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Research Article

EXTRACTION, IDENTIFICATION AND PHYTOCHEMICAL INVESTIGATION OF ETHYL ACETATE AND ACETONE FRACTIONS OF AQUEOUS EXTRACT OF *SESAMUM INDICUM* SEEDS

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Abstract

Objective: To identify the isolated compounds and asses the phytochemical properties of the possible functional molecules for therapeutic uses by screening the ethyl acetate and acetone fractions of water extract derived from sesame seeds (*Sesamum indicum*) *in vitro*. ***Methods:*** Fractions of ethyl acetate and acetone were extracted from the aqueous extract of *S.indicum* seed by liquid-liquid solvent extraction method and was screened for possible phytochemical, antibacterial and biomedical activities using specific standard *in vitro* methods. ***Results:*** The ethyl acetate and acetone fractions were subjected to various identification tests to identify the presence of possible flavonoid compound. The ethyl acetate fraction showed characteristic bands of flavonoids in the thin layer chromatography and ultra-violet spectroscopy. The ethyl acetate fraction was also potent antioxidant as it scavenged DPPH radical up to 70% at the concentration of 100µg/ml whereas the acetone fraction exhibited less activity. The ethyl acetate fraction showed moderate to good antimicrobial activities. In minimum bactericidal concentration test it exhibited better results in case of *S.aureus* than *E.coli*. The acetone fraction showed mild antimicrobial effect against some strains of microbes. The lethality index of the two fractions in brine shrimp indicated possible anticancer properties. The ethyl acetate fraction also showed stronger hemagglutination inhibition activity than the acetone fraction. ***Conclusion:*** The low activity of acetone fraction can be explained by the extraction procedure inefficiency. Since acetone is much soluble in water than ethyl acetate there was difficulty in separating the two layers that may had caused inadequate amount of polyphenols isolated by the procedure. Ethyl acetate fraction showed some potential therapeutic effects like antioxidant activity, antimicrobial activity, cytotoxic activity leading to anticancer property and hemagglutination inhibition activity indicating antiviral application. So, the isolated compound in this fraction may be used as future therapeutic tools if further therapeutic investigations are carried out.

Keywords: *Sesamum indicum,* TLC, HPLC, Antioxidant, Antimicrobial, Cytotoxicity, Hemagglutination.

Introduction

Sesame (*Sesamum indicum* L.) seeds have been grown in tropical regions throughout the world since prehistoric times. It is one of about 15 species of herbaceous plants of the genus 'Sesamum' native to Africa and Asia and is the most widely cultivated species for its nutritious seeds and oil. The seed has been called the "Queen of oilseed crops" because of high yield of oil and the quality of seed, oil and meal [1]. Sesame seeds are small. The size, form and colors vary with the thousands of varieties now known. The seeds are ovate, slightly flattened and somewhat thinner at the eye of the seed (hilum) than at the opposite end. The weights of the seeds are between 20 and 40 milligrams [2]. The seed contain 5.7% moisture, 20% crude protein, 3.7% ash, 3.2% crude fiber, 54% fat and 13.4% carbohydrate. It is also rich in phosphorous, iron, magnesium, manganese, zinc and vitamin B_1 [3]. Sesame seeds contain two unique substances, sesamin and sesamolin whence during refinement the two phenolic antioxidants, sesamol and sesaminol, are formed. Both of these substances belong to a group of special beneficial fibers called lignans [4]. Seeds and different parts of *S.indicum* plant were used in traditional medicine from ancient times. Sesame oil has been used in the treatment of insomnia and several chronic diseases including hepatitis,

diabetes and migraines. Dry flowers of sesame are used in curing alopecia, frostbite and constipation [5].

