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Original Article

QUANTIFICATION OF SEED OIL AND EVALUATION OF ANTIOXIDANT PROPERTIES IN THE WILD AND CULTIVATED SPECIES OF *SESAMUM* L. (PEDALIACEAE)

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ABSTRACT

Objective: Seed oil quantity and sesamin concentration in the wild and cultivated species of *Sesamum* from Kerala viz., *Sesamum indicum, S. malabaricum, S. radiatum, S. laciniatum, S. prostratum* and *S. alatum* were estimated in comparison with the commercially released variety, *S. indicum* var. CO1, along with the antioxidant properties of the species.

Methods: The seed oil extract in petroleum ether was assayed to determine sesamin concentration through High Performance Liquid Chromatography (HPLC). Free radical scavenging and reducing power activities of the oil were assessed by following standard procedures.

Results: The percentage of oil content was high (49.62%) in the cultivated species, compared to the wild taxa (23.25%). The concentration of sesamin was high in *S. radiatum* (6.52 mg/ g) at a retention time of 31 min. The study noticed high antioxidant activity of the seed oil of the wild species, *S. radiatum* and *S. malabaricum*, which was at par with that of the cultivated species, *S. indicum*.

Conclusion: The antioxidant assays indicates that the species of *Sesamum* as a natural source of antioxidant. This property prevalent in the wild species can be of immense use in the manufacturing of therapeutic as well as pharmaceutical combinations such as anti-cancerous and anti-inflammatory- drugs.

Keywords: *Sesamum*, HPLC, Sesamin, Antioxidant.

INTRODUCTION

Sesamum indicum (sesame) commonly known as 'Queen of Oil Seeds' belonging to the family Pedaliaceae, is one of the major oil crops cultivated in Asia from ancient times. The genus consists of both cultivated and wild species that exhibit a great deal of diversity [1]. The cultivated sesame seed is a good source of nutrients like iron, phosphorous, magnesium, calcium, manganese, copper and zinc besides its high oil and protein content. The oil content in the cultivated sesame was reported earlier [2, 3]. The oil is used for religious ceremonies, apart from its use in cooking, anointing, soap making, preparing antioxidants and as insecticides.

Sesamum oil has two major oil soluble lignans- sesamin and sesamolin which exist in relatively high contents as compared with other compounds [4]. Both the lignans have health promoting effects [5] and have strong antioxidant properties as well as a wide ranging effect on lipid metabolism [6-8]. Pioneer attempt to identify sesamin and sesamolin was that of Tocher [9]. Sesamin and sesamolin have the properties to inhibit free radical formation, reduce oxidative stress and protect neurons against oxidative stress [10]. Review of related literature revealed that the studies were mainly encircled on the cultivated species, *S. indicum*, while the reports of wild species were limited to *S. radiatum*, *S. alatum* and *S. angustifolium* [11-13]. Quantification of sesamin in the wild (*S. alatum*, *S. radiatum*, *S. angustifolium*) and cultivated, species of *Sesamum* were studied [14]. A simplified protocol for the quantification of sesamin and sesamolin in sesame seeds was suggested by Shirato-Yasumoto *et al.* [4]. High Performance Liquid Chromatography (HPLC) analysis of sesaminol glucosides and sesamol was carried out in sesame seeds [15, 16]. Photodiode and Flourescent detection HPLC methods have been developed for the analysis of sesamin, asarinin and sesamolin of sesame oil [17]. Sesamin and sesamolin contents were analysed in seeds of different sesame cultivars [18]. Both these substances have various pharmacological properties including antioxidant activity.

The antioxidant activity of many food plants is highly correlated with their phenolics [19, 20]. Phenolics are one of the groups of non-essential dietary components which help to inhibit atherosclerosis and cancer [21]. Bioactivity of phenolics is related to their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals [22, 23]. Various researchers have suggested the antioxidant property of *Sesamum indicum* [24-27]. Recent studies [28-31] revealed the antioxidant activity of *S. indicum* seed oil and i*n vitro* antioxidant activity of isolated sesamin from sesame oil [32]. However, attempts were not yet carried out to analyse the antioxidant properties of wild species of *Sesamum.* The present study is proposed to quantify sesamin, a lignan in the seed oil of the wild and cultivated species of *Sesamum* from Kerala and to determine the antioxidant properties of the species to unveil their significance in pharmaceutics.

