Phytochemical Constituent and *in Vitro* Antioxidant Studies of Crude Extracts of Selected Medicinal Plants

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Abstract

The crude methanolic extracts of five medicinal plants were screened for their antioxidant and free radical scavenging properties. The objective of the present study was the evaluation of antioxidant activity of methanolic extracts of *Ocimum sanctum* (OS), *Ocimum kilimandsacharicum* (OK), *Garcinia mangostana* (GM),*Swertia chirayata* (SC) & *Andrographis paniculata* (AP) done through various *in vitro* assays like (DPPH)· radical scavenging activity and ($FeCl_3$)assay. The overall antioxidant activity of *Swertia chirayata* was the strongest, followed in descending order by *Ocimum sanctum, Garcinia mangostana, Andrographis paniculata & Ocimum kilimandsacharicum.* Phytochemical analysis of plant extracts indicated the presence of major phytocompounds, including phenolics, alkaloids, glycosides, flavonoids, and tannins. The tested plant extracts showed promising antioxidant and free radical scavenging activity, thus justifying their traditional use.

KEYWORDS: - Methanolic extracts, Medicinal plants, Antioxidant activity, Phytochemical analysis, Phytocompounds

INTRODUCTION

The natural antioxidants extracted from herbs & spices having high antioxidant activities are gaining much importance nowadays. The first reports on antioxidant studies of seventy two medicinal plants appeared in the 50's [1, 2]. Natural antioxidants are used in many food products. Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, alzheimer's disease, and cancer have gained much importance during the last three decades [3]. Antioxidizing agents are substances capable to protect the organism against destructions caused by free radicals.

They can be divided into: primary antioxidants and secondary antioxidants. Most of the antioxidants used are primary antioxidants. They are phenolic compounds like phenol acids, flavonoids, anthocyanidins, lignans, tannins, coumarins etc.

Secondary antioxidants include metallic complex agents, singlet oxygen and others. Antioxidants help prevent the free radical damage that is associated with cancer and heart disease. Antioxidants can be found in most fruits, vegetables, culinary herbs and medicinal herbs [4, 5]. The preservative effect of many plant and herbs suggest the presence of antioxidative and antimicrobial constituents [6]. Plant's secondary metabolites have been referred to as phytochemicals. Phytochemicals are naturally occurring and biologically active plant compounds that have potential disease inhibiting capabilities. The medicinal

values of the plants lie in these phytochemicals, which produce definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds [6, 7].

It is believed that phytochemicals may be effective in preventing disease due to their antioxidant effect [8, 9]. Antioxidants protect other molecules (*in vivo*) from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the etiology of many diseases and in food deterioration and spoilage [10, 11]. Antioxidants protect food by delaying or inhibiting free radical oxidation [12].

Synthetic antioxidants like butylated hydroxytoulene, butylated hydroxyanisole and propyl gallate are being used in food industries [13]. According to some references herbal antioxidant level can be as high as 465 mmol per 100 g [14]. As indicated in another reference the consumption of smaller quantities of many phytochemicals may result in more health benefits than the consumption of larger quantities of fewer phytochemicals [15].

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds.

Plant essential oils and extracts have been used for thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate such plants used in traditional medicine scientifically, to improve the quality of healthcare. The antioxidant activity of these plant extracts has been previously reviewed and classified as strong, medium or weak [16-26].

MATERIALS AND METHODS:-

The objective of the present study was the evaluation of antioxidant activity of methanolic extracts of *Ocimum sanctum* (OS), *Ocimum kilimandsacharicum* (OK), *Garcinia mangostana* (GM), *Swertia chirayata* (SC) & *Andrographis paniculata* (AP) done through various *in vitro* assays like DPPH· radical scavenging activity and $FeCl_3$ assay.

EXPERIMENTAL SECTION: -

DPPH. FREE RADICAL SCAVENGING ACTIVITY –

The free radical scavenging effects of methanolic extracts of *Ocimum sanctum* (OS), *Ocimum kilimandsacharicum* (OK), *Garcinia mangostana* (GM), *Swertia chirayata* (SC) & *Andrographis paniculata* (AP) was determined by the DPPH assay. DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. The free radical scavenging of crude extracts was determined by the method of Ganesan et. al [27]. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. 0.1 mM solution of DPPH in methanol was prepared and 3 ml of this solution was added to the test tube containing 1 ml of sample solutions in methanol or water at different concentrations (10, 20, 40g/ ml). Thirty minutes later after incubation at room temperature, the absorbance was measured at 517 nm. The absorbance of the similar reaction mixtures of methanol or water (without plant extracts) with DPPH served as control.

