ISSN : 2347 - 8446 (Online)
ISSN : 2347 - 9817 (Print)

Analysis of Phytochemical and Antioxidant Activities of the Aqueous Extract of Some Medicinal Plant

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Abstract
Many oxidative stress related diseases are as a result of accumulation of free radicals in the body. A lot of researches are going on worldwide directed towards finding natural antioxidants of plants origins. [Family Asteraceae] aqueous crude extract.The objective of the present study was to evaluate the phytochemical constitution and antioxidant activity of methanolic extract of dried leaves of medicinally important herbs Ocimum sanctum, Mentha spicata. Qualitative analysis of phytochemical constituents' viz. tannins, phlobatannins, saponins, flavonoids, steroids, alkaloids, quinones, coumarin, terpenoids and cardiac glycosides and quantitative analysis of total phenolics, alkaloids, saponins and flavonoids was performed by the well-known tests protocol available in the literature. Quantitative analysis of phenolics, alkaloids, saponins and flavonoids had revealed that Mentha spicata possessed maximum phenolic (18.41 %). Antioxidant activity was determined by DPPH radical scavenging and reducing power assays. IC50 values obtained by DPPH activity for Mentha spicata crude extract was found to be 170µg/ml and reducing power was found to be maximum (1.92) at 1mg/ml concentration. The results suggest that Mentha spicata has promising antioxidant activity and could serve as potential source of natural antioxidants. The aims of this study were to evaluate in vitro antioxidant activities and to screen for phytochemical constituents of Helichrysum longifolium DC.

Keywords
Helichrysum longifolium DC, Mentha spicata,Ocimum sanctum,Reactive Oxygen Species, DPPH.

I. Introduction

We assessed the antioxidant potential and phytochemical constituents of crude aqueous extract of*Helichrysum longifolium* using tests involving inhibition of superoxide anions, DPPH, H_2O_2, NO and ABTS. The flavonoid, proanthocyanidin and phenolic contents of the extract were also determined using standard phytochemical reaction methods. Free radicals can initiate the oxidation of bio molecules, such as protein, lipid, amino acids and DNA which will lead to cell injury and can induce numerous diseases[1]. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage and oxidative stress is the main cause of several diseases: cancer, cataracts, age related diseases and Parkinson's disease. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases[2]. Many of these indigenous medicinal plants are used as spices and food plants. Phenolics have been known to possess a capacity to scavenge free radicals. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors. Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species (ROS) in the cellsThe use of this herb has been reported in Indian Traditional Systems of Medicine and its modern applications are receiving wide spread attention day by day. The plant has also been shown to reduce blood glucose levels, making it an effective treatment for diabetes. Mentha spicata is a perennial herb commonly known as mint and spearmint, belong to the family Lamiaceae. Mentha spicata have traditionally been used in folk medicine. The distinctive smell and flavor is a characteristic feature of Mentha Spp and it is due to the naturally occurring cyclic terpene alcohol called menthol.

II. Methods

Phenolics are especially common in leaves, flowering tissues and woody parts, such as stems and barks. Studies have shown that they play an important preventive role in the development of cancer, heart diseases and ageing related diseases. In the present study the phytochemical screening and antioxidant activities of four medicinally important herbs Ocimum sanctum (Tulsi), Mentha spicata (Pudina)[3]. These herbs were taken in different ways in our diet as Tulsi and Pudina as one of the tea components, Fenugreek as spice as well as green vegetable, Spinach as vegetable. . Ocimum sanctum belongs to the family, Lamiaceae. Tulsi has been widely known for its health promoting and medicinal value for thousands of years. Fresh leaves of selected herbs Ocimum sanctum, Mentha spicata and the specimen was authenticated by renowned taxonomist. The leaves were washed, cleaned and chopped into pieces and dried at 40 oC in thermostatically controlled oven until they attained a constant weight. The samples were then crushed into powder, using mechanical grinding machine, so as to enhance effective contact of solvent with sites on the plant materials.