Pharmacological studies have demonstrated that *S.indicum* posses potential antioxidant [6, 7, 8], antimicrobial [9], analgesic [10], antipyretic [11], anti-inflammatory [12], antinociceptive [13], antineoplastic [14, 15, 16], anti-hyperlipidemic [17, 18, 19, 20, 21], anti diabetic [22, 23], hepatoprotective [24], antihypertensive [25], anti-anemic [26], neuroprotective [27] activities. Many pharmacological and nutritious compounds have been isolated from *S.indicum* seed but there were no reported work of ethyl acetate and acetone extraction from the aqueous extract of *S.indicum* seeds. According to the property of chemical compound, flavones and polyphenols has a good solubility in ethyl acetate and acetone. So there is a rational possibility to isolate flavonoid or polyphenols by the liquid-liquid extraction method using ethyl acetate and acetone from *S.indicum* seeds. So, the present investigations were carried out to identify the isolated compounds and asses the phytochemical properties of the possible functional molecules for therapeutic uses by screening the fractions of water extracts derived from sesame seeds (*Sesamum indicum*) *in vitro*.

Materials and Methods

Plant collection and identification

The fresh seeds of sesame (*S.indicum*) were purchased from the B.D.R. market, Uttara, Dhaka during July, 2013 identified by the taxonomist of the Bangladesh National Herbarium, Mirpur, Dhaka as *Sesamum indicum* L. A voucher specimen (Accession

Preparation of Chloroform Fraction from Aqueous Extract of S.indicum Seeds

Shade dried powdered black sesame seeds were used for extraction. In our study we used the catechin extraction method to prepare the ethyl acetate and acetone extract. The extract was prepared by using a liquid-liquid solvent extraction method [28] with some modifications.

Preparation of ethyl acetate fraction:

150 gm of powder sesame seed was measured and added with 1000ml of distilled water in a beaker and heated to 80ºc using hot plate. The content of the beaker was then cooled and filtered using cloth and filter paper. The filtrate was placed into separating funnel and allowed to settle for some time. Five washings with 200ml (5x40ml) chloroform were done and the aqueous layer was collected in a beaker. Chloroform is much denser than water and insoluble in it. So, the lower part was caffeine and others dissolved in chloroform and upper part was the undissolved remaining concentrate. This aqueous layer was the washed with 200ml ethyl acetate for five times. The lower layer was collected and evaporated. The extract was then stored at -80ºc for further use.

Preparation of acetone fraction: 150 gm of powder sesame seed was measured and added with 1000ml of distilled water in a beaker and heated to 95ºc using hot plate. The content of the beaker was then cooled and filtered using cloth and filter paper. The filtrate was placed into separating funnel and allowed to settle for some time. Six washings with 240ml dichloromethane were done and the aqueous layer was collected in a beaker. Dichloromethane is much denser than water and insoluble in it. So, the lower part was caffeine and others dissolved in CH_2Cl_2 and upper part was the undissolved remaining concentrate. This aqueous layer was the washed with 240ml acetone for four times. The lower layer was collected and evaporated. The extract was then stored at -80ºc for further use.

TLC analysis

The extracts were analyzed TLC to identify isolated compound. TLC was done using three solvent systems. The best result was obtained from solvent system-2 (chloroform: ethyl acetate: formic acid- 5:4:1) [29]. After development of TLC plates, they were exposed to UV light. For charring the plates were sprayed with 10% sulphuric acid solution, dried and then heated to 80-90ºC. This allowed the spots to be visible. For detection of flavanoids the plates were dipped into 0.04% DPPH solution and dried while keeping in a dark place. For detection of polyphenols the plates were washed with Folin-ciocalteu reagent and dried.

Preliminary Phytochemical Investigation

The ethyl acetate fraction of Aq. seed extract was qualitatively tested for the detection of any kind of secondary metabolites like alkaloid (Wagner Test), anthraquinone (Borntrager's Test), cardiac glycosides (Keller-kiliani Test), flavanoids (NaOH Test), steroids and terpenoids (Liebermann- Burchardt Test) [30].

