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Antioxidant activity in different plant parts of *Sesamum indicum*

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Abstract
Sesame is one of the world`s important oil crops. It is primary marketable products are whole seeds, seed oil. However the Plant leaves shoot and roots are majorly generated agro-industrial waste. The present study emphasizes on these parts as a potent antioxidant agents. The study also reveals in vitro grown sesame plant prominence antioxidant activity against field plant. The organic and extracts were selected to highlight efficiency. It was found that methanol extract gives highest antioxidant property with DPPH and ABTS assay. The load of shoot is major in left-over substantial of plant. The results indicate great potential in shoot with IC_{50}ug/ml of 8.14and 46.1 antioxidant activities by DPPH and ABTS method respectively. The study demonstrates the efficacy of sesame byproducts as potent source of bioactive compounds.

Keywords: sesame, antioxidant, DPPH, ABTS, methanol

1. Introduction
Sesame is a member of Pedaliaceae family. It is an annual shrub. It is oldest oilseed plant known to world. Commonly called as Til. Seeds are rich in oil (50%), Protein (18-20%).Around 60-65 countries of the world produce sesame seed. Asian and African countries are the major producers of Sesame. The top five producers account for around 70% of the production. India produces a wide variety of Sesame seeds varying in color from white to red to black, with oil content varying from 40 to 50 %. The major sesame cultivating states are Gujrat, Rajsthan, Madhya Pradesh, Uttar Pardesh, Tamil Nadu and Maharashtra. In Maharashtra Area (M.ha) 8.80 1.74 0.132.Popularverities are Gauri, Madhavi, YLM-11, YLM-17 [1].

Free radicals are highly reactive unstable & byproduct compounds of metabolic function in the human body.Most free radicals come from the oxygen atom are called reactive oxygen species (ROS). These are introduced in the body from both exogenous and endogenous sources such as pollutants, drugs. [2]. These radicals play deleterious effects in body resulting in a condition known as oxidation stress. Human body is protected from oxidative damage of free radicals through complex defense systems which are known as Antioxidants [3].

Studies indicated that antioxidant activity of much plant is highly co-related to their total phenolics [4, 5]. There is growing interests in natural sources as antioxidant compounds due to adverse effects of synthetic antioxidants.

Therefore the purpose of present study was to assess the antioxidant activity from *in vitro* grown sesame plant extract and comparing it with field plant. The study also offer benefit in using shoot, root and leaf in extract preparation which is a waste product and create negative impact on environment.

2. Material and Methods
2.1 Procuration of Plant
The sesame plant is obtained from Pimpalgaon Walan at post Wanegaon Taluka Phulambri Dist. Aurangabad, Maharashtra, India.

2.2 Authentication of Plant
The selected plant is authenticated from Department of Botany, Dr. B. A. U. Aurangabad. The Authentication Number of 0582 is awarded to genuine sample.

2.3 Selection of Sample
The Agro waste product of sesame is selected. It will help in reducing environmental pollution load as well as bring about advantage to Pharmaceutical Industry. Root, Shoot and leaves are considered from in vitro cultured and field plant for evaluation of Antioxidant Activity.

2.4 Extract Preparation
The Aqueous, Organic (Methanol, Ethanol) is prepared by considering 0.06/0.016 g of sample in pre sterile Distilled Water. It is incubated on rotary shaker in dark at 25^0C for 24 hours. The extract is filtered through Buckner Funnel and concentrated to dryness in evaporator.

2.5 DPPH antioxidant Assay
This assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH [6]. A dilution series of each extract was (0.001-0.03 mg/ml) prepared. DPPH reagent prepared in methanol (5 mg/100 ml, 2.0 ml) was added to each test sample (1.5 ml) and mixed with 0.5 ml of methanol. The mixture was allowed to stand for 10 min in the dark and absorbance was measured at 517 nm.

L- Ascorbic acid is used as reference standards. The capacity to scavenge DPPH radical was calculated by following equation 1:

$$\text{Scavenging activity (\%)} = [1-(As/A0)] \times 100$$

Where A0 is absorbance of the control and as is the absorbance in presence of extracts or standard. The results were plotted as the % of scavenging activity against concentration of the sample and scavenging activity express in IC_{50}.

