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ANTIMICROBIAL EFFICACY, PHYSICO- NUTRITIONAL PROFILE OF *SESAMUM INDICUM L.* SEED AND SEED OIL INDIGENOUS TO KPK, PAKISTAN

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ABSTRACT

The ever increasing dietary demands and the scarcity of edible oil sources have led to the need for the exploration of new sources in order to meet the local demands. Blessed with nature's tremendous sources there is a dire need of exploring the non-traditional food and oil sources in Pakistan. The present study was undertaken to evaluate the physicochemical, nutritional, tocopherols' composition and antimicrobial efficacy of the essential oils from two black *Sesamum* indicum seed locally grown in Khyber Pakhtunkhwa, Pakistan. About four fungal species (*Phythium Ultimum, Paecilomyces Lilacinus ,Rhizopus stlonifer and Tricoderma Harzanium*) and four bacterial strains (*Xnythomonas campestris,Proteus Mirabilis, Escherichia Coli, Bacillus Cereus*) were tested against different n-hexane and sesame oil fractions. Results showed good antimicrobial properties of the oils against all the pathogens. The proximate composition showed the seed cakes of both the varieties to be a good source of oil, protein, dietary fiber, ash. Both of the varieties showed to be good sources minerals. The intra variety difference in the nutrient composition was significant only in the percent oil, zinc, and copper.

The physicochemical tests of the oils from both varieties revealed that refractive index, surface tension, density, specific gravity, saponification value, and iodine number fell well within the international standards for edible oils. The fatty acids composition showed the oils to be a rich sources of oleic acid, linolenic acid, and linoleic acid with an excellent proportion of unsaturated to saturated fatty acids. Intra variety differences were significant in oleic acid, elaidic acid, linoleic acid, and total unsaturated fatty acids. Tococpherol profile indicated the oils were rich sources of tocopherols though the variety S-17 appreciably greater proportion of β+γ and total tocopherols. These differences may be attributed to the genetic makeup or genotype response to the local climate. Both of the oils showed good DPPH free radical scavenging properties. The study conclude that indigenous variety of *Sesamum Indicum* possess excellent nutritional composition along antioxidant and antimicrobial potential making it ideal as food with utility in the development of variety of natural compounds.

Keywords: *Sesamum Indicum*, Proximate and Elemental Composition, Tocopherol profile, physicochemical properties, Fatty acid Composition, DPPH free radical Scavenging, Antimicrobial activities.

1. INTRODUCTION:

The genus S*esamum Indicum,* locally known as sesame seeds, til or benniseed, belongs to the *Pedaliaceae* family.It is one of the most ancient oilseeds crop known to mankind. It consists of about 36 species and is the most reputable folk medicinal plant in Africa and Asia [1, 2]. In Pakistan, it is grown in 65 districts as irrigated as well as rainfed crop. During 2001-2002, it was cultivated on an area of 136,000 hectares with national average seed yield of 513 kg/ha. The area under this crop was 90,700 ha with total production of 41,000 tons and yield of 452 kg/ha in 2008-09. The seed contain 50-60 percent oil and 22 percent protein. Its oil is semi-drying type and suitable for human health. Its seed is mostly used in confectionary items whereas; good quality oil is also used for medicinal purposes [3]. Sesame plays an important role in human nutrition and is grown primarily for its oil-rich seeds. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes [4]. After the extraction of oil, the cake is mostly used for livestock feed or as a manure. Its color varies from cream-white to charcoal-black but it is mainly white or black [5].