MATERIALS AND METHODS

Materials

The cultivated species, *S. indicum* and five wild species viz., *S. radiatum, S. malabaricum, S. prostratum, S. laciniatum and S. alatum,* were collected from different localities in the state of Kerala. The specimens were identified with the help of Floras [33-39] and herbarium of Jawaharlal Nehru Botanical Garden and Research Institute, Thiruvananthapuram, Botanical Survey of India, Coimbatore, Royal Botanic Garden, Kew and Department of Botany, University of Kerala. The voucher specimens were deposited in the Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram (table1). Seeds of the commercial variety, *S. indicum* var. CO1 procured from Tamil Nadu Agricultural University, Coimbatore, were included for comparison.

Preparation of oil extract

Soxhlet extraction method was used to extract oil from 5 g of the seeds of all the taxa in 100 ml petroleum ether (60^{o} - 80^{o}C). Extraction was continued for six hours without interruption. Extraction flask was cooled and petroleum ether was allowed to evaporate in the water bath until no odour of ether remained. Weight of the oil was recorded and percentage of oil in the ground sample was calculated as shown below.

$$\text{Percentage (\%)of oil in the ground sample} = \frac{\text{Weight of oil (g)}}{\text{Weight of the sample (g)}} \text{X } 100$$

Quantification of sesamin by HPLC (High Performance Liquid Chromatography) and determination of antioxidant activity by

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, hydroxyl radical, superoxide free radical, nitric oxide radical scavenging assays and reducing power activity assay were carried out in the extracted seed oil.

Table 1: Details of *Sesamum* germ plasm collection

OTUs	Voucher number	Place of collection	Altitude (m)	Latitude/longtitude
S. indicum	KUBH 5883	Kollam	54m	08^{0}52.460'N/076^{0}46.535'E
S. radiatum	KUBH 5899	Kollam	70 m	08^{0}57.816'N/076^{0}50.0607'E
S. malabaricum	KUBH 5908	Kottayam	12 m	09^{0}45.068'N/076^{0}54.719'E
S. laciniatum	KUBH 5914	Malappuram	75 m	11^{0}084.391'N/075^{0}53.530'E
S. prostratum	KUBH 5916	Palakkad	102 m	10^{0}49.654'N/076^{0}40.984'E
S. alatum	KUBH 5917	Kollam	13 m	08^{0}53.532'N/076^{0}36.178'E

Quantification of sesamin using HPLC

The extracted oil samples of both the cultivated and wild taxa of *Sesamum* were subjected to HPLC. The sample containing 10 µg/ ml oil in HPLC grade methanol was sonicated and filtered using the syringe. The standard sesamin (Sigma Chemical Company, St. Louis MO 63178) was prepared at 100 µg/ ml in methanol.

The HPLC system (Shimadzu Prominence UFLC, LC 20 AD) was connected with a diode array detector (SPD-M20A). Chromatographic analysis was performed on C-18 G 250 mm x 4.6 mm x 5µm reverse phase column. The mobile phase was methanol: water (70:30) with a flow rate of 0.5 ml/ min. The D_2 & W PDA (Deuterium and Tungsten Photo Diode Array) detector is used with a wavelength of 290 nm. 5 µl volume each of the sample and the standard was injected for the analysis. The concentration of the sample (µg/ ml) was calculated by the formula,

$$\text{Concentration of sample} = \frac{\left(\frac{\text{Area of sample}}{\text{Area of standard}}\right) \text{X Concentration of standard}}{\text{Weight of the sample (g)}}$$

Total phenol and flavanoid content

The amount of total phenol content in the seed oil was determined following the modified method of Singleton and Rossi [40]. The samples were dissolved in distilled water and added 0.5 ml Folin-Ciocalteu reagent. 1 ml of 20% sodium carbonate was then added to the mixture and kept for 90 min. Absorbance was recorded at 760 nm against the standard catechin (mg CE/ g).