1. Preparation of extract

The powdered plant material (200 g) was extracted thrice in distilled water (5.5 L; 27°C-30°C) on shaker (Stuart Scientific Orbital Shaker, UK) for 48 hours. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper[4]. The filtrate of aqueous extract obtained was quickly frozen at -40°C and dried for 48 h using a freeze dryer (Savant Refrigerated vapor Trap, RV T41404, USA) to give a yield of 30 g of dry extract. The resulting extract was reconstituted with distilled water to give desired concentrations used in this study. 10 g of each powdered leaves were placed in conical flask and 100 ml of methanol was added and plugged with cotton. The powder material was extracted with methanol for 24 hours at room temperature with continuous stirring[5]. After 24 hours the supernatant was collected by filtration

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and the solvent was evaporated to make the crude extract. The residues obtained were stored in airtight bottles in a refrigerator for further use.

III. In Vitro Antioxidant Activity

The DPPH radical scavenging method was used to evaluate the antioxidant property. The antioxidant activity was compared with that of the natural antioxidant, ascorbic acid. The concentrations of the plant extracts required to scavenge DPPH showed a dose dependent response. The antioxidant activity of each sample was expressed in terms of IC50, and was calculated from the graph after plotting inhibition percentage against extract concentration PPH assay was carried out after making some modifications in the standard protocol. 1.5 ml of 0.1 mM DPPH solution was mixed with 1.5 ml of various concentrations (10 to 500 µg/ml) of leaf extract[6]. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

Inhibition (%) = [(Acontrol-Atest)/Acontrol] × 100

Where Acontrol is the absorbance of the control (L-Ascorbic acid) and Atest is the absorbance of reaction mixture samples (in the presence of sample). All tests were run in triplicates (n=3), and average values were calculated.

A. Chemicals

All chemicals were of highest purity (≥99.0%). Ferric chloride, HCl, Dragendorff's reagent, magnesium metal strips, methanol, gallic acid, commercial saponins were purchased from BDH, England, blood agar from Biolab, South Africa, chloroform, H2SO4, Folin-Ciocalteu reagent, Na2CO3, vanillin, aluminium chloride, potassium acetate, phosphate buffer, K3Fe(CN)6, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), thiocyanate (FTC), butylated hydroxyl toluene (BHT), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS), potassium persulphate, sodium nitroprusside, hydrogen peroxide, sulfanilic acid, glacial acetic acid, naphthylethylenediamine dichloride, potassium metabisulphite (PMS), NADH were all purchased from Merck, USA.

B. Phytochemical screening of the plant extract

A small portion of the dry extract was used for the phytochemical tests for compounds which include tannins, flavonoids, alkaloids, saponins, and steroids in accordance with the methods of [17,18] with little modifications. Exactly 1.0 g of plant extract was dissolved in10 ml of distilled water and filtered (using Whatman No 1 filter paper) A blue colouration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of tannins in the extract[7,8]. Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. A millilitre of the filtrate was treated with few drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloid. About 0.2 g of the extract was dissolved in 2 ml of methanol and heated.

IV. Results

The phytochemical analysis conducted on *H. longifolium* extract revealed the presence of tannins, flavonoids, steroids and saponins. The total phenol content of the aqueous leaf extract was 0.499 mg gallic acid equivalent/g of extract power. The total phenolic content of the aqueous leaf extract was 0.499 mg gallic acid equivalent/g of extract powder. The total flavonoid and proanthocyanidin contents of the plant were 0.705 and 0.005 mg gallic acid equivalent/g of extract powder respectively[9].

Table 1
The phytochemical components of *H. longifolium* based on the preliminary aqueous crude leaf extract screening

Phytochemical Compounds	Presence	Extract equivalent of Gallic (mg/g)
Tannins	+	ND
Flavonoids	++	ND
Steroids	+++	ND
Alkaloids	-	ND
Saponins	+	ND
Total phenol	+++	0.499
Total flavonoids	+++	0.705
Total proanthocyanidin	+	0.005

+++ = appreciable amount (positive within 5 mins.); ++ = moderate amount (positive after 5 mins. but within 10 mins); + = trace amount (positive after 10 mins. but within 15 mins); - = completely absent.