Sesame seed oil has been used as healing oil for thousands of years and has been enjoyed by mankind since the dawn of civilization. Sesame seed (*Sesamum indicum L.*) is an oilseed with a chemical composition of about 50-52% oil, 17-19% protein and 16-18% carbohydrate [6]. Some seeds contain about 42-54 % quality oil, 22-25 % protein, 20-25 % carbohydrates and 4-6%ash. The hull contains large quantities of oxalic acid, crude fiber, calcium and other minerals [7]. However properly dehulled seeds contain less than 0.25% oxalic acid [8]. Sesame seed contains antioxidants which inhibit the development of rancidity in the oil. In the food industry, where synthetic antioxidants are used extensively, there is an increasing demand for more of these natural products [9]. Sesame plants have shown to synthesize variety of active secondary metabolites. Some phenolic compounds have also been found in the essential oils. The seed oils possess established potent insecticidal and antimicrobial activities making it suitable for their use in some pharmaceuticals, alternative medicines and natural therapies [10-12]

The oil seed is renowned for its versatility and stability. From edible oil in traditional cooking and baking , to salad oils , confectionary items, manufacturing of shortenings and margarine, and the excellent flavors that it instill in the food sesame seed oil is being termed as the queen of the oil seed crops [13]. The oil fraction shows a remarkable stability to oxidation. This could be attributed to endogenous antioxidants namely lignins and tocopherols. The seed is rich in protein and the protein has disable amino acid profile with good nutritional value similar to soybean . Sesame seed is approximately 50 percent oil out of which 35% is monounsaturated fatty acids and 44% polyunsaturated fatty acids [14-16].

Differences in the chemical and nutritional composition are a natural phenomena attributed to climatic and ecological conditions, agricultural practices, and ecotypes. There is a paucity of knowledge regarding the nutritional and chemical composition of the indigenous varieties of sesame seeds grown under the existing conditions of KPK, Pakistan. The present study was an effort to analyze the nutritional composition of the whole seeds and physicochemical properties, antioxidant, antimicrobial potential of the seed oil from the *sesamum indicum* cultivars commonly grown in KPK.

2. MATERIALS AND METHODS

2.1 Procurement of Seeds

Seeds of approved local varieties were procured from the fields around Agriculture University, Peshawar. The seeds were separated and were washed several times with water to remove any plant and foreign material. Later the seeds were dried in an electric oven until constant weight was achieved. The dried seeds were stored in airtight jars in triplicates for further analysis.

2.2 Oil Extraction

Dried seeds were grinded in an electric grinder. The ground seed powders were soaked in n-hexane for 24 hours in three replicates. After soaking for a day the extract was filtered through filter paper (Whatman 42) and were then concentrated /distilled in rotary evaporator under reduced pressure and 40-45^{o}c. Percent oil was calculated and the oil and cake were directly used for quality parameters.

2.3 Proximate Composition

Using AOAC official methods total protein content of the seed residues was estimated by a micro Kjedlhal apparatus. Crude oil was estimated by soxhlet method while crude fiber and ash contents were determined as per standard methods. For minerals analysis samples were first subjected to acid digestion and later were analyzed by atomic absorption spectrophotometer (AAS) methods [17].

2.4 Mineral Content Determination

For minerals analysis samples were first subjected to acid digestion and later were analyzed by atomic absorption spectrophotometer (AAS) methods [19].

2.5 Oil Analysis

The physical and chemical parameters of the crude oil were determined as per AOAC official methods .Perfective index, density, specific gravity, surface tension, acid value, free fatty acid value, saponification value, iodine value, color and peroxide value were carried out.[17,18]

2.6 Analysis of Fatty acids by Mass Spectrophotometer

About 25-40 mg of the oil samples were taken in AMEs tubes to which 1.5 ml methanolic Sodium hydroxide (0.5 N) was added. Stoppard with screw capes the mixture was heated in a boiling water bath for 05 minutes. After cooling 2.5 ml BF_3 (10 % in MeOH) was added and the tubes were heated again in boiling water bath for 30 minutes. Later 5 ml brine solution + 1 ml n-hexane was added. The tubes were shaken vigorously on vortex and the upper (hexane) layer was taken through pasture pipette. This step was repeated twice and was filtered through 45 µm membrane filter. The filtrates were transferred to GC vial. The esterified oils were analyzed for the fatty acids by Shimadzu GC-MS- QP 2010 Plus Gas Chromatography Mass Spectrometry. Using a capillary column TRB FFAP (30 m x 0.25mm i.d) at column temperature set as 50 ^{o}C – 220 ^{o}C. Helium was used as the carrier gas flow rate of 77.1 ml/min with column flow at 3.29 ml/min at split ratio of 20.0. The peaks eluted were identified by comparison of their retention time with those of the standard methyl esters (FAMEs standard mix, 37 components, Sigma Aldrich) analyzed under the same conditions.

