargularia daemia*

INTRODUCTION

Free radicals donate to more than one hundred disorders in humans counting atherosclerosis, arthritis, ischemia and reperfusion damage of numerous tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996). Free radicals due to ecological pollutants, radiation, chemicals, toxins, profound fried and spicy foods as well as corporeal stress, cause exhaustion of immune system antioxidants, modify in gene expression and persuade abnormal proteins. Oxidation development is one of the most imperative routs for producing free radicals in food, drugs and still living systems. Catalase and hydroperoxidase enzymes change hydrogen peroxide and hydroperoxides to nonradical forms and purpose as natural antioxidants in human body. Owing to depletion of immune system natural antioxidants in dissimilar maladies, overwhelming antioxidants as free radical scavengers may be essential (Halliwell, 1994; Kuhnan, 1976; Kumpulainen and Salonen, 1999; Younes, 1981). At present available synthetic antioxidants similar to butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been supposed to cause or punctual negative health effects. Consequently, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Furthermore, these synthetic antioxidants also show low solubility and reasonable antioxidant activity (Barlow, 1990; Branen, 1975). Recently there has been an increase of interest in the therapeutic potentials of medicinal plants as antioxidants in dropping such free radical induced tissue injury. Polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995). A number of confirmations suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski *et al.,* 1987). An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the occurrence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva *et al.,* 2002). In particular, despite extensive use of wild plants as medicines in Iran, the prose contains few reports of antioxidant activity and chemical composition of these plants. In current study, we carried out a systematic record of the relative free radical scavenging activity in selected medicinal plant species, which are being used traditionally: The leaves of *Metha pulegiam* (Lamiacea) and seeds of *Pargularia deamia* (Apocynaceae). We have also found the relationship of total flavonoid and phenol contents with antioxidant activity. In the longer term, plant species (or their active constituents) recognized as having high levels of antioxidant activity *in vitro* may be of value in the design of additional studies to unravel novel treatment strategy for disorders connected with free radicals induced tissue damage. Besides well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements (Schuler, 1990). Also many other plant species have been investigated in the search for novel antioxidants (Chu, 2000; Koleva *et al.,* 2002; Mantle *et al.,* 2000; Oke and Hamburger, 2002) but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned the antioxidant activity of

plants might be due to their phenolic compounds (Cook and Samman, 1996).

MATERIALS AND METHODS

Selection of Plant material and extraction

The leaves of *Metha pulegiam* and seeds of *Pargularia deamia* were collected from Mirzapur region UP, Indiain the month of July 2019. The extraction process was done by hot extraction method with soxhlet apparatus using ethanol as a solvent. This procedure was continued until the solvent became clear and collected the sample in container. After the extraction, the extract was kept in heating mantle for evaporating the solvent until the extract given in crude form.

Qualitative phytochemical analysis of plant extract

Table 1: Flavonoid and phenol contents in the studied plant extracts

Plant species	Flavonoid (mg/g)	Phenol (mg/g)
Mantha pulegium	56±4.41	278.4±71
Pargularia daemia	80.3±3.7	25.3±7

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

Table 2: Comparison of DPPH radical scavenging activity of the plant extracts and those of BHT and quercetin

Plant species	Concentration (mg/ml)	Scavenging %
Mantha pulegium	0.1	91.3±0.31
Pargularia daemia	0.7	90.3±0.5
BHT	0.4	92±0.7
Quercetin	0.030	94.6±0.8

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

Table 3: Phytochemical evaluations of different Ethanolic extracts of leaves of *Metha pulegiam* and seeds of *Pargularia deamia*

S. No.	Experiment	Ethanolic Extracts	
		Metha pulegiam	*Pargularia deamia*
Test for Carbohydrates			
1	Molisch's Test	-ve	+ve
2	Fehling's Test	-ve	+ve
3	Benedict's Test	-ve	+ve
Test for Protein & Amino acids			
4	Biuret's Test	-ve	-ve
5	Ninhydrin Test	-ve	-ve
Test for Glycosides			
6	BorntragerTest	+ve	+ve
7	Killer killaniTest	+ve	+ve
Test for Alkaloids			
8	Mayer's Test	-ve	+ve
9	Hager's Test	-ve	+ve
10	Wagner's Test	-ve	+ve
Test for Saponins			
11	Froth Test	-ve	-ve
Test for Flavonoids			
12	Lead acetate	-ve	+ve
13	Alkaline reagent test	-ve	+ve
Test for Triterpenoids and Steroids			
14	Libermann-Burchard Test	-ve	+ve
15	Salkowski Test	-ve	+ve
Test for Tannin and Phenolic compounds			
16	Ferric Chloride Test	-ve	+ve
17	Gelatin Test	-ve	+ve
18	Lead Acetate Test	-ve	+ve

