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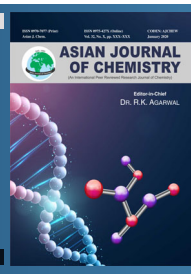

in vitro Screening of *Sesamum indicum* Seeds for Antioxidant, Phytochemical and Biological Properties

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Herbal medicines has been the most cost-effective and valuable medical practice to cure diseases and emphasize modern health care treatment. The present research was conducted to assess the biological activities of 10 fractions obtained from methanolic extract which was derived from dried seeds of *Sesamum indicum*. Antioxidant activity was assessed using DPPH radical scavenging, total antioxidant capacity and total reducing power assays. Highest free radical scavenging activity (80.3 ± 1.36%), total antioxidant capacity (104.7 ± 4.04 µg AAE/mg) and ferric reducing power activity (238.76 ± 1.23 µg AAE/mg) was shown by fraction SE. Fraction SE showed the highest phenolic contents (63.72 ± 1.5 µg GAE/mg) while fraction SG sample showed highest flavonoid contents (54.62 ± 2.61 µg QE QE/mg). Antibacterial activity was performed against four selected bacterial strains including *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogens* and *Bacillus subtilis*. Highest inhibition was shown by fraction SD (11 ± 1.04 mm) against *Staphylococcus aureus*, fraction SH against *Bacillus subtilis* (11 ± 1.06 mm) and fraction SB against *Escherichia coli*. All fractions were found inactive against the selected fungal strains. While performing antileishmanial activity, fraction SC showed highest percent mortality (78%) of *Leishmania tropica*. In brine shrimp lethality bioassay, fraction SG showed significant LD_{50} value (23.48 µg/mL).

Keywords: *Sesamum indicum*, Antioxidant assay, Antimicrobial assay, Antileishmanial assay, Cytotoxic assay.

INTRODUCTION

In human's body as result of oxidation, electron transfers from a substance to oxidizing agent resulting formation of free radicals. These radicals further start chain reaction being most reactive and unstable products of metabolism process. Reactive oxygen species (ROS) belongs to the most common free radical like hydroxyl and superoxide anion, in addition to non-free radical (*e.g.* H_2O_2 species) and the singlet oxygen come from oxygen atom. Exposure of body to ROS occur due to the exogenous sources like pollutants and endogenic sources like diseases, *etc*. [1]. Normally, the reductants in the body detoxify free radicals and there exist an equilibrium between reductants and ROS produced. However, poor antioxidant defence and/or overrun of ROS may certainly affect and induce oxidative damage to DNA, lipids and proteins which may ultimately cause many different chronic diseases, like aging, cancer, diabetes and various degenerative diseases in humans [2]. Polyphenols naturally found in vegetables, fruits and plants are significant source of natural antioxidants because they exhibit reducing activity and act as a singlet oxygen quenchers, metal chelators and hydrogen donors [3,4].

Plants holding unlimited potential to produce secondary metabolites with their versatile role in managing miscellaneous human diseases, always grasping interest of scientists [5,6]. In folk's medicine, herbal plants acting as a cheaper source of therapeutic remedies play a vital role in primary healthcare [7]. Bioresources of a plant could be an alternative to antimicrobials in the production of new bioactive compounds with identified structure. These compunds could lead to synthesize patent medicine with low toxicity or improved activity [8].

Sesame (*Sesamum indicum*) belongs to Pedaliaceae family is the ancient plant cultivated in the world and primarily grown for oil extraction from its seeds [9]. In India, sesame oil is the

vital ingredient in remedies of Ayuverdic while in Chinese medicines it is used in preventing aging and enhancing energy [10]. These activities are linked to certain bioactive ingredients of seeds including phytosterols, phenylpropanoids which include lignans like sesamol, sesamin and sesamolin, vital minerals, tocopherols and polyunsaturated fatty acids [11]. Furthermore, protection from the species of reactive oxygen and oxidative rancidity to maintain oil quality is also provided by these phytochemicals [12,13].