UV-Visible Spectrometric Scanning of Ethyl acetate and Acetone Fractions of Aqueous Extract

Ultraviolet (UV) spectroscopy scanning [31] of the diluted samples was performed within 200nm to 400nm using a Lambda UV spectrometer (Shimadzu, Japan).

HPLC Analysis

Filtered ethyl acetate and acetone extracts were injected for HPLC analysis according to the method [33] with

some modifications. The HPLC instrument was Shimadzu Prominence high-performance liquid chromatograph from Japan. A reversed phase column was used. The mobile phase consists of acetonitrile to water (10:90 v/v) and the separation was performed by using isocratic mode at 30^{o} C temperature, elution performed at a flow rate of 1 ml/min. The 30µl sample of extract and standard was run for 10min. and detection was done at 265 nm by UV-Visible (PDA) detector.

DPPH Free Radical Scavenging Assay

The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the samples [35]. Different concentrations of the ethyl acetate fraction and acetone fraction (20, 40, 60, 80 & 100µg/ml, in respective solvents) were prepared and 100 µl of DPPH solution was added. Different concentrations of L-Ascorbic acid (20-100 µg/ml) were used as the standard antioxidant. After 30 min at room temperature, the absorbance values were measured at 517 nm on a spectrophotometer and expressed into percentage of antioxidant activity using the following equation: DPPH antiradical scavenging capacity (%) = (Absorbance of sample – Absorbance of blank) × 100/Absorbance of blank. DPPH solution plus water was used as a control. IC_{50}values denote the concentration of the sample required to scavenge 50% of DPPH radicals. The results were expressed as mean ± standard deviations.

Determination of Total Phenolic Content

The total phenolic content of chloroform fraction was determined using Folin-Ciocalteu method using salicylic acid as standard [36] with some modifications. The samples were oxidized with 10% Folin-Ciocalteu reagent and were neutralized with 700 mM sodium carbonate solution. The absorbance of the sample was measured at 765 nm after 60 minutes. A calibration curve of salicylic acid was prepared. The total phenolic content was calculated as salicylic acid equivalent by the following equation: T = C x V /M, where, T is the total phenolic content in $mg{\cdot}g^{-1}$ of the extracts as SAE, C is the concentration of salicylic acid established from the calibration curve in mg/ml, V is the volume of the extract solution in ml and M is the weight of the extract in g. The estimation of the phenolic compounds was carried out in triplicate. The results were expressed as mean ± standard deviations.

Antimicrobial Screening

The antibacterial activity was carried out by the disc diffusion method [37] using 100µL of suspension containing ~10^3 CFU/mL of microorganism spread on nutrient agar medium (Himedia, India). Different strains of bacteria (gram positive; gram negative) and fungi were used to carry out this assay. Dried and sterilized filter paper discs (6 mm diameter), impregnated with 10 mg/ml of ethyl acetate extract and 10 mg/ml of acetone extract were placed gently on the previously marked zones in the agar plates. Standard disc of Ciprofloxacin (30 µg/disc) was used as positive control. After incubation at 37^{0}C for 24 hours, the antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm. The results were expressed as mean ± standard deviations.

Minimum Bactericidal Concentration (MBC) Test of Ethyl acetate fraction

The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium [38]. 1ml of *S.aureus* and *E.coli* was taken into two separate screw test tubes from the stock then made up to 10ml stock solution with normal saline. Then 6 dilutions were run in the screw test tube for the MBC test. For the ethyl acetate crude, a stock solution of 500µg/µl was made in normal saline. In an eppendrof tube 2 ml of bacterial solution was taken from the 6^{th} dilution stock solution. 100µl of ethyl acetate crude stock was added to it and mixed with vortex and the mixture was serially diluted with normal saline in another three eppendrof tubes. A fifth eppendrof was used as control. The nutrient agar was poured into the petri dishes and left to cool and solidify. Using a sterile spreader the 100µl bacteria solution each eppendrof tube were taken and carefully swabbed on the agar plates so that the bacteria were uniformly distributed everywhere. The plates were left to rest for some time and then place in an inverted position in an incubator at 37ºC for 24 hrs. After the incubation time the plates were removed from the incubator and the formed bacteria colonies were counted. Each experiment was carried out 2 times and was correlated against the controls.