2.6 ABTS radical scavenging assay

This assay is based on the ability of the antioxidants to scavenge the blue-green ABTS radical compared to the scavenging ability. [7]. The ABTS radical was produced by the reaction between 1.8 mM ABTS and 0.63 mM $K_2S_2O_8$ solution, stored in the dark at room temperature for 16 hrs. Before measure the absorbance of the test samples, the ABTS solution was diluted with methanol to obtain the absorbance of 0.900±0.020. The test extracts (50 µL) or standards (L-Ascorbic acid) are allowed to react with 950 µL of the ABTS solution. Absorbance was taken after 6 min at 734 nm. Scavenging activity of ABTS was calculated using equation 1. The percentage of scavenging activity was plotted against the concentration of extracts and scavenging activity express in IC_{50}.

2.7 Statistical Analysis

The mean ± standard error of the mean (SEM), Standard Deviation are determined and tested using analysis of variance (ANOVA). Interquartile range includes values between 25th percentile (Quartile 1) and 75th percentile (Quartile 3).The symmetry of distribution was defined by maximum and minimum. Discrepancies with $p < 0.05$ were considered significant.

3. Results

The sesame seed leftover after oil extraction can be used as a good quality fodder [8], but the plant byproduct remains a major concern. In the present study bioactivity of shoot, root and leaf agro wastes are tested against antioxidant activity. Aqueous and organic (Ethanol/Methanol) extracts are prepared from sesame plant parts. The results reveal that Methanol extract is having highest antioxidant activity enhancing power (Refer Graph).

Sesame plant is having phenolic compounds. Phenolic compounds have the ability to scavenge free radicals by hydrogen donation or electron donation and against oxidative damages. DPPH radical one of the few commercially available stable organic nitrogen radical. DPPH radical scavenging model is widely used common method to evaluate antioxidant activity. This assay is based on hydrogen donating ability or radical scavenging ability of extract in alcoholic medium and it yields color change from purple to yellow [9, 10]. Among the standards, highest DPPH radical scavenging activity was shown by methanol extract of shoot with mean of 12.26 and standard deviation 5.35 and calculated standard error of 3.09. The Interquartile range from I to III quartile is 9.23, 14.32 respectively. The Maximum range of data is 18.32 to Minimum is 8.14. The present study reveals in vitro cultured plant exhibit effective free radical scavenging activity than field plant. The extract shows significant activity ($p > 0.05$) in agreement with standard. (Refer Table 02). The lowest IC50 value of shoot supports highest antioxidant activity among test samples.

ABTS assay based on electron transfer ability of the test sample with long life and reactive radical anion 2, 2-azinobis (3 - ethylbenzothiazoline - 6-sulfonic acid). ABTS is applicable for both lipophilic and hydrophilic compounds analysis. In this assay, ABTS oxidize by persulfate and form green intense color radical cation ABTS which has a characteristic spectrum at max 734 nm [10, 11]. The ABTS radical cation-scavenging activities of sesame plant extracts obtained from shoot, root and leaf are shown in Table 01. Methanol root and shoot extracts exhibit good scavenging activity than methanol leaf extract. The statistical analysis reveals mean of 107.43 with standard deviation 55.62 and standard error 32.11. The symmetry of data defined by Interquartile range is 83.84 (I Quartile) and 138.0 (III Quartile). The maximum value is 154.6 to minimum is counted 46.1. The *p* value supports statistical significance of data ($p < 0.05$) shown in Table 02.

4. Table Legends

Table01. Different parts as root shoot and leaves are collected to find out antioxidant activity of *Sesamum indicum.* The radical scavenging activity is recorded by DPPH and ABTS assay.

Table02. Statistical Analysis is done of DPPH and ABTS assay is performed by ANOVA Test.

Table 01: Antioxidant Activity in *Sesamum indicum* Plant Parts.

Contents	DPPH IC_{50} (ug/ml)	ABTS IC_{50} (ug/ml)
Plant Root	10.33	154.61
Plant Leaf	18.32	121.58
Plant Shoot	8.14	46.1

Table 02: Statistical Analysis of Data by ANOVA Test

Statistics Applied	DPPH Assay	ABTS Assay
Mean	12.26333333	107.43
Standard Error	3.09361888	32.1131951
Mode	0	0
Median	10.33	121.58
First Quartile	9.235	83.84
Third Quartile	14.325	138.095
Variance	28.71143333	3093.7719
Standard Deviation	5.35830508	55.6216855
Skewness	1.41227431	-1.0706991
Range	10.18	108.51
Minimum	8.14	46.1
Maximum	18.32	154.61

5. Figure Legends

Figure1. It explains antioxidant activity of *Sesamum indicum* by DPPH and ABTS procedure. The values are estimated in IC_{50} (ug/ml).