Total flavanoid content was estimated by the modified method of Chiang *et al.* [41]. 250 µl of the seed oil samples were mixed with 1.5 ml distilled waters, 150 µl 10% aluminium chloride, 75 µl 5% sodium nitrite and 2 ml of sodium hydroxide. The absorbance was measured at 510 nm using quercetin (mg QE/ g) as standard.

Determination of antioxidant activity

DPPH radical scavenging assay

DPPH radical scavenging activity was determined following Gezer *et al.* [42]. Different concentrations of oil extractions (1 mg/ ml - 5 mg/ ml) were prepared with methanol as test solutions. Then 2 ml of 2 mM DPPH (Sigma-Aldrich) solution in methanol was added to 1 ml of prepared test solution. The tubes were incubated for 30 min and read the absorbance at 517 nm using UV-Vis spectrophotometer (UV-1700 Pharmaspec, Shimadzu). A solution comprising all the reagents except the extract was treated as the control. The DPPH radical scavenging activity inhibition percentage was calculated by the formula,

$$\text{Percentage (\%) of inhibition} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \text{X } 100$$

Where,

$A_{control}$ is the absorbance of the control reaction; A_{sample} is the absorbance of the sample.

The half maximum inhibitory concentration (IC_{50}) value for each sample was calculated to denote the concentration of the sample required to decrease the absorbance at 517 nm by 50%.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was determined according to the modified method of Halliwell *et al.* [43]. 1 ml of the reaction mixture containing 100 µl 2-deoxy-2-ribose (28 mM in 20 mM potassium dihydrogen phosphate-potassium hydroxide (KH_2PO_4—KOH) buffers, p^H 7.4), 200 µl of ferric chloride (100µm), 200 µl Ethylenediaminetetraaceticacid (EDTA, 100 µm), 100 µl hydrogen peroxide (1.0 mM), 100 µl ascorbic acid (100 µm) and various concentrations (6.25, 12.5, 25, 50 and100 µg/ ml) of the test sample was analysed for the assay. After incubation for 1h at 37°C, 1 ml of 2.8% TCA (Tri Chloro Acetic acid) and 1 ml aqueous TBA (Tri Barbituric Acid) was added subsequently to 0.5 ml of the reaction mixture. Then the mixture was incubated at 90°C for 15 min to develop a pink colour. The absorbance was measured at 532 nm against an appropriate blank solution after cooling.

Super oxide free radical scavenging assay

The superoxide scavenging assay was done by Elmastas *et al.* method [44]. The reaction mixture consisting of 1 ml of Nitro Blue Tetrazolium (NBT) solution (1.56 mM in phosphate buffer, p^H 7.4), 1 ml Nicotinamide adenine dinucleotide (NADH) solution (468 mM NADH reduced nicotinamide adenine dinucleotide in phosphate buffer p^H 7.4) and 1 ml of different concentrations of the samples were mixed together. Then 60 mM Phenazine Methosulfate (PMS) solution was added and incubated at 25°C for 5 min. The absorbance was measured at 560 nm against the blank sample and then compared with the standard ascorbic acid.

Nitric oxide scavenging assay

Nitric oxide scavenging activity was measured spectrophotometrically [45]. Sodium nitro prusside (5 mM) in phosphate buffered saline (p^{H}7.4) was mixed with different concentrations of the methanolic extract (6.25-100 µg/ ml) and incubated at 25°C for 30 min. A control without the test compound, but an equivalent amount of methanol was taken. After 30 min, 1.5 ml of the incubated solution were taken and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthyl ethylene diamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with N-1 naphathyl ethylene diamine dihydrochloride was measured at 546 nm. Scavenging activity was measured with reference to the standard.