Brine Shrimp Lethality Test

Brine shrimp lethality test [39] was carried out to investigate the possible cytotoxic effect of ethyl acetate and acetone fractions. Brine shrimp eggs were hatched in a shallow rectangular dish filled with artificial seawater provided with light and aeration. We allowed 2 days (48h) for the eggs to hatch and mature as nauplii. Samples were prepared by dissolving 20 mg of the sample extract and pure caffeine in 2 ml of hot water. Dilution of this stock solution gives the series of concentrations required for testing. 6 vials for each sample were taken. The no.6 vial was used as control (nauplii without sample). To each sample vial ten shrimps were transferred using a Pasteur pipette, and artificial seawater was added to make a total volume of 5 ml. The nauplii were counted against a lighted background. Counting for the chronic LC_{50} began 24

hour after initiation of tests [40]. Nauplii were considered dead if they were lying immobile at the bottom of the vials and the percentage of deaths at each concentration and at the control were determined.

Hemagglutination Inhibition Assay

Hemagglutination activity of ethyl acetate and acetone fractions were tested against human erythrocyte blood group A+ (positive) as previously described [41] with some modifications. Stock solution of the test samples was prepared at concentration of 10 mg/ml and each solution was serially diluted. Fresh blood was collected from healthy persons, centrifuged and the erythrocytes were separated. 2% erythrocyte suspension was prepared in phosphate buffer (pH 7.4). 100µl of sample was placed in the first well and then this was subsequently diluted two fold up to the 8th well. 100µl of the RBC suspension was added to all the wells and was incubated for one hour at 4°C. After incubation, the results were noted. Smooth button formation in bottom indicated negative activity, while a rough granular deposition at bottom showed positive activity. The intensity of hemagglutination was determined from the extent of deposition.

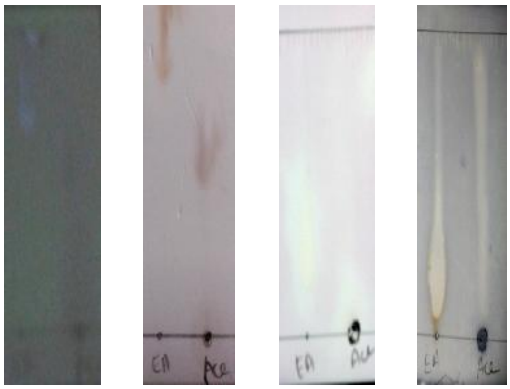
Statistical analysis

Statistical comparisons were performed with using Microsoft Excel 2007. Mean values ± SD were calculated for the parameters where applicable.

Results

TLC Analysis

TLC analysis was done as described in materials and methods. The plate was observed under UV light (indicated as 1). It showed two spots, one of pure caffeine and the other of isolated chloroform fraction with same R_f value and color. After charring of the TLC plate with sulfuric acid (indicated as 2) has visualized the two spots. After being soaked into DPPH and FC solution, plate 3 and 4 showed moderate yellow color



1 2 3 4

Figure 1: Chromatograms developed after TLC.

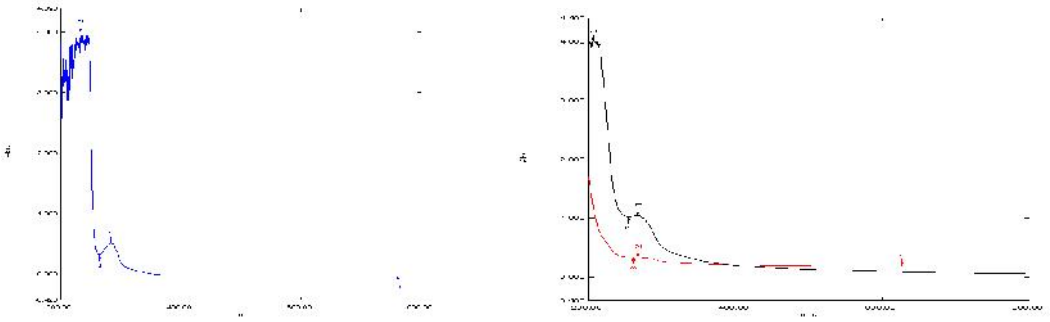
Preliminary Phytochemical Investigation of Ethyl Acetate Fraction: Preliminary phytochemical screening showed the presence of flavonoid in the ethyl acetate fraction. Other phytochemicals were absent.