2.7 Determination of Tocopherols

Determination of the tocopherol isomers (α-,β-,γ-, and σ-) in the *sesemumindicum*oil samples was performed by a modified HPLC procedure.Tocopherol Standards α-tocopherol (M.W – 430.7) and γ- tocopherol (≥ 96% HPLC) T 1782 were purchased from sigma Aldrich USA while delta – tocopherol lot: LC 1802V and rac - β - tocopherol (90 + %) lot: LC 18838V were procured from Supelco. USA. Stock reagents for derivatization and for mobile phase were of HPLC grade and were procured from Merck.

Samples were prepared by weighing exactly 0.1 of oil samples in test tubes with Teflon caps. About 0.05g of ascorbic acid was added followed by the addition to 5ml of 90% ethanol and 0.5ml of 80% aqueous (W/V) potassium hydroxide. The contents were thoroughly mixed by vortexing on a vertex machine for 1-2 minutes. The tubes were purged with nitrogen and tightly capped. The tubes were then incubated at 70^{o}C for 30 minutes in a water bath with occasional mixing and vortexing. Later the test tubes were cooled room temperature. After cooling 3ml of deionized water and 50ml n-hexane were added and the tubes were again vortexed for 30 seconds following centrifuging for 10 minutes. The top hexane layers were separated by Pasteur pipettes in separate tubes and the aqueous layers were re-extracted with the same procedure. These hexane layers were evaporated till dried with nitrogen streaming.

Instrumentation and Chromatographic Conditions

About 01ml of the mobile phase was added to the tubes and were transferred to HPLC vials. About 20µl of the sample volume was injected into a C-18 column (25 x 4.6 nm; Supelco Inc.) into Perkin Elmer series 200 HPLC with auto- sampler, pump, column oven and UV / VIAS detector with a binary mixture of acetonitrile: MeOH (50 : 50 V/V) mobile phase at a run time of 12 minutes and flow rate of 10ml/L/min. Detection of the respective tocopherols was performed at 29.5nm and were identified by comparison of their retention times with those of the standards. Quantification of the tocopherols was performed by comparison of the peak areas of the respective standards as per external standard calibration. The retention times of β- tocopherol and γ-tocopherols were same therefore the concentration of β- and γ- tocopherols were determined as sum of both the isomers (β+ γ).

2.8 Antimicrobial Assay

The extracted oils of *Sesemum Indicum* were tested against different strains for anti-infective potential. Four types of bacteria; *Bacillus Subtitles Escherichia Coli, Proteus*, and *Xanthomonas* and four pathogenic fungi including *Phytium Ultimum, Paecilomyces Lilacinus , Rhizopus Stolonifer, and Tricoderma Harzianum* were tested. The pure microbial strains were obtained from the chemistry lab at the department of Agricultural Chemistry, KPK Agriculture University Peshawar. Agar Well Diffusion was used for fungi and streaking method was used for bacteria.

2.9 DPPH Radical Scavenging Activity

DPPH radical scavenging activity was by following the method of Rauf et al [20]. About 25mg of oil was dissolved in distilled methanol and was diluted up to 50 ml. from the stock solution 10, 20, 40, 60, 80, 100, mg/ ml were prepared. About 5 ml of each solution was added along 1 ml of 0.01 M of DPPH solution in a test tube. They were kept in a dark for test minutes along a control (5 methanol+ 1 ml DPPH solution). After the incubation the antioxidant activity was measured using Optima U-V- Visible Spectrophotometer at wave length of 517 nm. The experiments were performed in three replicates. Quercetin was taken as a standard. Percent radical scavenging activity was calculated as:

% DPPH= Control Absorbance- Extract Absorbance × 100/ Control Absorbance

2.10 Statistical Analysis

Results obtained from various experiments were statistically analyzed by IBM SPSS version 19. Results were expressed as percentage or mean± SD. Statistical significance between the two varieties was determined through one way analysis of variance (ANOVA) for all the means. Results were considered significantly different when the values were less than 0.05 ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

Result of the proximate composition of the sesame seeds is given in **Table – 1**. The total protein content of both varieties was 18.17 and 18.30 respectively. The crude fiber was (10.37 & 11.09%) , crude ash (4. 48 & 4.78%) were found to be insignificantly different. The variety S-17 contained significantly higher percent oil than Til-93. Mineral composition of the seeds indicated calcium and iron of both varieties were almost same. S-17 contained higher concentration of copper and til-93 contained higher zinc levels. The oil content of the oil was found to be lower than being reported by Yermanos et al, however, it fell within the standards of CODEX Alimentarius standards for sesame seed oil [21, 22]. These differences may be attributed to the differences in the agro climatic conditions and agricultural practices. The proximate values and mineral content of the seed were comparable to the findings of other studies [23-25].

3.2 Physico Chemical Characteristics of the Oils

The physical analysis of sesame seed oils (**Table-2**) revealed that both varieties possessed oils with low refractive index, surface tension, density, specific gravity, moisture, and viscosity. These oils had higher mean saponification values and iodine numbers while the peroxide values, free fatty acid values and total acid number were quite low. These oil extracts exhibited good physicochemical properties indicative of being useful with diverse industrial applications. Borchani found the refractive index value of Sesame similar being found in the current study [26]. The results are in accordance with the findings of other researchers who reported exactly the same values for surface tension, specific gravity, and viscosity [26-29]. The saponification values of our study are in strong agreement with those of Nzikou *et al,* Dossa et al and CODEX standards [30, 31, 22]. Results of the acid number, iodine number, and peroxide values of our study were in compliance with Nzikou et al [30].

3.3 Fatty Acids Composition

Fatty acid composition from the selected sesame seed oils contained appreciable amounts of stearic acid, palmitic acid, oleic acid, elaidic acid. Differences in the oleic acid, linoleic acid and linolenic acid were significantly different between the studied varieties. The values of the palmitic acid, stearic acid and linoleic acid of our study fell well within the CODEX standards. Ali and Majeed , however, reported lower values for stearic acid, higher values for oleic acid and similar values for palmitic acid [22, 32]. The studied varieties contained much higher concentrations of linoleic acid and lower concentrations of oleic acid as recommended by the CODEX standards [22]. Variations in the fatty acid content may be attributed to variation in the soil condition, type of variety, and climatic conditions in which the tested seeds were grown. Of the selected varieties S-17 contained much higher beta+ gamma and total tocopherols. However both varieties contained appreciable amounts of tocopherols which fell within the CODEX standards [22].

3.4 Antimicrobial Bioassays

3.4.1 Antifungal activities of the oils

The mean antifungal activity of both sesame oils against various fungal species (Table-4) showed remarkable improvement in the inhibition when the ratio of oil in the n-hexane increased. The trend of the zone of growth of the fungal followed the pattern of:

Paecilomyces Lilacinus> Phytium Ultimum> Tricoderma Harzianum> Rhizopus Stolonifer

These activities showed that sesame oil have good antifungal properties. Results of the current study are in strong agreement with the findings of other researchers indicating antifungal effectiveness of the locally grown variety [33-35].