Reducing power assay

Reducing power was determined according to the modified method of Gulcin *et al.* [46]. Different concentrations of seed oil were mixed with 2.5 ml each of 200 mM phosphate buffer (p^H 6.6) and 2.5 ml of 1% potassium ferric cyanide. The mixture was incubated for 20 min at 50°C. Then, 2.5 ml of 10% Tri chloro acetic acid was added and the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and then added 1 ml of 0.1% ferric chloride and read the absorbance at 700 nm.

Statistical analysis

Data obtained was analysed using SPSS (Statistical Program for Social Sciences) version 7.5. One-way analysis of variance (ANOVA)

was used to compare the means of phenol and flavanoid content at p<0.05 significance level.

RESULTS

The percentage of oil content varied between 10.44% to 49.62% (table 2) in the species analysed. The cultivated taxa had the highest oil yield compared to the wild species. Among the wild, *S. radiatum* and *S. malabaricum* were superior in the quantity of oil, whereas, *S. laciniatum* showed the lowest percentage (10.44%). The oil content in the commercial variety, CO1 was higher (35.87%) though it was not at par with the landrace of *S. indicum* (49.62 %). The HPLC chromatograms of seed oil from the wild and cultivated taxa of *Sesamum* obtained with PDA (Photo Diode Array) detector were shown in fig.1a, 1b. The chromatographic peaks were determined by comparing the retention time of the standard sesamin at 31.157 min.

The peak at 31 min retention time was observed in the seed oil of all the species except in *S. alatum*. It was observed that the concentration of sesamin was high in *S. radiatum* (6.52 mg/ g) compared with the cultivated variety *S. indicum* var. CO1 (2.07 mg/ g).