Table 1: Result of preliminary phytochemical investigation of secondary metabolites

Phytochemical Test	Result
Alkaloid	-
Anthra-quinone	-
Cardiac Glycoside	-
Flavonoid	+
Steroid & Terpenoid	-

UV-Visible Spectrometric Scanning of Ethyl acetate and Acetone Fractions of Aqueous Extract

The fig showed the λmax for ethyl acetate fraction (blue line) and for acetone crude (black line). Ethyl acetate crude gave maximum absorbance at 283 nm whereas acetone crude gave maximum absorbance at 263 nm.



(a) **(b)**

Figure 2: Wavelength vs. Absorbance graph of Ethyl acetate fraction (a) and acetone fraction (b- red line) of aqueous extract (red line).

HPLC Analysis of Ethyl acetate fraction

The ethyl acetate fraction showed a single chromatogram in HPLC analysis and the retention time was 3.770 min. The chromatogram is showed below:

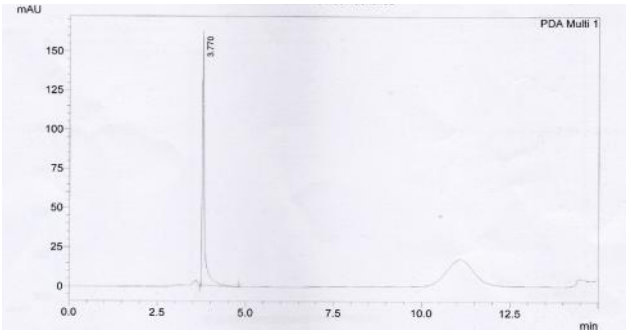


Figure 3: HPLC chromatogram of Ethyl acetate fraction with retention time 3.770 min.

DPPH Free Radical Scavenging Assay

From the analyses of Figure-4, we can conclude that the scavenging effect of ethyl acetate and acetone fraction increased with increased concentration. From the linear regression equations, the IC_{50} values of ascorbic acid, ethyl acetate fraction and acetone fraction were found to be 36.38µg/ml, 34µg/ml and 125.64µg/ml respectively.

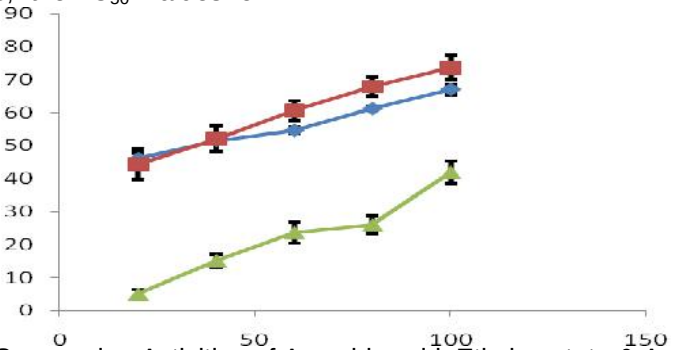


Figure 4: Comparison of Mean Scavenging Activities of Ascorbic acid, Ethyl acetate & Acetone Fractions of Aqueous Seed Extract [Ascorbic Acid: blue line; Ethyl acetate fraction: red line and Acetone fraction: green line].