Moreover lignans of Sesame exhibit numerous pharmacological properties such as antimicrobial property, antiproliferative activity, antioxidant activity, antihypertensive effects, decreasing cholesterol level and to increase hepatic fatty acid oxidation enzymes [14-20]. Keeping in mind these properties, the present study was conducted to evaluate phytochemical, antioxidant, antifungal, antibacterial, brine shrimp lethality and antileishmanial activity of different fractions of *Sesame indicum* seeds.

EXPERIMENTAL

Plant collection and extraction: *Sesame indicum* plants were collected from Multan, Pakistan in August 2019 and shade dried. They were documented and identified with the help of Flora of Pakistan and also by comparing them with herbarium specimen [21]. Specimens were submitted to the Herbarium of Quaid-e-Azam University and voucher number was obtained. Seeds weighing 2.5 kg were taken, washed with distilled water, shade dried, ground and powder was macerated, filtered and oily filtrate was evaporated under reduced pressure.

Fractionation: Column chromatography was used for fractionation of crude extracts. To start with, a glass column was packed with silica (Merck Cat No.1.07734.1000) slurry prepared in chloroform. Sample weighing 125 g was dissolved in mobile phase and poured on the top of column and its outlet was opened. Solvents of different polarity were passed *i.e.* 1% $CH_3OH/CHCl_3$, 2% $CH_3OH/CHCl_3$, 3% $CH_3OH/CHCl_3$, 5% $CH_3OH/CHCl_3$, 9% $CH_3OH/CHCl_3$, 12% $CH_3OH/CHCl_3$, 15% $CH_3OH/CHCl_3$, 20% $CH_3OH/CHCl_3$, 25% $CH_3OH/CHCl_3$ and 30% $CH_3OH/CHCl_3$. The collected fractions were observed constantly by TLC. Ten fractions were obtained and named as SB, SC, SD, SE, SF, SG, SH, SI, SJ, SK.

Antioxidant assays

DPPH method: DPPH scavenging activity was calculated following the method described previously by Clarke *et al.* [22]. A 20 µL plant fraction, 180 µL of DPPH was taken in microplate and final volume was made 200 µL. After 1 h incubation at 37 ºC, absorbance reading was taken at 517 nm. Methanol and ascorbic acid were used as negative and positive control, respectively. Experiment was performed in triplicate and scavenging percentage was calculated by using percentage inhibition formula.

Phosphomolybdenum method: Total antioxidant capacity was measured by phosphomolybdenum method previously described by Ullah *et al.* [23]. Briefly, 1 mL reagent was taken in Eppendorf tube, 0.1 mL fraction was added, mixture was incubated at 95 ºC for 90 min and absorbance was taken at 695 nm. Total antioxidant capacity of all fractions (SB-SK) was calculated as ascorbic acid equivalent.

Potassium ferricyanide method: To determine reducing power of all fractions, a method reported by Ullah *et al.* [23] was followed. Briefly, 100 µL fraction, 400 µL buffer and 500 µL $K_3Fe(CN)_6$ was added to Eppendorf tube and mixture was incubated at 50 ºC for 20 min. Then, 500 µL trichloroacetic acid was added, mixture was centrifuged at 3000 rpm for 10 min, 100 µL supernatant was taken in separate microplate, $FeCl_3$ (0.1%) was added, 20 µL distilled water was also added and absorbance was taken at 630 nm wavelength.

Phytochemical analysis: Total phenolic contents of all fractions were determined using Folin-Ciocalteu reagent with little modifications [22]. Each 20 µL sample was taken in microplate, 90 µL Folin-Ciocalteu reagent was added, mixture was incubated at room temperature for 5 min, then 90 µL of 6% Na_2CO_3 solution was added to the reaction mixture and incubated for 1 h again and absorbance was taken at 715 nm. Phenolic contents were measured as gallic acid equivalent.

