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FREE RADICAL SCAVENGING CAPACITY AND ANTIOXIDANT ACTIVITY OF *ONOSMA BRACTEATUM*

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ABSTRACT

Damage to cells caused by free radicals is alleged to engage in recreating a fundamental task in the aging progression as well as in disease progression. Antioxidants are our initial line of resistance aligned with free radical damage, furthermore are decisive for maintaining optimal healthiness with happiness. The necessitate intended for antioxidants become yet additional crucial with enlarged revelation to free radicals. Free radical revelation can enhance by pollution, cigarette smoke, drugs, illness, stress, and even exercise. Since so many factors can put in to oxidative stress, individual assessment of susceptibility becomes important. As a fraction of a vigorous lifestyle also a well-balanced, wholesome diet, antioxidant supplementation is at the moment being renowned as an imperative means of recuperating free radical protection. There has been mounting interest in the advantageous health effects of overwhelming plants. Chiefly, the occurrence of phenolic antioxidants is supposed to have the defensive mechanisms. The antioxidant activity of the methanolic extract of Onosma bracteatum was evaluated. Methanolic extract be evaluate for its reducing power, hydrogen peroxide scavenging activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity. High content of saponin, tannin and alkaloids may account for the antioxidant activity of the plant. This Study shows the impending of the methanolic extract of Onosma bracteatum as a natural antioxidant.

Key words: *Antioxidant, DPPH, Reducing power, Scavenging activity*

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INTRODUCTION

Free radicals causes cell damage and appears to be there a foremost giver to aging in addition to to degenerative diseases of aging like as cancer, cataracts, cardiovascular disease, brain dysfunction as well as immune system decline[1]. On the whole, free radicals included in the pathogenesis of at slightest 50 diseases[2,3]. Providentially, free radical configuration is inhibited logically by an assortment of valuable compounds recognized as antioxidants. It is as the accessibility of antioxidants is inadequate to facilitate this injure that can turn out to be growing also devastating. These are electrically charged molecules, i.e., they have an unpaired electron, which causes them to look out along with incarcerate electrons commencing former substances in command to counterbalance themselves. Even though the preliminary assault source the free radical turns out to be neutralized, an additional free radical is twisted in the progression, causing a series rejoinder to take place. Moreover in anticipation of successive free radicals are deactivated, thousands of free radical reactions be able to take place within seconds of the preliminary reaction. Antioxidants are proficient of stabilizing, or deactivating, free radicals earlier than they assault cells. Antioxidants are enormously decisive for maintaining optimal cellular plus systemic health along with well-being. Hence a lot of consideration has been focused on the use of antioxidants, particularly natural antioxidants to reduce peroxidation furthermore to defend from damage owing to free radicals.

The therapeutic property of plants has been investigated, in the brightness of topical methodical development, in the course of the world outstanding to their compelling pharmacological actions moreover to profitable viability. A enormous amount of pungent, peppery, medical along with other plants enclose chemical compounds, exhibiting antioxidant properties. Numerous antioxidant compounds acquire antiinflammatory, antiatherosclerotic, antitumor, anticarcinogenic,, antibacterial with antiviral activities to a superior or slighter extent[4]. Natural antioxidants like as flavonoids, phenolics, tannins, saponin and terpenoids are originate in a variety of plants[5]. They can diminish the admittance of oxidants along with other detrimental molecules owed to their capability to forage oxygen-nitrogen-derived free radicals by donating hydrogen atom or an electron, chelating metal catalysts, activating antioxidant enzymes, as well as inhibiting oxidases[6]. In current decades, incredible attention have significantly augmented in verdict of natural substances (i.e. antioxidants) in attendance to medicinal plants to reinstate synthetic antioxidants, which are being constrained due to their side effects.

Onosma bracteatum, belongs to family Boraginaceae. It is generally known as Gaozaban, Gojihva and Sedge . It is a average sized perennial herb and the stems are as many, simple, hairy, arising from a importunate cluster of radical leaves, which are lanceolate and with conspicuous hairy pallid bases. The drug is worn as a tonic, demulcent, alterative, refrigerant, as well as diuretic, furthermore it is constructive as a spasmolytic. Flowers are hermaphrodite i.e. they have both male along with female organs and they are pollinated by Insects. It is useful in rheumatism, alterative, diuretic, syphilis, leprosy along with heart diseases. It is one of such therapeutic plant recognized traditionally in Ayurveda for the dealing of asthma as well as bronchitis[7,8].

MATERIALS AND METHODS

Collection:

Authentic samples: Various market samples of *Onosma bracteatum* were procured from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of March, 2010.