Table 2: DPPH Free Radical Scavenging activity

Sample Name	IC_{50} (µg/ml)
Ascorbic Acid	36.38
Ethyl acetate crude	34.00
Acetone crude	125.64

Total Phenolic Content

In case of polyphenolic content a standard curve was used where the equation is y = 0.044x + 0.024, R^2 = 0.972. From the standard curve, the total phenolic compounds as Salicylic acid equivalent (SAE) of the ethyl acetate and acetone fraction was 40.67 mg/g and 38.17 mg/g respectively.

Table 3: Total Phenolic Content of ethyl acetate and acetone fraction

Sample Name	Total phenolic content (mg/g SAE)
Ethyl acetate crude	40.67±2.32
Acetone crude	38.17±6.33

Data are presented as mean values ± standard error of mean (±SD).

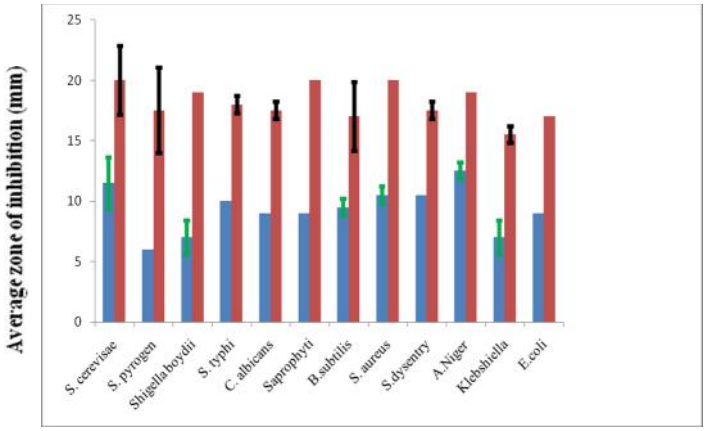
Antimicrobial Assay

The results showed that the ethyl acetate fraction isolated from the seeds of *S.indicum* has moderate to good antimicrobial effect against the tested strains of bacteria and fungi. The negative control (pure ethyl acetate) showed no antimicrobial effects.

Table 4: Zone of Inhibition for different species of bacteria and fungi for ethyl acetate fraction
*Data are presented as mean values ± SD.

Species	Ethyl acetate fraction (10mg/ml)	Positive control(30µg/µl)
S. cerevisae	11.5±2.12	20 ±2.83
S. pyrogen	6±0	17.5±3.54
Shigella boydii	7±1.41	19±0
S. typhi	10±0	18±0.71
C. albicans	9±0	17.5±0.71
Saprophyti	9±0	20±0
B.subtilis	9.5±0.71	17±2.83
S. aureus	10.5±0.71	20±0
S.dysentry	10.5±0	17.5±0.71
A.niger	12.5±0.71	19 ±0
Klebshiella	7±1.41	15.5±0.71
E.coli	9±0	17±0

Figure 5: Zone of inhibition for Ethyl acetate fraction. The values were expressed as mean ± SD. Blue bars represent values of ethyl acetate crude; red bars represent values of positive control ciprofloaxacin.



The acetone fraction exhibited mild antimicrobial activity against some strains of microbes. Here also negative control had no effect.

Table 5: Zone of Inhibition for different species of bacteria and fungi for acetone fraction

Species	Acetone crude(10mg/ml)	Positive control(30µg/µl)
S. cerevisae	6.5±0.71	20.5 ±4.95
Betahemolytic streptococcus	-	23.5±3.54
Shigella boydii	8.5±0.71	21.5±0.71
S. typhi	8±0	19±1.41
Pseudomonas	6±0.71	20.5±0.71
B.subtilis	6.5±0.71	20.5±2.12
S. paratyphi	6.5±1.21	19.5±0.71
S.dysentry	6±0	17.5±0.71
Klebshiella	-	18±2.83
E.coli	-	22.5±7.79