In order to determine total flavonoid contents, aluminium chloride colorimetric method was used [24]. Mixture was prepared in microplate by mixing 20 microliter of sample, 10 µL of potassium acetate, 10 µL of $AlCl_3$ (10%) and 160 µL distilled water to make the final volume of 200 µL. Reaction mixture was incubated at room temperature for 0.5 h and absorbance was taken at 415 nm. Total flavonoid contents were measured quercetin equivalent.

Antibacterial activity: Disc diffusion method of Bhakt *et al.* [25] was used with slight modification to determine the antibacterial activity of all fractions from SB to SK. Four bacterial strains, two Gram negative *E. coli* (ATCC 87121) and *E. aerogens* (ATCC 13048) and two Gram positive, *S. aureus* (ATCC 6538) and *B. subtilis* (ATCC 6633) were used. Experiments were performed in triplicate.

Antifungal activity: Disc Diffusion method described by Bhakt *et al.* [25] was also used for antifungal activity. Four different strains, Mucor species FCBP 0300, *Aspergillus flavis* FCBP 066, *Fusarium solani* FCBP 0064 and *Aspergillus niger* FCBP 0198 were used for antifungal activity. Experiments were performed in triplicate.

***in vitro* Antileishmanial activity:** To determine antileishmanial activity, Khan *et al.* [26] method with slight modification was followed. The Leishmania tropica KWH23 strain was used. The procedure was carried out in triplicate and percentage mortality was calculated for all samples.

Brine shrimp lethality assay: All the plant fractions were evaluated for cytotoxicity effect using brine shrimp lethality test as defined by Mclaughlin *et al.* [27]. Stock solutions of all fractions along with three dilutions of 1000, 500 and 250 µg/mL of each fraction were prepared. Doxorubicin was used positive control while DMSO was selected as a negative control. Lethal dose was calculated using table curve software.

RESULTS AND DISCUSSION

Antioxidant assays: DPPH free radical % scavenging of all fractions showed that sample SE have significant percent inhibition 80.3 ± 1.36%, followed by sample SD having 79 ±

1.72% inhibition while least percent inhibition was observed in sample SI *i.e*. 0.7 ± 0.1%. Present DPPH results are also agreed with another study tested on different plant extracts. Crude methanolic extracts of *Rhaponticum carthamoides* and *Melilotus officinalis* showed 87% and 75% inhibition while *Salvia pratensis* had 80.3% inhibition. Further the lowest % inhibition was that of *Lavandula angustifolia* and *Echinacea purpurea*, *i.e.* 35.4% and 6.8%, respectively [28]. Antioxidant potential was calculated as % scavenging/mg (Fig. 1).

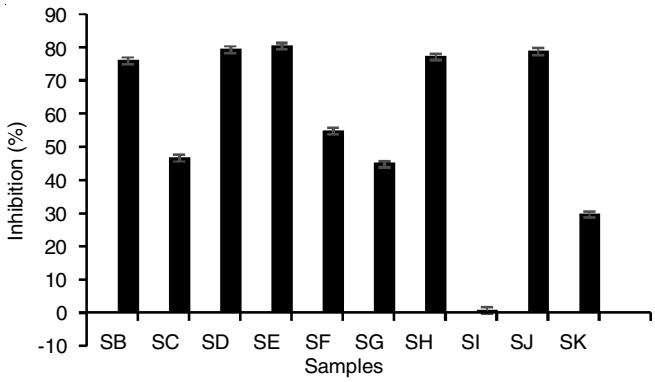

Fig. 1. DPPH free radical assay of all fractions (SB-SK) from *Sesamum indicum*

Total antioxidant capacity assay: Results indicate that the highest value of total antioxidant capacity was shown by sample SE *i.e*. 104.74 ± 4.04 µgAAE/mg while the lowest value was shown by sample SH (55.9 ± 3.45 µgAAE/mg). Similarly, a study was performed to test antioxidant activity of black and white sesame seeds [29], which strongly agreed with present results (Fig. 2).