Ethanolic extracts of leaves of *Metha pulegiam* and seeds of *Pargularia deamia* were subjected to the preliminary phytochemical analysis (Jain, *et. al.,* 2012 & Jain, *et. al.,* 2019). The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Test for carbohydrates

Molisch's test: In a test tube containing extract of drug, added two drop of freshly prepared 20% alcoholic solution of α- napthol and mixed concentrated sulphuric acid along the sides of the test tube. If carbohydrate present purple color or reddish violet color produce at the junction between two liquids.

Benedict's test: In a test tube containing extract of drug add benedict's solution, mix well, boiled the mixture vigorously for two minutes and then cooled. Formation of red precipitate due to presence of carbohydrates.

Barfoed's test: The barfoed's solution added to 0.5 ml of solution under examination, heated to boil. Formation of red precipitate of copper oxide was indicated the presence of carbohydrates.

Anthrone test: To the two ml of anthrone test solution, add the extract of drug. A green or blue colour indicated the presence of carbohydrate.

Test for alkaloids

Dragendorff's Test: Few mg of extract of the drug dissolved in 5 ml of water added 2 M hydrochloric acid until an acid reaction occurred; 1 ml of dragendorff's reagent (potassium bismuth iodide solution) was added an orange red precipitate indicated the presence of alkaloids.

Wagner's test: Acidify the extract of drug with 1.5 % v/v of hydrochloric acid and added a few drop of Wagner's reagent (iodine potassium iodide solution). Formations of reddish brown precipitate indicated the presence of alkaloids.

Mayer's Test: Two ml of extract solution was treated with 2 - 3 drops of Mayer's reagent was added (potassium mercuric iodide solution) formation of dull white precipitate indicated the presence of alkaloid.

Hager's Test: Extract of the drug solution was treated with 3 ml of Hager's reagent (saturated solution of picric acid) formation of yellow precipitate confirmed the presence of alkaloids.

Test for glycosides

Legal's test: Extract solution dissolved in pyridine then sodium nitroprusside solution was added to it and made alkaline. Pink red colour indicated the presence of glycosides.

Baljet's test: To the drug extract, sodium picrate solution was added, yellow to orange colour was indicated the presence of glycosides.

Borntrager's test: Few ml of dilute sulphuric acid solution, the test solution of extract was added. It was filtered and the filtrate was boiled with ether or chloroform. Then organic layer was separated to which ammonia was added, pink, red or violet colour was produced in orange layer confirmed the presence of glycosides.

Keller Kiliani test: Methanolic extract was dissolved in glacial acetic acid containing trace of ferric chloride one ml concentrated sulphuric acid was added carefully by the side of the test tube. A blue colour in the acetic acid layer and red colour at the junction of the two liquid indicated the presence of glycosides.

Test of saponins

1 ml of alcoholic extract was diluted with 20 ml distilled water and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins.

Test for flavonoids

Shinoda test: In the test tube containing alcoholic extract of the drug added 5 - 10 drops of dil. hydrochloric acid followed by the small piece of magnesium. In presence of flavonoids a pink, reddish pink or brown color was produced.

Test for tannins

To the sample of the extract, ferric chloride solution was added appearance of dark blue or greenish black colour indicated the presence of tannins.

Test for protein and amino acid

Biuret's test: To 2 - 3 ml of the extract of drug added in 1 ml of 40 % sodium hydroxide solutions and 2 drops of 1 % copper sulphate solution mix thoroughly, a purplish - violet or pinkish - violet colour produced that indicates the presence of proteins.

Ninhydrin's test: Two drops of freshly prepared 0.2 % ninhydrin reagent was added to the extract and heated to boiling for 1 - 2 min. and allow cooling. A blue colour developed that indicating the presence of proteins, peptides or amino acids.