3.4.2 Antibacterial Potentials of the oils

The antibacterial activity of sesame oils against various bacterial species (Table-5) indicated that the zone of inhibition in the lowest fraction (2:1) was the lowest among all whereas with the increase of the crude oil the inhibition potential increased appreciably. The work of Soureshjani et al and Oginsola and Fasola are comparable to the current study where *Sesamum indicum* oil produce significantly affected against *Pseudomonas aeroginosa and Proteus mirabilis [36-37]*. The trend of inhibition among the selected strains followed the following trend:

Escherichia Coli >Proteus Mirabilis> Bacillus Cereus> Xynthomonas Campestris

DPPH Free Radical Scavenging Activity of BGS Oil

Observed DPPH scavenging activity (mg/mL DPPH scavenging) demonstrated by sesame seed oilsat the tested doses of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml respectively against the standard Quercetin demonstrated good results (Figure-5). It was observed that the extracted oil exhibited overall excellent antioxidant activity which are in close compliance with the findings of another study in which individual antioxidants extracted from the whole sesame seeds showed similar results[38]. This showed that local varieties of Sesame seeds possessed antimutagenic activity. This percent inhibition of DPPH might be attributed to the number of inherent anti oxidants and phenolic compounds that can scavenge free radicals. This can be the strongest area of the utility of these oils in a variety of natural formulations.

Table 1: Proximate Composition of *Sesamum Indicum L.* Seed

Proximate values (g/100g)			
Parameters	Til-93	S-17	P-Level
Moisture (w/w %)	4.66±2.081	3.99±1.09	0.08
Percent oil (w/w %)	47.86 ±0.19	49.83±0.56	0.041*
Crude protein %	18.17±6.091	18.30±1.43	0.29
Crude fiber %	10.37±0.26	11.09±0.029	0.065
Ash %	4.48±0.52	4.78±1.93	0.095
Carbohydrates %	28.88±2.37	27.65±1.30	0.075
Mineral (mg/100mg)			
Calcium	374.4±7.99	381.3±0.03	0.090
Iron	3.01±0.08	3.09±0.01	0.069
Copper	39.57±3.84	40.07±0.002	0.05*
Zinc	15.08±6.87	12.04±0.02	0.040*

*Values are different significantly at p≤0.05

Table 2: Physico Chemical Characteristics of the Oils

Parameters	Til-93	S-17	P-level
Color	Light yellow	Light Yellow	-----------------
Refractive index at 25^{0}C	1.47±0.71	1.39±0.07	0.21 [a]
Density (20^{0}C Kg/m^3)	1.92±0.036	1.94±0.18	0.08 [a]
Specific gravity	1.27±0.052	1.32±1.40	0.07 [a]
Surface tension	1.003±0.004	1.029±1.03	0.21 [a]
Viscosity (21^{0}C)	60.36±0.351	60.89±0.19	0.20 [a]
Kinematic viscosity (mm^2/Sec)	38.32± 0.93	37.96±0.73	0.071 [a]
Acid value (mg KoH/g)	3.7±1.5	3.08±0.67	0.086 [a]
Free fatty acid (g/100g)	1.66±0.577	1.46±0.56	0.19 [a]
Saponification value (mg KoH/g of oil)	196.3±1.527	197.8±0.28	0.071
Iodine value (g of I_2 /100g)	115.6±1.527	115.9±2.01	0.091[a]
Peroxide value (meg of O_2 kg of oil)	2.3±0.576	3.02±0.07	0.19[a]

*Values are means of three replicates; [a] figure carrying similar alphabets are not different significantly (P> or =0.05)