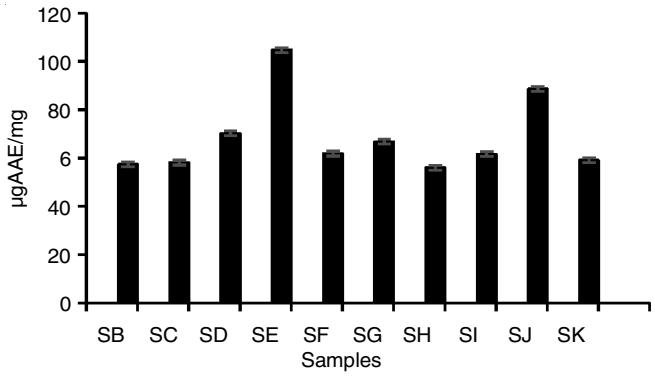

Fig. 2. Total antioxidant capacity of all fractions (SB-SK) from *Sesamum indicum* seeds

Reducing power assay: According to the results, sample SE have highest reducing power of (238.26 ± 1.23 µg AAE/mg) and the lowest reducing power was observed in sample SI (64.18 ± 2.24 µg AAE/mg). These results are in agreement to study of reducing power of methanolic extract of leaves and flowers of *Lippia alba* [30]. Similarly in another study, methanolic extract of *Buddleja officindis* was assayed for reducing power assay. Highest value was observed (284.19 µmol Fe(II)/g) (Fig. 3).

Phytochemical screening: Phenolic content was found maximum for sample SE (63.72 ± 1.50 µg GAE/mg) and minimum for sample SK (15.98 ± 0.03 µg GAE/mg extract). Shahidi *et al*. [13] studied the phenolic content of ethanolic extract of white sesame seeds and it was found to be 29.7mg/g. Similar study carried out to estimate total phenolic content of different extracts of *Anabasis aretioides* Coss. & Moq. Phenolic content was 101.85 mg/g in case of methanolic extract while phenolic content was high in case of chloroform extract (196.6 mg/g) and ethyl acetate extract (134.82 mg/g) [31] (Fig. 4).

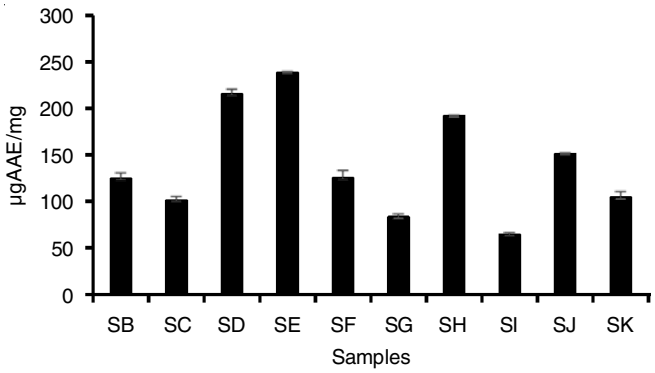

Fig. 3. Reducing power assay of all fractions (SB-SK) from *Sesamum indicum* seeds

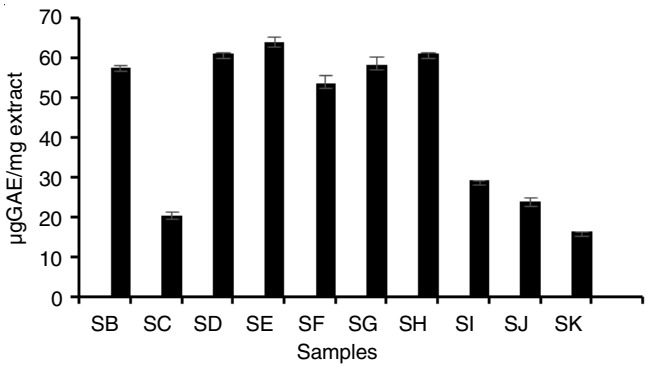

Fig. 4. Total phenolic content of all fractions (SB-SK) from *Sesamum indicum* seeds

Maximum flavonoid contents were found in sample SG (54.62 ± 2.61 µg EQ/mg) while least flavonoid content was found in SK sample (43.67 ± 1.53 µg EQ/mg) and sample SI (44.88 ± 4.11 µg EQ/mg). Present results strongly agreed with the outcomes achieved by quantification of flavonoid contents in methanolic extract of seed and pulp. With value of 74.6 µg/mg for pulp and 67.78 µg/mg for seeds [32]. The present study was also in compliance with previous research in which flavonoid content of chloroform extract of *Merremia borneensis* was observed (Fig. 5).