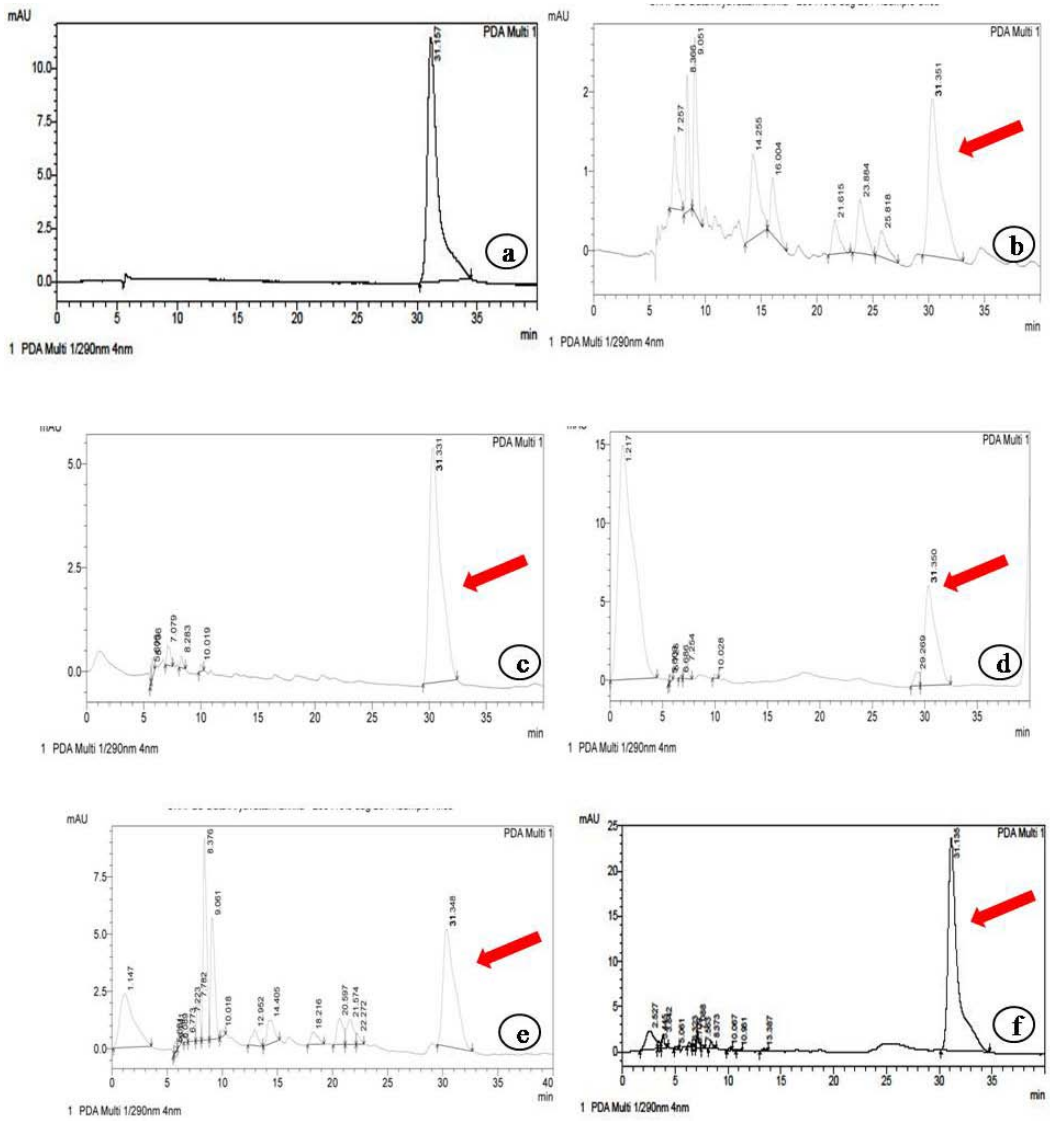

Fig. 1a: HPLC analysis of *Sesamum* seed oil. a: Standard sesamin; b: *S. indicum* var. CO1; c: *S. indicum*; d: *S. radiatum*; e: *S. malabaricum*; f: *S. laciniatum*

Table 2: Oil percentage, total phenol, flavanoid content and sesamin concentration of seed oil in the genus *Sesamum*

S. No.	Taxa	Percentage of oil (%)	Total phenol content (mg CE/g)	Total flavanoid content (mg QE/g)	Concentration of sesamin (mg/g)
1.	*S. indicum* var. CO1	35.87	1.453±0.004[b]	0.250±0.003[b]	2.07
2.	*S. indicum*	49.62	1.938 ±0.005[f]	0.559±0.005[g]	5.49
3.	*S. radiatum*	23.25	1.473±0.01[b]	0.431±0.003[c]	6.52
4.	*S. malabaricum*	21.67	0.31±0.02[a]	0.104±0.001[a]	5.29
5.	*S. laciniatum*	10.44	1.601±0.001[c]	0.468±0.002[d]	2.05
6.	*S. prostratum*	11.74	1.689±0.005[d]	0.513±0.0008[e]	2.03
7.	*S. alatum*	16.11	1.778±0.004[e]	0.537±0.001[g]	-