The percentage inhibition of lipid peroxide at the initial stage of oxidation showed antioxidant activity of 87% compared to those of BHT (84.6%) and gallic acid (96%). Also, the percentage inhibition of malondialdehyde by the extract showed percentage inhibition of 78% comparable to those of BHT (72.24%) and Gallic (94.82%). The total flavonoid and proanthocyanidin contents of the plant were 0.705 and 0.005 mg gallic equivalent/g of extract powder respectively with reference to standard curve ($Y = 0.0067x+0.0132$, $r^2 = 0.999$). These phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities of this plant extract used in this study.

A. Quantitative analysis of selected species

Medicinal plants constitute the group of plants mainly used for health care[10]. Use of them as traditional medicine is known since time immemorial. Chemicals present universally in all the plants can be classified as primary and secondary metabolites. Primary metabolites include proteins, amino acids, sugars, purines and pyrimidines of nucleic acids, chlorophylls etc., while secondary phytochemicals as alkaloids to terpenoids and acetogenins to different phenols. These are chemically and taxonomically extremely diverse compounds with obscure function.

Table 2: Total Phenolics, alkaloids, saponins and flavonoids in selected flora

Methanolic extracts	phenolic (%)	Alka-loids %	Saponins %	Flavonoids %
Ocimum sanctum	17.66	0.65	0.50	9.88
Spinacia Oleracea	18.00	1.22	5.22	16.98

B. DPPH radical scavenging activity and Total Antioxidant Capacity

The *in vitro* antioxidant assay of the plant extract reveals significant antioxidant potential compared with standard BHT and gallic

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ISSN : 2347 - 8446 (Online)
ISSN : 2347 - 9817 (Print)

acid (final concentration of 0.02% w/v). DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts[11]. Fig 1 shows DPPH radical scavenging activities of the extracts depended not only on plant type but also upon the extraction solvent.

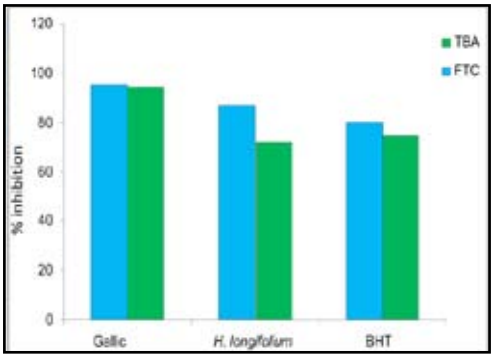

Fig. 1: Antioxidant properties of plant extract

In general, DPPH scavenging activities increased with increasing phenolic components such as flavonoids, phenolic acids, and phenolic diterpenes. The percentage inhibition of lipid peroxide at the initial stage of oxidation showed antioxidant activity of 87% compared to BHT (84.6%) and gallic acid (96%), and the percentage inhibition of malondialdehyde by the extract showed percentage inhibition of 78% compared to both BHT (72.24%) and gallic (94.82%). Antioxidant activity in the form of IC50 values of different extracts were calculated[12,13].Highest antioxidant activity was given by Mentha spicata extract at the concentration of 170µg/ml among all the methanolic leafs which is found to be more than even the ascorbic acid while activity of Ocimum sanctum was found to close to the standard. Thus it is clear that polyphenolic antioxidants in leaves of selected plants play an important role as bioactive principles and the scavenging effect can be attributed to the presence of active phytoconstituents in them.

V. The Reducing Power Potentials of The Extract

Figure2 shows the reducing power potentials of the aqueous extract of the test plant in comparison with a standard BHT at 700 nm. The reducing capacity of the extract, another significant indicator of antioxidant activity was also found to be appreciable[14]. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's blue at 700 nm. Increasing absorbance indicates an increase in reductive ability[15]. The results show that there was increase in reducing power of the plant extract as the extract concentration increases.