Xanthoprotein test: To the extract in a test tube, add conc. nitric acid. A white precipitate was obtained and upon heating turns to yellow and cool the solution carefully. Added 20 % of sodium hydroxide solution in excess orange colour indicated presence of aromatic amino acid.

Millon's test: The small quantity of extract of the drug dissolved in distilled water added 5 - 6 drop of millon's reagent. A white precipitate was formed which turned red on heating, indicated the presence of proteins.

Lead acetate test: The extract was taken and two ml of 40 % sodium hydroxide solution was added and boiled, glacial acetic acid was added and cooled than added 1 ml of lead acetate solution, gray black precipitate was formed which indicated presence of sulphur containing amino acid.

Test of fats or fixed oils

Using sodium hydroxide: The extract was mixed in one ml 1 % of copper sulphate solution then added 10 % sodium

hydroxide solution a clear blue solution was obtain which showed glycerin present in sample.

Using sodium hydrogen sulphate: The extract was taken in test tube added a pinch of sodium hydrogen sulphate pungent odour was formed which showed glycerin present in sample.

Saponification: Four ml of 2 % sodium carbonate solution was taken and the extract was added. Shaked vigorously and boiled. A clean soapy solution was formed cooled and added few drops of conc. HCl and observed that fatty separate out and float up.

Determination of total phenol

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipette into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark in the 50 ml flask and left to react for 30 min for colour development. This was measured at 505 nm.

Determination of total flavonoids

10 g of the plant sample was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

***In-vitro* antioxidant activity of both extracts**

DPPH radical-scavenging activity

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Koleva *et al.,* 2002). Different concentrations of each herbal extract were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. BHT and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50 % of DPPH free radicals.

RESULT AND DISCUSSION

Flavonoid and total phenol contents of the extracts

It has been documented that flavonoids show antioxidant activity and their effects on human nourishment and health are considerable. The mechanisms of action of flavonoids are during scavenging or chelating process (Cook and Samman, 1996). Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992). The flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve equation: y = 0.0067x + 0.0132, r2 = 0.999) were between 56±4.4 and 80.3±3.7 (Table 1). The flavonoid contents in the extracts of *Metha pulegiam* (56 ± 4.4 mg g^{-1}) and *Pargularia deamia* (80.3 ± 3.7 mg g^{-1}). Table 1 also show the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 0.05x + 0.0545, r2= 0.9873). The total phenol varied from 25.3±7 to 278.4±7 mg g^{-1} in the extract powder. *Metha pulegiam* with total phenol contents of 278.4±7 mg g^{-1} had the highest amount among the plants in this study. The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants (Das and Pereira, 1990; Younes, 1981). According to our study, the high contents of these phytochemicals in *M Metha pulegiam* can explain its high radical scavenging activity.

Antioxidant activity

Free radicals are concerned in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants during their scavenging power are helpful for the management of those diseases. DPPH stable free radical method is an easy, rapid and receptive way to survey the antioxidant activity of a precise compound or plant extracts (Koleva *et al.,* 2002). IC_{50} of the standard compounds, BHT and quercetin were 0.044 and 0.01 mg ml^{-1}, respectively. The uppermost radical scavenging activity was showed by *Metha pulegiam* with IC_{50}=0.018 mg ml^{-1} which is higher than that of BHT ($P<0.05$). The radical scavenging activity in the plant extracts decreased in the subsequent order: *Metha pulegiam* > *Pargularia deamia*. Most of the plants extracts at dissimilar concentrations exhibited more than 70 % scavenging activity (Table 2). The radical scavenging effect of *Metha pulegiam* at 0.1 mg ml^{-1} was similar to BHT at 0.4 mg ml^{-1}. Therefore, the antioxidant effect of *Metha pulegiam* was 4 times greater than that of the synthetic antioxidant, BHT.

CONCLUSION

The result of the current study showed that the extract of *Metha pulegiam*, which hold highest amount of flavonoid and phenolic compounds, exhibited the maximum antioxidant activity. The high scavenging property of *Metha pulegiam* may be due to hydroxyl groups accessible in the phenolic compounds' chemical structure that can offer the necessary component as a radical scavenger. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a probable anticipatory intervention for the diseases (Gyamfi et al., 1999). All of the extracts in this research exhibited dissimilar amount of antioxidant activity. *Metha pulegiam* extract showed a higher potency than Nutritional Biochemistry BHT in scavenging of DPPH free radical. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract.

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