Table 3: Fatty Acids and Tocopherols Profile of *Sesamum Indicum L.* Seed Oils

Fatty Acids	Concentrations Percent of mg/kg^{-1}		
	Til-93	S-17	P-Level
Hexanoic acid C6:0	0.013±0.191	0.013±0.08	ns
Capric Acid C_{10}:0	0.013±0.021	0.016±0/03	ns
Lauric Acid C12:0	0.013±0.0145	0.014±0.03	ns
Myristic Acid C_{14}:0	0.067±0.52	0.070±o.o4	ns
Palmitic Acid C16:0	11.482±0.83	11.93±0.012	ns
Palmitoleic Acid C_{16}:1	0.207±0.65	0.29±0.02	ns
Stearic Acid C_{18}:0	5.808±0.296	4.98±0.03	ns
Oleic Acid C_{18}:1c	30.238±0.037	33.58±0.04	0.048*
Elaidic acid C18:1n9	1.860±2.001	1.20±0.02	0.053*
Lenoleic Acid C_{18}:2c	48.431±0.051	49.29±0.030	0.045*
q-Linolenic acid C_{18}:3n6	5.936±0.921	4.988±0.091	O.032*
Linolenic acid C_{18}:3n3	0.482±0.062	0.501±0.032	ns
Arachidic Acid C_{20}:0	0.709±0.871	0.679±0.09	ns
Behenic Acid C22:0	1.129±0.091	0.982±0.03	ns
Lignoceric acid C_{24}:0	0.067±0.30	0.059±0.19	ns
Total Saturated acid	38.39±0.023	39.08±0.56	ns
Total Unsaturated acid	85.087±0.078	83.78±0.014	O.039*
18 / 16 ratio	7.792±0.035	7.45±1.091	ns
20 – 24 ratio	1.905±0.057	1.861±0.891	ns
Tocopherol Content mg/kg^{-1}			
α-tocoperol	3.7±0.04	4.03±0.03	ns
β+ γ tocopherol	675±0.03	798.26±0.02	0.036*
δ-tocoperol	4.87±0.07	4.81±0.02	Ns
Total tocopherol	683.78±2.71	806.38±1.98	0.021*

ns non-significant variation; * Variations between the varieties is significantly different at P≤0.05