Antimicrobial assays: All samples showed variable activities against all strains. Slight activity was shown by all samples. Maximum zone of inhibition was found in sample SH (11.5 mm) against *B. subtilis*, followed by SD (11 ± 1.04 mm) against *S. aureus*, then SB (11 ± 0.29 mm) against *E. coli*, SE (9 ± 1.05 mm) against *S. aureus*. The average zone of inhibition of all fractions showed that SB fraction have highest zone of inhibition *i.e*. 8 mm against four strains while that of fraction SJ has the lowest average zone of inhibition. Comparatively all fractions showed significant activity against *S. aureus*. This antibacterial

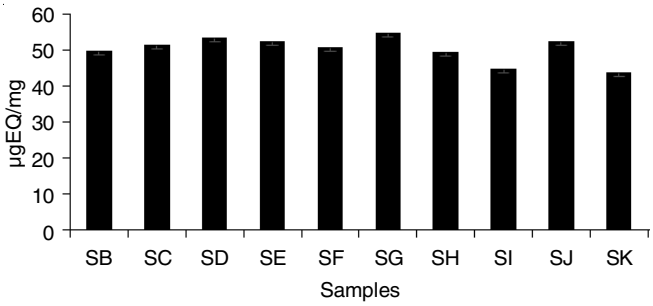

Fig. 5. Total flavonoid content of all fractions (SB-SK) from *Sesamum indicum* seeds

activity relates with one of the study of antibacterial activity of *Sesame radiatum* against *S. aureus* in which mild activity was observed against *S. aureus* strain [33]. Similar study has been carried out on antibacterial activity of *Sesamum indicum* on which methanolic extract of *Sesamum indicum* showed the highest activity against *E. coli* and *S. aureus* [34]. Standard drug was cefotaxime and 2.5 µL solution of cefotaxime was applied with a conc. of 10 µg/disc (Fig. 6).

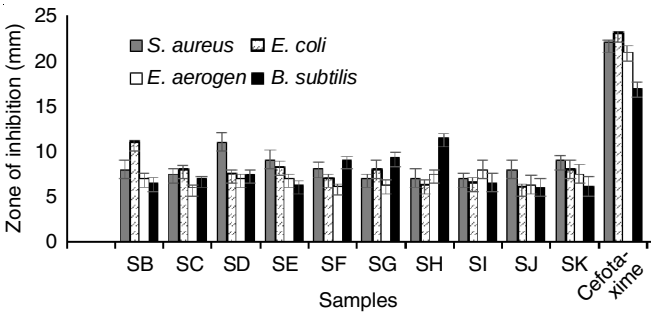

Fig. 6. Antibacterial assay of all fractions (SB-SK) of *Sesamum indicum*

According to the results, maximum zone of inhibition was shown by samples SI and SJ (7 mm) against *F. solani* and no significant antifungal activity of fractions was observed against other fungal strains. Contrary to this study, *Sesamum indicum* methanolic fractions exhibited less activity against *A. flavus*, *A. niger* and mucor specie but mild activity against *F. solani*. These results agree with one of study of antifungal activity of some plant extracts against clinical infectious agents [35] (Fig. 7).