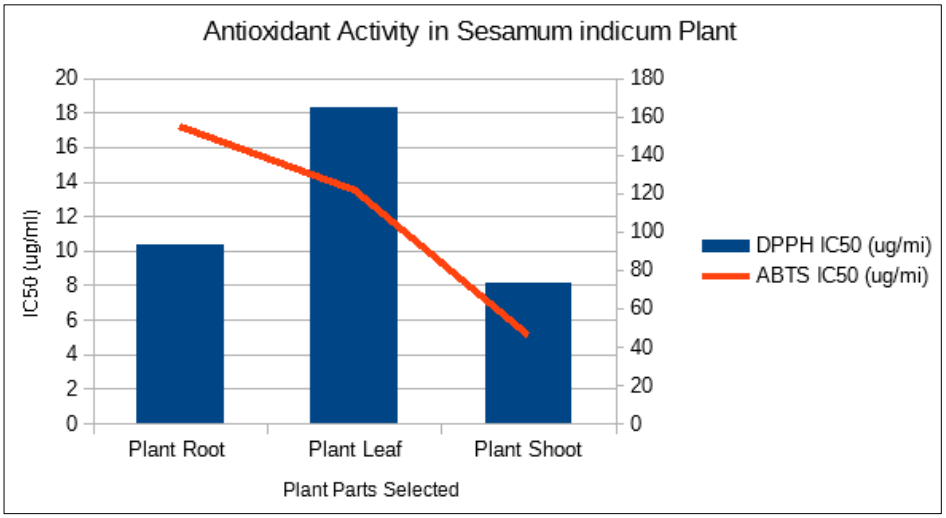

Fig 1: Antioxidant activity of *Sesamum indicum* by DPPH and ABTS Method

6. Discussion

Many experimental investigations have demonstrated that a number of secondary metabolites such as polyphenol compounds extracted from medicinal and aromatic plants possess a high antioxidant potential due to their hydroxyl groups and protect more efficiently against some free radical-related diseases [12]. Our work is unique in the sense utilizing agricultural waste which is root, shoot or leaf of sesame plant, simultaneous demonstrating rich antioxidant activity of it. Sesame is commonly grown cash crop and thus generating load of wastes which can be benefited in pharmaceutically important bioactive molecules generation.

In today's environment stress drugs and diet generate excessive free radical in human body and cause imbalance in homeostatic phenomenon between oxidants and antioxidants. The hydroxyl radical is an extremely reactive free radical from in biological systems and has been implicated as a highly damaging species capable of damaging biomolecule of the living cells [13]. Therefore there is constant requirement for new bioactive compound discovery in this area.

The Methanol extract shows highest antioxidant activity which is in agreement with cited literatures [14] which codes that methanol is best extract to scavenge DPPH free radical. Although ethanol was more effective in extraction of phenolic compound, but regarding the antioxidant activity of methanol extract, it is more than that of the ethanol one and that of ethanol extract is more than that of the aqueous one and could scavenge DPPH free radical. In general, DPPH free radical scavenging method is more suitable in an organic medium than in an aqueous one [15].

The formation of the ABTS radical cation takes place almost instantaneously after adding ammonium per sulfate to an ABTS solution. The scavenging ability of peroxides against ABTS radicals was concentration dependent. A more appropriate format for the assay is decolourisation technique in that the radical is generated directly in stable form prior to reaction with putative antioxidants [16]. Our results are compared with previously reported ABTS radical cation-scavenging activity of sesame seeds and seeds cake [17]. It was reported that ABTS radical cation- scavenging activity of In vitro cultured Sesame plant's methanol extract shows more radical scavenging activity than field plant.

7. Conclusion

The Properties of *Sesamumindicum* L. extract shows that it is having higher antioxidant activity. The investigations suggest that methanol is choice of solvent for promising radical scavenging ability. On comparing different parts, shoot gives highest antioxidation characteristic therefore it can acts as a bioactive component and can be applicable in food and pharmaceutical industry. Thus agricultural waste can be used to develop potential value added ingredient. However further work is needed to define the optimum dietary combination for obtaining the greatest stability in resultant product.

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