Values with different superscript are significantly different at p<0.05 level

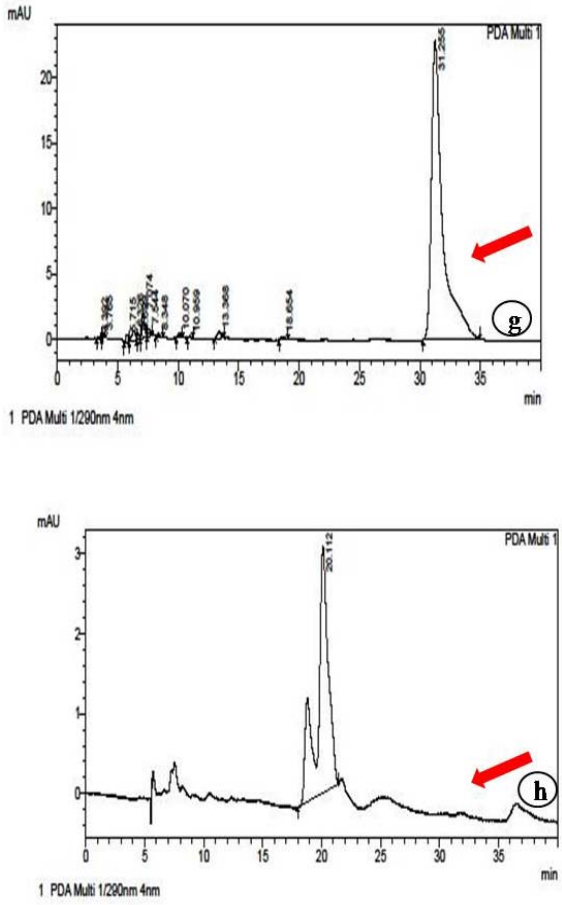

Fig. 1b: HPLC analysis of *Sesamum* seed oil. g: *S. prostratum*; h: *S. alatum*

The total phenolics and flavanoid contents of the seed oil determined were tabulated (table 2). Estimated phenol and flavanoid contents were high in the landrace of *S. indicum* (1.938 ±0.005 mg CE/ g of extract and 0.559±0.005 mg QE/ g respectively). Among the wild species, phenol content (1.778±0.004 mg CE/ g of extract) and flavanoid content (0.537±0.001 mg QE/ g) was high in *S. alatum,* whereas, it was low in *S. malabaricum* (0.31±0.02 mg CE/ g of extract and 0.104±0.001 mg QE/ g respectively).

Data obtained in different scavenging assays viz., DPPH, hydroxyl scavenging, nitric oxide scavenging, superoxide scavenging and reducing power activity were used to assess the antioxidant property of the seed oil (fig. 2-4). The DPPH free radical scavenging assay of the genus showed variations in their IC_{50} value compared with the standard BHT (Butylated Hydroxy Toluene). The IC_{50} value was low in *S. radiatum* (2.69 µg/ ml) and high in *S. prostratum* (4.67 µg/ ml). Hydroxyl radical scavenging activity of the seed oil compared with the standard ascorbic acid was found to be high in *S. malabaricum* with a low IC_{50} value (16.98 µg/ ml).

However, the activity of *S. indicum* var. CO1 and the land race of *S. indicum* were low with high IC_{50} value. Antioxidant studies with the superoxide scavenging assay varied in the species compared with the standard (ascorbic acid) (fig. 3). The low IC_{50} value was noticed in *S. malabaricum* (12.56 µg/ ml) whereas, the cultivated *S. indicum* (22.66 µg/ ml) as well as the variety CO1 (30.39 µg/ ml) had less ability to scavenge superoxide anion. Nitric oxide scavenging activity was found to be higher in the wild species compared to the cultivated taxa. However, *S. radiatum* (10.43 µg/ ml) had lesser scavenging activity than the standard gallic acid (11.25 µg/ ml).

The reducing power activity of the sample increased as its absorbance increases (fig. 4). It was found that the absorbance of *S. radiatum* (0.187 to 0.483) was higher than the standard ascorbic acid.

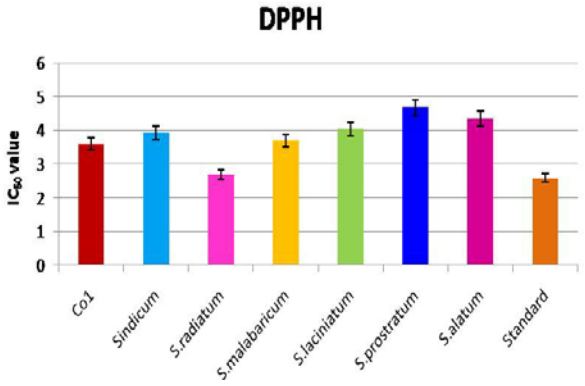

Fig. 2: DPPH assay with IC_{50} value in the *Sesamum* seed oil

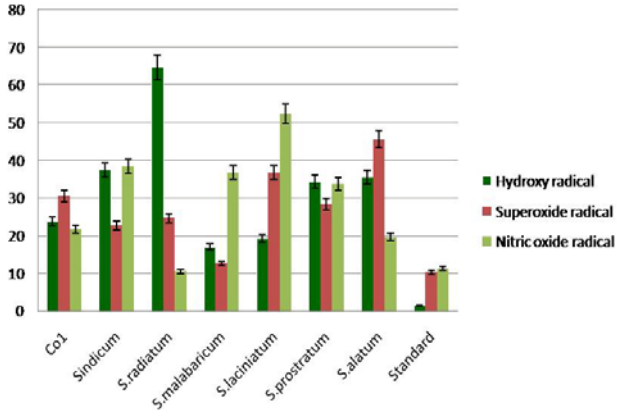

Fig. 3: IC_{50} value of *Sesamum* seed oil in hydroxyl radical, superoxide radical and nitric oxide radical scavenging activity