Identification:

All the samples were authenticated and were given identification number. The identification was as follows:

These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Processing of plant materials:

During the course of the study each sample was screened for its foreign matter and milled, before use.

Experimental details:

Present studies were performed on *Onosma bracteatum* for the following studies-.

1. Phytochemical test of plant extract
2. Antioxidant Potentials of Methanolic extract of plant

1. PHYTOCHEMICAL SCREENING

Phytochemical screening was performed using standard procedure:

TEST FOR REDUCING SUGARS (FEHLINGS TEST)

The aqueous ethanol extract (0.5gm in 5 ml of water) was added to boiling fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction.

TEST FOR TERPENOIDES (SALKOWSKI TEST)

To 0.5 gm each of the extract was added to 2ml of chloroform. Concentrated sulphuric acid (3ml) was carefully added to form a layer. Reddish brown coloration of the interface indicates the presence of terpenoides.

TEST FOR FLAVONOIDES

4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated Hydrochloride acid was added and red colour was observed for flavonoids and orange color for flavons.

TEST FOR TANNINS

About 0.5 g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration.

TEST FOR SAPONINS

To 0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously. And observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

TEST FOR ALKALOIDS

Alkaloids solutions produce white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's regent.

The alcoholic extract was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagent. The sample was then observed for the turbidity or yellow precipitation.

2. ANTIOXIDANT ACTIVITY

Preparation of test extracts

All the test plant sample and their adulterants were milled and refluxed in ethanol for 36 h, filtered, concentrated to dryness *in vacuo*. A portion of ethanolic extract was further successively extracted in pet. ether, benzene, chloroform, alcohol and water, concentrated and stored at minimum temperature, until used.

Preparation of DPPH

DPPH (2, 2'-diphenyl-1-picrylhydrazl, $C_{18}H_{12}N_5O_6$; Hi media) 0.8 mg was dissolved in 10 ml methanol to obtain a concentration of 0.08 mg/ml for antioxidative (qualitative and quantitative) assay.

Qualitative assay

Each successive extract (10 mg) was dissolved in 10 ml of its suitable solvent to get a concentration of 1 mg/ml and from this, 0.25µl was taken with the help of micropipette, applied on silica gel G coated plates. These circular spots were sprayed with DPPH solution, allowed to stand for 30 min. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced, and the changes in colour (from deep- violet to light- yellow on white) were recorded at 517 nm on a UV spectrophotometer (Varian Cary PCB 150, Water Peltier System).

Quantitative assay

A concentration of 1 mg/ml of ethanolic extract of each test sample was prepared to obtain different concentrations (10^2µg to 10^{-3} µg/ ml). Each diluted solution (2.5 ml each) was mixed with DPPH (2.5ml). The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured. Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured. The UV absorbance was recorded at 517 nm. The

experiment was done in triplicate and the average absorption was noted for each concentration. Data were processed using EXCEL and concentration that cause 50% reduction in absorbance (RC_{50}) was calculated. The same procedure was also followed for the standards- quercetin and ascorbic acid.

RESULTS AND DISCUSSION

CONCENTRATION (µg/ml)	O.D (nm)
0.001	0.593
0.01	0.570
0.1	0.564
1	0.535
10	0.490
100	0.468
1000	0.447

Table 1: Showing Optical density of *Onosma bracteatum* on different concentrations

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In current presentation, attempts have been made to come across for the methanolic extract which has the potentials as equivalent to antioxidant agents as the methanolic extracts of *O.bracteatum* shows the antioxidant activity which is as analogous to ascorbic acid. All the way through the present investigation it was showed that the maximum optical density comes out to be 0.593 nm which is at the concentration 10^{-3} µg/ml and the smallest optical density is 0.447 nm which is at the concentration 10^{3} µg/ml where as the other shows comparable O.D at different concentrations i.e. 0.570 nm at 10^{-2}µg/ml, 0.564 nm at 10^{-1} µg/ml, 0.535 nm at 1µg/ml, 0.490 nm at 10^{1} µg/ml, 0.468 nm at 10^{2} µg/ml.