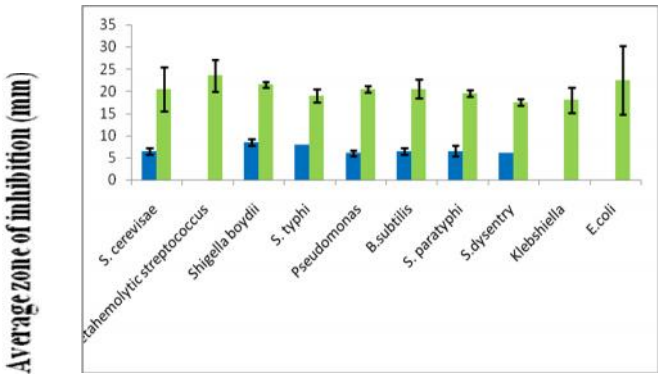


Figure 6: Zone of inhibition for Acetone fraction. The values were expressed as mean ± SD. Blue bars represent values of acetone fraction; green bars represent values of positive control ciprofloaxacin.

Minimum Bactericidal Concentration (MBC) Test of Ethyl acetate fraction

The MBC test was carried out only with the ethyl acetate crude since it exhibited good antimicrobial activity against microbes. The highest concentration of the sample that was taken was 50mg/ml and was serially diluted. *E.coli* and *S.aureus* were used as test microorganisms. The bacterial concentration at which (30-300) bacterial colonies were formed was used as test concentration. The results are given below:

Table 6: MBC Test on *E.coli* with ethyl acetate fraction

Species	Minimum bactericidal concentration (CFU)				
	500µg/µl	**250µg/µl**	**125µg/µl**	**62.5µg/µl**	**Control**
E.coli	24.5 ± 0.71	61.5 ± 7.78	89.5 ± 3.54	141±22.63	295 ± 7.07
S.aureus	1.5 ± 2.12	6.5 ± 4.95	12.5 ± 6.36	115 ± 8.48	177.5 ± 10.61

*Data are presented as mean values ± SD.

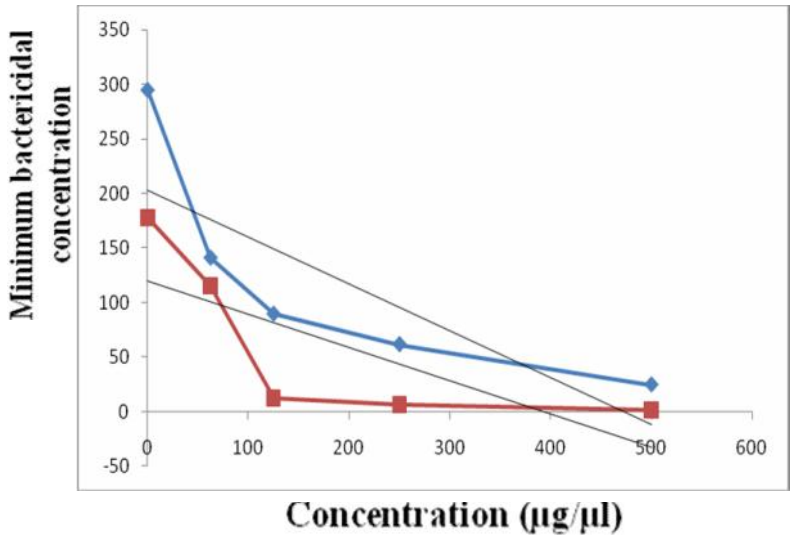


Figure 7: Concentration vs. bacterial count graph of ethyl acetate fraction; X axis represents concentration of sample; Y axis represents minimum bacterial count; blue line represents *E.coli* with regression equation: y = -0.429x +202.7 with R^2 = 0.646; red line represents *S.aureus* with regression equation: y = -0.304x +119.6 with R^2 = 0.571**.**

The graph above showed that the bacterial colony forming units were increasing in numbers with the decrease of sample concentration. Ethyl acetate fraction showed 50% bactericidal concentration against *E.coli* at the concentration of 355.9µg/µl and against *S.aureus* at the concentration of 228.9µg/µl.