$FeCl_3$ ASSAY-

The Fe^{+++} reducing power of methanolic extracts of *Ocimum sanctum* (OS), *Ocimum kilimandsacharicum* (OK), *Garcinia mangostana* (GM), *Swertia chirayata* (SC) & *Andrographis paniculata* (AP) was determined according to the method of Oyaizu [28].1.0 ml of methanol or water containing different concentrations of extracts (10, 20, 40g/ ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1% w/v). Reaction mixture was incubated at 50^0C for 20 min. After incubation, 2.5 ml of trichloroacetic acid (10%) was added and centrifuged (650g) for 10 min. From the upper layer, 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1% w/v in water). Absorbance of all the sample solutions was measured at 700 nm. The absorbance of the similar reaction mixtures without plant extracts served as control.

RESULTS AND DISCUSSION:-

The capability of the extracts to scavenge the DPPH radical was calculated using the following equation.

DPPH Scavenged (%) = A (cont) - A (test) X 100/ A (cont)

A (cont) – Absorbance of control

A (test) – Absorbance of the test extracts

From the percentage DPPH· free radical scavenging values, it was found that the methanolic extract of *S. chirayata* was the most efficient scavenger followed by methanolic extracts of *O. sanctum*, *G. mangostana, A. paniculata & O. kilimandsacharicum* at 10g/ ml, 20g/ ml & 40 g/ ml concentration with maximum scavenging at 40 g/ ml conc. of the extract. The present work revealed that all the extracts possess considerable antioxidant potential.

From the percentage scavenging values in $FeCl_3$ assay, it was found that the methanolic extract of *G. mangostana* was the most effective for the reduction of iron (III) followed by methanolic extracts of *S. chirayata*, *A. paniculata, O. sanctum & O. kilimandsacharicum* at 10g/ ml, 20g/ ml & 40 g/ ml concentration with maximum activity at 40 g/ ml conc. of the extract.

The results of the DPPH· free radical scavenging assay suggest that components within the extracts are capable of scavenging free radicals via electron or hydrogen donating mechanisms and thus should be potent enough to prevent the initiation of deleterious free radical mediated chain reactions, presumably because of its phytochemical constituents. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. And higher absorbance of the reaction mixture in $FeCl_3$ assay indicates greater reducing power. All the absorbance values in both the assays were compared to the control system (without extract) used.

TABLE 1:- PHARMACOGNOSTIC FEATURES OF THE MEDICINAL PLANTS

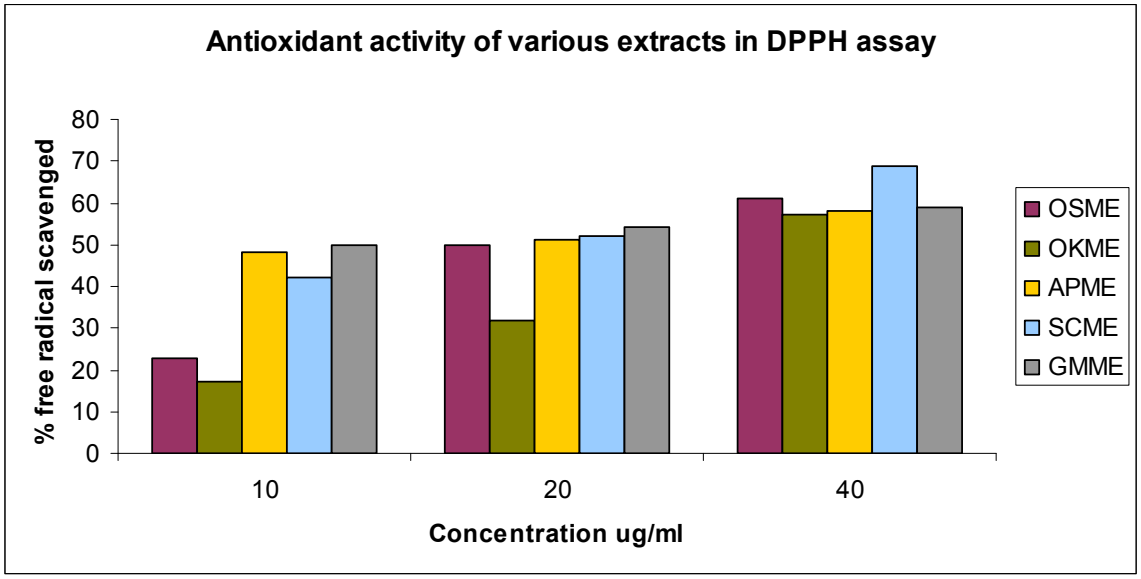
S.no.	Plant name/ Family	Common Name	Part used	Medicinal properties
1	*Ocimum sanctum*/ Lamiaceae	Tulsi	Whole plant	antistress, analgesic, antipyretic, anti-inflammatory, antihypertensive, CNS depressant, radio protective activities etc
2	*Ocimum kilimandsacharicum* / Lamiaceae	Kapur tulsi	Whole plant	antistress, analgesic, antipyretic, anti-inflammatory, antihypertensive, CNS depressant, radio protective activities etc
3	*Garcinia mangostana*/ Guttiferae	Mangosteen	Fruit	anti-inflammatory, CNS depressant, anti-Alzheimer, anti-arthritis, anti-Parkinson's etc
4	*Swertia chirayata*/ Gentianaceae	Chirayita	Whole plant	anti-inflammatory, anti-helmintic, astringent, antimalarial, CNS depressant etc
5	*Andrographis paniculata*/ Acanthaceae	Kalmegh	Whole plant	anti-fertility, CNS depressant, anti-inflammatory, anti- HIV, anti-cancer activities, etc