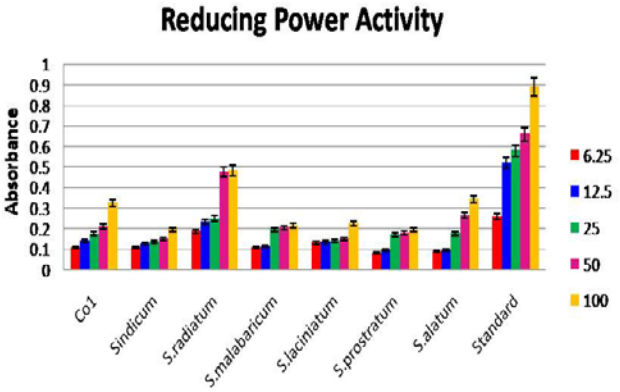

Fig. 4: Reducing power activity of *Sesamum* seed oil

DISCUSSION

Seed oil extracted from plant sources has a rich history of use by local people, as a source of food, energy, medicine and for cosmetic applications [47]. Previous reports [48, 6] pointed out that the presence of sesamin, sesamol, sesamolin and tocopherol in sesame oil are responsible for its beneficial health effects. The current study evaluated the oil quantity, sesamin content and antioxidant properties of the seed oil extracted from the wild and cultivated species of *Sesamum.*

Determination of oil content in plants is important, because it predicts the profitability of plants as a potential source of oil [49]. Analysis of the data (table 2) revealed that the seed oil content was higher in the cultivated species compared to that of the wild.

Comparatively low oil content in the wild species may be due to the presence of the rough seed coat that prevents the complete extraction of the oil. Hiremath *et al.* [13] also had the opinion that the seed oil content was higher in the cultivated species compared to the wild (*S. mulayanum*, *S. capense*, *S. laciniatum*, *S. latifolium*, *S. occidentale*, *S. schinzianum*) ones. High percentage of oil (23.25%) noticed during the investigation in the wild species, *S. radiatum* points out its significance in industrial utility. More or less the same quantity was reported in the Nigerian accessions of *S. radiatum* [11]. The study found out that the landrace of *S. indicum* (49.62%) was superior than the commercially released variety CO1 (35.87%) in the percentage of oil content, which is in concordance with the earlier reports [2, 3]. This was in sync with the report of Kamal-Eldin *et al.* [12] in the species *S. alatum*, *S. radiatum* and *S. indicum*. The oil content in wild species can possibly be increased by selection made for thinner seed coat [50].

High Performance Liquid Chromatography (HPLC) analysis noticed variations in the quantity of sesamin in the species studied. It ranged from 2.03 mg/ g (*S. prostratum*) to 6.52 mg/ g (*S. radiatum*) in the wild species. But in the cultivated species, the sesamin content was higher in the landrace than the variety CO1. More or less the same quantity of sesamin present in *S. malabaricum* and *S. indicum* revealed their close relationship. Interrelationship between *S. prostratum* and *S. laciniatum* was also evident in the study. The species, *S. alatum,* was unique by the absence of the lignan, sesamin. Variation in the content of sesamin may be due to either the diversity in the genotype or in the altitudinal variation. A range of 0.82 to 11.05 mg/ g sesamin content was observed in sesame cultivars of China [18]. The study is in conformity with Willianson *et al.* [51] who reported variations in sesamin content in the diverse genotypes of sesame which was influenced by genetic, environmental and geographical factors.