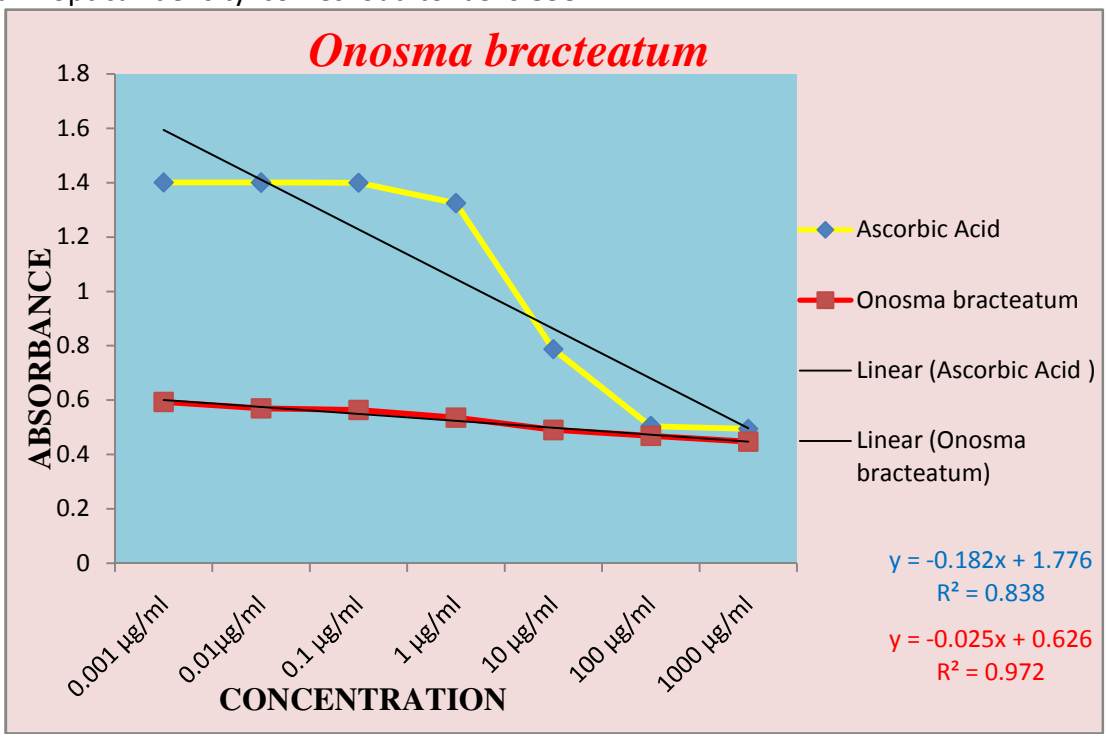

Fig 1: Graph showing Antioxidant Activity of *Onosma bracteatum* at different concentration.

In the current investigations antioxidant activity of *Onosma bracteatum* shows considerable activity associated with the DPPH assay method wherever the regression line perceptible shows the efficacy of it as it has the potentials which are equivalent to ascorbic acid. The antioxidant activity of *Onosma*

bracteatum methanolic extract using DPPH assay method shows generous activity which is as similar to standard ascorbic acid. The straight line showed Y= -0.182x+1.776 & regression = 0.838 whereas, in above drug the straight line is Y= -0.025x+0.626 & regression = 0.972.

Onosma bracteatum						
TEST	**Reducing Sugar**	**Saponin**	**Tannin**	**Terpenoides**	**Flavonoides**	**Alkaloides**
	-	**+**	**+**	**-**	**-ve**	**+**

Table 2: Showing phytochemical screening results of *Onosma bracteatum*.

The phytochemical screening of *Onosma bracteatum* shows the occurrence of Saponin, Tannin and Alkaloids whereas it shows the absence of flavonoids respectively. The screening of the *Onosma bracteatum* make only a a small amount of differences in the constituent of the hard-edged plants. The drug shows the substantiation of strong antioxidant activity complementary or in a less important amount. The existence of alkaloids in this plant is credible to be meticulous for the free radical scavenging effects pragmatic.

CONCLUSION

For their plausible antioxidant activity, the extract of *Onosma bracteatum* was subjected to screening. The consequent test systems, specifically free radical scavenging along with reducing power, was used for the chemical analysis. It was used for observing the radical scavenging effects of extracts. As antioxidants put in protons to these radicals, the absorbance of these radicals decreases. The decrease in absorbance is betrothed as a evaluate of the scope of radical scavenging. Free radical scavenging capacity of the extract as well as standard (Ascorbic Acid) was observed. It was also observed that the phytochemical screening of this plant comprises a few differences in which alkaloids, saponin and tannin were present in large amount. The incident of these compounds will exhibit the antioxidant activity along with it promotes a drug for conduct of an assortment of disease. The incidence of these compounds in the plants is likely to be accountable for the free radical scavenging effects pragmatic. As a consequence, this type of studies suggested that these vegetation obtain antioxidant activities which can counterbalance the oxidative damage induced by the vermin.

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