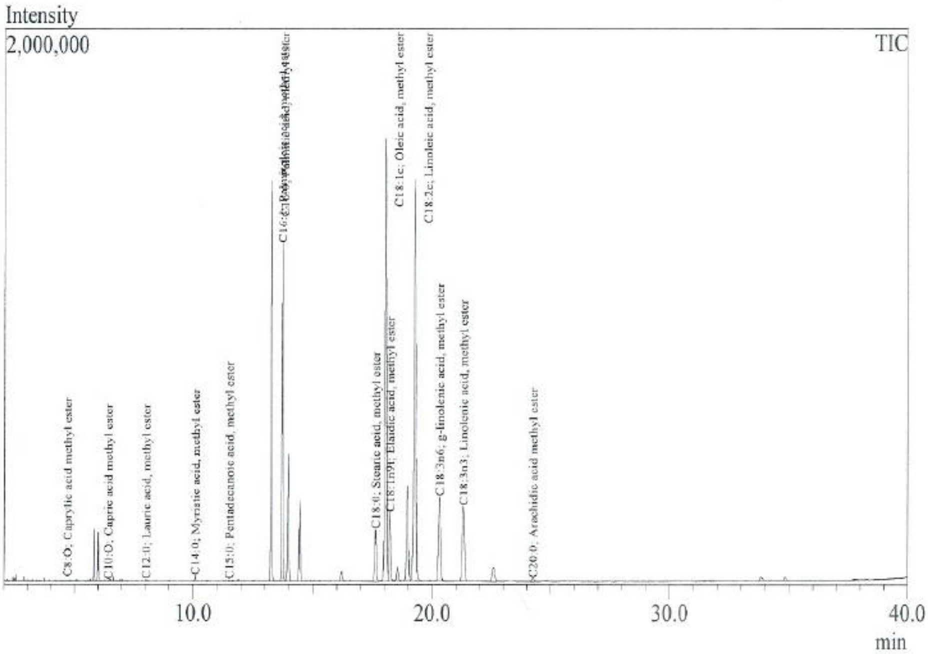

Figure 1: HPLC Chromatogram of fatty acids in Sesame Variety Til-93

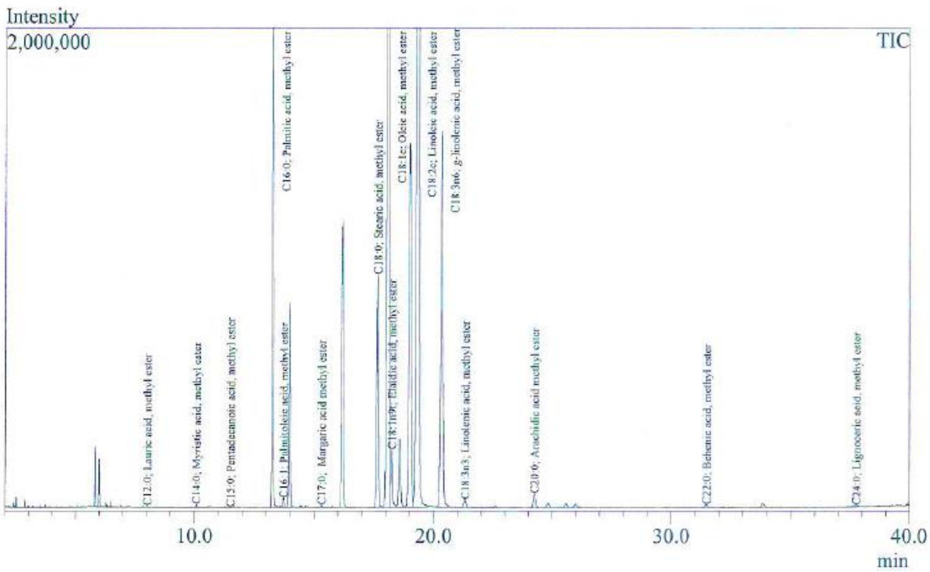

Figure 2: HPLC Chromatogram of fatty acids in Sesame variety S-17l

Figure 3: Growth inhibition zones developed by *Sesemum Indicum L.* Seed oils Til-93 and S-17 respectively against fungal strains

Table 4: Mean Antifungal Assays of *Sesamum Indicum L.* Seed Oils

Zone of Growth(mm) by various Fungal Species with n-hexane: oils concentrations						
Concetration(mg/µl)	**2:1**	**4:4**	**5:3**	**6:2**	**8:0**	**Control**
Phytium Ultimum	**18.1±1.25**	**16±2.6**	**13.3±0.57**	**11±1**	**8±0.9**	**21.5±4.82**
Paecilomyces Lilacinus	**19±1.7**	**17.6±2.0**	**14.6±4.7**	**10±1**	**8.3±1.5**	**23.1±3.2**
Rhizopus Stolonifer	**16.6±1.52**	**12.6±1.5**	**11±2**	**9.3±1.52**	**9±1**	**25±2.6**
Tricoderma Harzianum	**17.6±1.52**	**15.3±3**	**12.6±1.52**	**9±1**	**8.3±1.5**	**25±2**
STANDARD	**0**	**0**	**0**	**0**	**0**	

Table 5: Mean Antibacterial Bioassays of the *Sesamum Indicum L.* Seed Oils

Zone of Inhibition(mm) by various Bacterial Species with n-hexane-to-oils concentrations						
Concetration(mg/µl)	**2:1**	**4:4**	**5:3**	**6:2**	**8:0**	**Standard**
Xynthomonas Campestris	**2.0±1**	**3.3±2.08**	**3.6±1.5**	**5.3±0.5**	**7.6±1.5**	**10±0.081**
Proteus Mirabilis	**2.3±0.5**	**3.6±0.5**	**4.66±1.1**	**7±1**	**8.3±0.57**	**6.46±0.368**
Escherichia Coli	**5±2.6**	**6.3±4.5**	**7.6±2.08**	**8.6±2.3**	**9.6±3.5**	**10.3±0.44**
Bacillus Cereus	**3.6±2.08**	**5.3±3.05**	**6.6±1.5**	**7.33±0.5**	**8±1**	**8±0.81**
CONTROL	**0**	**0**	**0**	**0**	**0**	