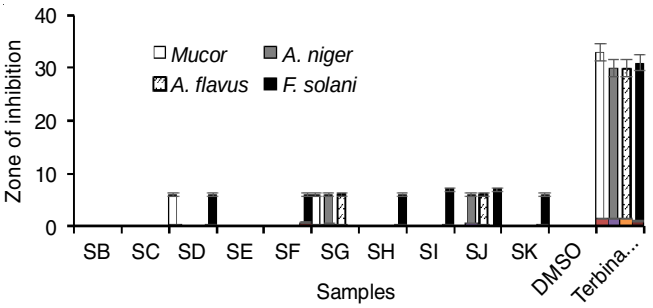

Fig. 7. Antifungal assay of all fractions (SB-SK) from *Sesamum indicum*

Antileishmanial activity: Present results revealed that highest antileishmanial activity was shown by sample SC *i.e.* 78 ± 2.21% at concentration of 50 µg/mL followed by sample SJ with percent mortality of 75 ± 2.71%. Lowest antileishmanial activity was found in sample SK *i.e.*18 ± 2.06%. Present study equates with a study in which antileishmanial activity of leaves of *Calophyllum brasiliense* at different concentration was performed [36]. A similar study was conducted on evaluation antileishmanial assay of alkaloids (indole) from *Peschiera australis* [37] (Fig. 8).

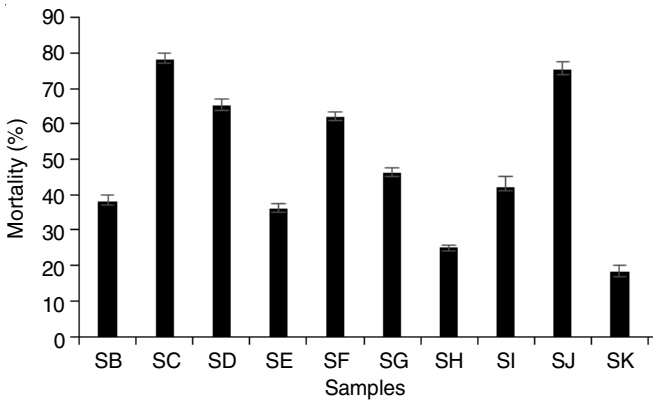

Fig. 8. Antileishmanial activity of fractions from *Sesamum indicum* seeds

Brine shrimp lethality bioassay: Results revealed the highest LD_{50} in case of sample SG (23.48 µg/mL) followed by sample SK (47.8 µg/mL). Lowest LD_{50} values were shown by sample SF (800 µg/mL) and SE (799.1 µg/mL). A study was conducted for determination of cytotoxic activity of plant material of *Croton bonplandianum* in which ethanolic fraction at the concentration of 46.7 mg/L indicated highest cytotoxicity with LD_{50} [38]. Similarly in another study, *Picralima nitida* (apoceanacea) extract of seeds was subjected to brine shrimp lethality and methanolic extracts showed better cytotoxic activity with LC_{50} 317 µg/mL [39]. This assay was performed in triplicate and the values are expressed as mean (Table-1).

TABLE-1
RESULTS OF BRINE SHRIMP LETHALITY ASSAY OF FRACTIONS OF *Sesamum indicum*

Sample code	200 µg/mL	100 µg/mL	50 µg/mL	LD_{50} (µg/mL)
SB	50	10	0	200
SC	0	0	20	49.08
SD	50	10	0	200
SE	70	80	90	799.1
SF	30	20	10	800
SG	100	60	10	23.48
SH	70	70	60	49.26
SI	90	70	0	99.15
SJ	100	90	10	56.84
SK	10	0	10	47.8
Doxorubicin	90	85	70	1.98

Conclusion

Based on the obtained results, it can be seen that the sample SG having high total flavonoid contents of 54.62 QE µg/mg have high potential towards cytotoxic activity. Similarly, sample SE having high amount of phenolic content (63.72 µg GAE/mg) showed better antioxidant activity than other samples. Furthermore, sample SC shown high antileiscmanial activity

related to its high flavoinoid contents. However, no significant antibacterial and antifungal activity was observed in the samples of methanolic extract. Conclusively, this variety of sesame seeds should be investigated further due to its high medicinal value.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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