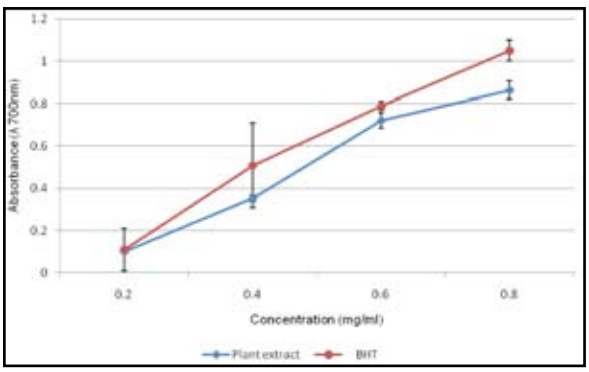

Fig 2: Reducing power activities of the aqueous extract of H. longifoliumin comparison with a standard (BHT) at λ = 700 nm. BHT: Butylated hydroxyl toluene[16].

VI. Discussion

Alkaloid was not detected in this study plant. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity, and their absence in this plant tend to lower the risk of poisoning by the plant. Antioxidants block the action of free radicals which have been implicated in the pathogenesis of many diseases and in the aging process. An important role is being played by free radicals in governing the various biological processes which are necessary for the body.They have their role in implicating cell-signaling mechanism occurring in our body. This shows that free radicals are necessary but at the same time harmful for the body.The result of DPPH scavenging activity assay in this study indicates that the plant was potently active. It was a woody climber belonging to Ranunculaceae family and whole plant is used as medicine for different problems. Powdered plant material was found to have alkaloid 0.86%w/w, total phenol 0.72 %w/w, tannin 8.72 %w/w, flavonoids 0.56 %w/w and saponin 2.86 %w/w were present in the aerial parts. Phytochemical studies on methanolic and ethyl acetate extracts of leaves of Anogeissus leiocarpus and showed that the plant contains alkaloids (152.0 ± 0.1 mg/g), phenolics (1294.81 ± 3.0 mg/g), flavonoids (330.7 ± 3.0 mg/g) in the methanol extra ct and alkaloids (80.20 ± 0.0 mg/g), phenolics (616.5 ± 4.4 mg/g), flavonoids (202.5 ± 4 .0 mg/g) in the ethyl acetate extract respectively .The methanol extract of the leaves of the plant Leucas aspera has been tested for the determination of antioxidant activity by reducing assay and found that reducing power increases with the increase in concentration of the crude extract. Free radical scavenging potential of the different extracts of leaves of Oroxylum indicum (L.) Vent. (Bignoniaceae), one of the widely used medicinal plant, was evaluated in vitro by using diphenylpicryl- hydrazyl (DPPH) assay. The results were expressed as IC50. Ascorbic acid was used as standard showed an IC50 of 24.0 ug/mL, whereas, the crude ethyl acetate (I), methanolic (II) and water (III) extracts of leaves of O. indicum showed IC50 values of 49.0, 55.0 and 42.5 respectively at 100 mg/mL concentration[15,16]. Nitric oxide (NO) is a reactive free radical produced by phagocytes and endothelial cells, to yield more reactive species such as peroxynitrite which can be decomposed to form OH radical. The level of nitric oxide was significantly reduced in this study by the crude extract. Since NO plays a crucial role in the pathogenesis of inflammation, this may explain the use of *H. longifolium* for the treatment of inflammation and for wound healing.Plants with antioxidant activities have been reported to possess free radical scavenging activity. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism.

VII. Conclusion

Our findings provide evidence that the crude aqueous extract of *H. longifolium* is a potential source of natural antioxidants, and this justified its uses in folkloric medicines. Phytochemical screening of methanolic extracts of herbs Ocimum sanctum, Mentha spicata results obtained in this investigation indicate that Mentha spicata leaf extract, rich in phenolics exhibited highest antioxidant and reducing activities. Total phenolic content had positive correlation

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with antioxidant capacity. It was observed that the leaf extract contained high level of phenolic content that might have accounted for the strong activity observed against DPPH radicals. This study affirms the *in vitro* antioxidant potential of crude extract of the leaf of *Helichrysum longifolium*, with results comparable to those of the standard compounds such as gallic acid and butylated hydroxyl toluene (BHT). Further studies are needed to clarify the *in vivo* potential of this plant in the management of human diseases resulting from oxidative stress and this is a subject of investigation in our group.

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