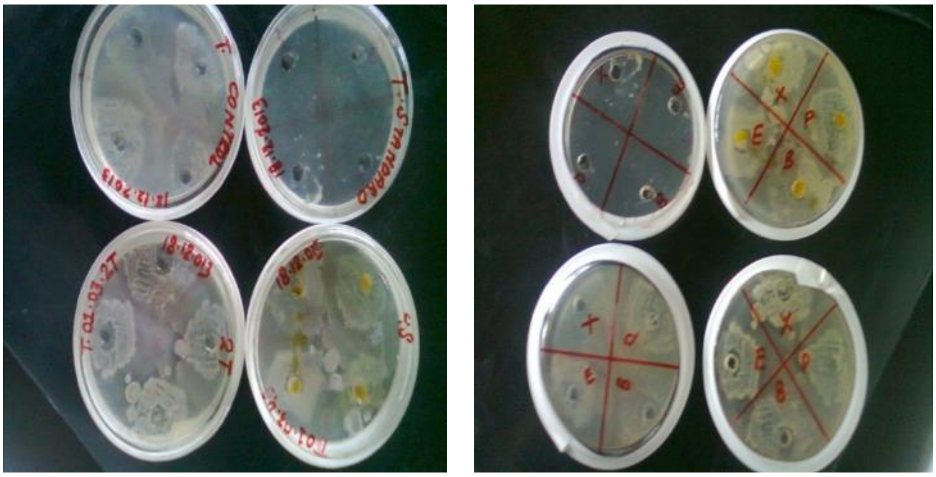

Figure 4: Growth inhibition zones developed by *Sesemum Indicum L.* Seed oils Til-93 and S-17 respectively against bacterial strains

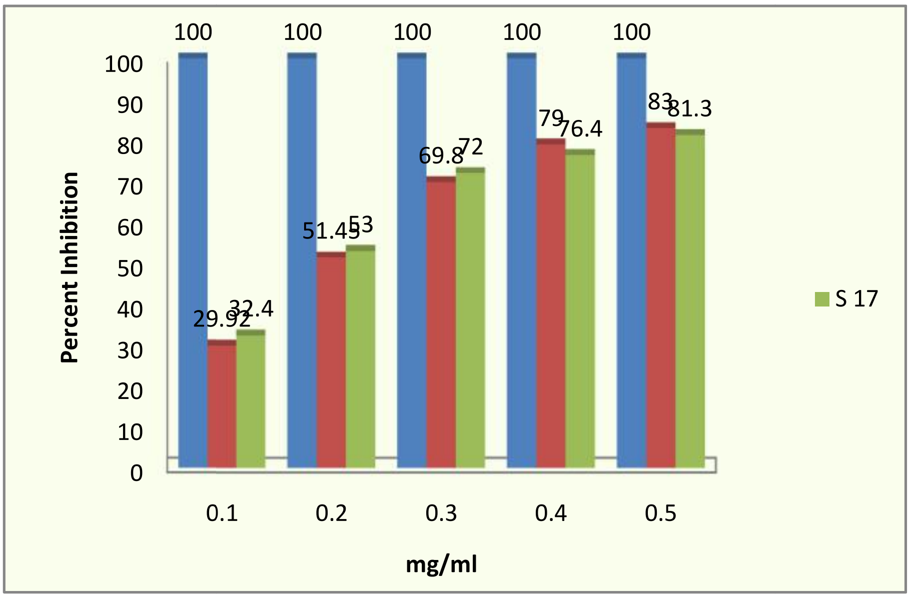

Figure-5: DPPH Free radical Scavenging of the *Sesamum Indicum L* Seed Oil

CONCLUSION

It can be concluded from the finding of the current study that local varieties of *sesemum indicum* can be an excellent potential sources of fortification in dietary lipids due to its higher level of unsaturated fatty acids as well due to its high protein and fiber content the pomace/cake can be used as a dietary fiber preparation source in human diets (unpublished work of the authors). Due to its physico-chemical properties it can be considered as a new and valuable source of edible oil having prolonged shelf life. It can be industrially utilitized in the manufacturing of margarine, soap , perfumery, and in paints and coating industry. Due to its antimicrobial properties the oil can be used as an antibacterial and antifungal agents in pharmaceutical preparations along that it can be utilized for blending with other conventional edible oils and in the preparation of nutraceuticals for health promotion.

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