Phenolic compounds such as flavanoids, phenolic acids and tannins have an important role in stabilizing lipid oxidation and are related to antioxidant activity [52]. Total phenol and flavanoid content of sample extract estimated was higher in *S. indicum* landrace than in the var. CO1 whereas, low in *S. malabaricum* (table 2). Among the wild species, *S. alatum* had high phenol and flavanoid content, though the lignan sesamin was absent. This may be due to the presence of other lignans such as sesamolin and sesamol. Better content of polyphenols and flavanoids were reported in white sesame seed extract [28].

2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals are scavenged by antioxidants to prove the ability to act as donors of hydrogen atom. The free radical scavenging activity of oil in the species of *Sesamum* was evident from the graph given (fig. 2). The high absorbance in *S. radiatum* was obvious by the low IC_{50} value (2.69 µg/ ml), that reflects its high DPPH scavenging antioxidant activity, which was compared with IC_{50} values (3.93 µg/ ml) of standard BHT. The IC_{50} value of sesame oil extract as 0.026 mg/ ml was reported [30]. The high antioxidant activity has been reflected by lower IC_{50} value in species like *Ipomoea batatas* [53], *Dioscorea alata* [54] and *Armoracia rusticana* [55]. The activity of cultivated seed oil in the present investigation was supported by the report of Farhoosh *et al.* [56] as it showed almost similar IC_{50} value ie; 5.219 µg/ ml.

Hydroxyl radicals, the quick initiators of lipid oxidation process are highly reactive and cause damage to biomolecules. The hydroxyl radical scavenging activity of the seed oil in the current study ranged from 16.98 µg/ ml (*S. malabaricum*) to 64.66 µg/ ml (*S. radiatum*) (fig. 3). At the minimal oil concentration, the hydroxyl radical scavenging activity was high in *S. malabaricum* and were compared with the standard ascorbic acid. As the concentration of oil increases, a decrease in absorbance as well as an increase in the percentage of inhibition was noticed. The same phenomenon was observed in *Macrolepiota mastoidea* [57]. The ability of the sesame oil to prevent assault on deoxyribose degradation by hydroxyl radical and that can be related to their antioxidant property [58].

Superoxide scavenging activity of *Sesamum* oil was observed high in *S. malabaricum* with low IC_{50} value (12.56 µg/ ml). Superoxide anions generate dangerous hydroxyl radicals as well as singlet oxygen, which will produce oxidative stress [59, 60]. Comparatively high IC_{50} value and low superoxide scavenging activity observed in var. CO1 and *S. indicum* suggest the lowest antioxidant activity of the species. However, the wild species, *S. malabaricum* was found to be more potent scavenger of superoxide anion than the other species. In the case of nitric oxide scavenging activity, minimum concentration of oil extract competes with oxygen to react with nitric oxide and inhibits the nitride formation. The scavenging activity of the extract was based on their ability to prevent the formation of nitrite ions [61]. The present study revealed that nitric oxide scavenging activity was very potent in *S. radiatum* (10.43 µg/ ml) and very low in *S. laciniatum* (52.38 µg/ ml).

Reducing power of the oil indicates its significant antioxidant property. This activity is considered as a defense mechanism, which is related to the ability of antioxidant reagents to transfer an electron or hydrogen atoms to oxidants or free radicals [62]. Present investigation noticed an increase in antioxidant activity as the concentration of the oil increases. Thus, the reducing power activity of *S. radiatum* oil was observed to be high (0.483) at a concentration of 100 µg/ ml, when compared with the standard ascorbic acid (fig. 4). The lowest absorbance was shown by *S. prostratum* (0.193) at 100 µg/ ml concentrations indicates its poor antioxidant capacity.

CONCLUSION

Even though, the oil percentage was higher in landrace of *S. indicum,* analysis of antioxidant property revealed the superiority of the wild species. The study suggests the significance of the wild species, particularly *S. radiatum* and *S. malabaricum* with high antioxidant property, in the improvement of cultivated taxa, *S. indicum*. The study also recommends the incorporation of wild species in the production of therapeutic and pharmaceutical products due to their high antioxidant property.

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CONFLICT OF INTERESTS

Declared None

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