TABLE 2:- RESULTS OF PHYTOCHEMICAL ANALYSIS

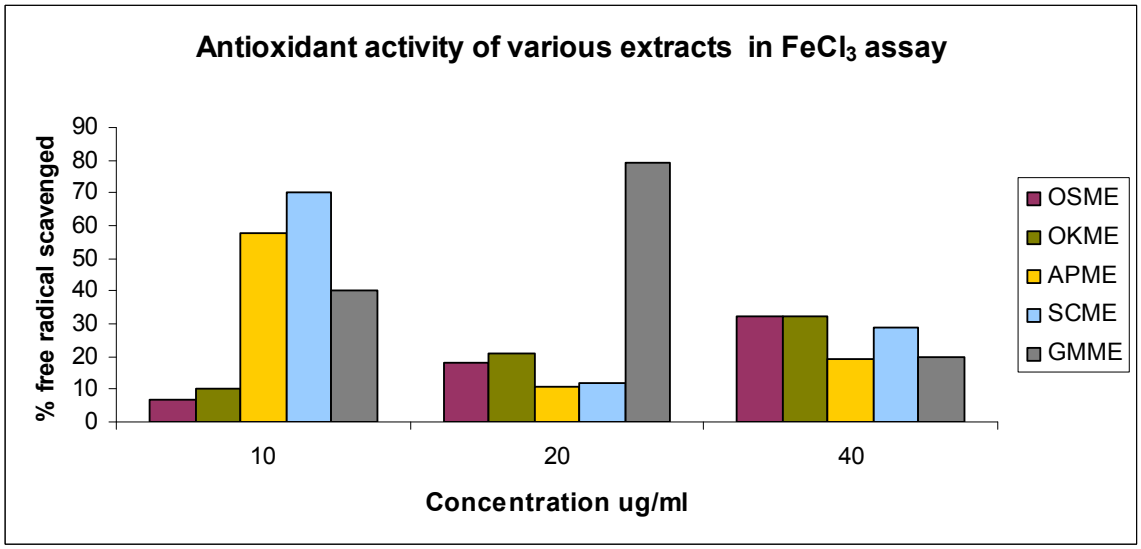
S.no.	Plant Constituents	Test / Reagent	OS	OK	GM	SC	AP	GG
1	Sterols	Salkowski Reaction, Lieberman Burchard reaction	A	A	A	P	P	A
2	Alkaloids	Dragendorff's Reagent, Mayer's reagent, Wagner's reagent, Hager's reagent	P	P	A	A	A	A
3	Tannins	Ferric chloride test, Lead acetate test	P	P	P	P	P	A
4	Flavonoids	Shinoda test	A	A	A	P	P	P
5	Carbohydrates	Molisch's test, Barfoed's test	A	A	P	P	P	P
6	Proteins	Xanthoproteic test, Biuret test	A	A	A	A	A	A
7	Amino acids	Ninhydrin test	A	A	A	A	A	A
8	Saponins	Foam test	P	P	A	A	A	P
9	Glycosides	General test	P	P	A	A	A	P
10	Phenolic	Ferric chloride test, Lead acetate test	P	P	P	P	P	A

Where (OS) - *Ocimum sanctum*; (OK) - *Ocimum kilimandsacharicum*; (GM) - *Garcinia mangostana;* (GM) - *Swertia chirayata* (SC) & (AP) *Andrographis paniculata*
P indicates presence of constituent; A indicates absence of constituent

GRAPH 1:- ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS IN DPPH ASSAY



GRAPH 2:- ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS IN $FeCl_3$ ASSAY



Where OSME - Methanolic extract of *O. sanctum,* OKME- Methanolic extract of *O. kilimandsacharicum,* APME -Methanolic extract of *A. paniculata,* SCME -Methanolic extract of *Swertia chirayata &* GMME- Methanolic extract of *G. mangostana*

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