Brine shrimp lethality test

In brine shrimp lethality bioassay, percentage of mortality increased gradually with the increase in concentration of the test samples. Using the linear regression equations, y = -11.42x + 103.3 where R^2 = 0.979 and y = -7.42x + 86 where R^2 = 0.965, the LC_{50} values of ethyl acetate crude and acetone crude were determined respectively. The LC_{50} values for ethyl acetate crude and acetone crude were 4.67 mg/ml and 4.85 mg/ml respectively.

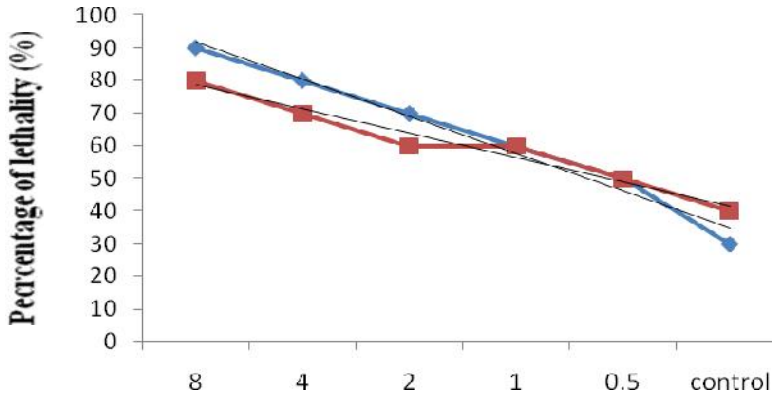


Figure 8: Concentration vs. percentage of lethality graph of Ethyl acetate fraction and acetone fraction [Ethyl acetate fraction: blue line; acetone fraction: red line].

Hemagglutination Inhibition Assay

Ethyl acetate fraction exhibited hemagglutination inhibition activity potentially from highest concentration 10mg/ml to 0.0781 mg/ml i.e. it has potential binding capacity with human erythrocytes whereas, acetone fraction exhibited less hemagglutination inhibition activity at the concentrations 10mg/ml to 0.625 mg/ml.

Discussions

The plate held under UV lamp, revealed two spots, each one for ethyl acetate fraction (light violet color) and acetone fraction (deep violet color). Single chromatogram indicated the isolation of a single compound by the isolation method. Also the violet color of the spots seen under UV lamp indicated the presence of flavonoids [42]. Once the plate was sprayed with 10% sulfuric acid and heated, the spot for ethyl acetate gave pink color and later became light brown in color when cooled. The flavonoid catechin also gave pink colored spot when sprayed with vanillin or sulfuric acid and it also gave spot at the upper end of TLC plate [43]. The spot for ethyl acetate fraction was also situated near the end of TLC plate with R_f value 0.94. So, the compound isolated by ethyl acetate might be catechin like flavonoid. The spot for acetone crude became brown in color after charring and did not showed any pink colored spot like ethyl acetate crude and had different R_f value (0.71) than ethyl acetate crude. The formation of yellow and white color spots on the plates after being exposed to DPPH solution and Folin-Ciocalteu reagent respectively indicated that the isolated compounds had antioxidant activity like flavonoids.

Preliminary phytochemical screening was done to detect any kind of secondary metabolites like alkaloid, flavonoid, steroid, reducing sugar etc in the ethyl acetate and acetone fraction. From the tests it was concluded that the ethyl acetate fraction might be flavonoid because it gave positive result for the flavonoid test only. The yellow color formed during the addition of NaOH became colorless when diluted HCl was added. This confirms the presence of flavonoid. On the other hand, the acetone fraction did not showed any results.

The spectrum of wavelength vs. absorbance for ethyl acetate fraction and acetone fraction was obtained from the UV-Visible spectrophotometer and the maximum wave length (λ_{max}) for ethyl acetate crude was 283 nm and the λ_{max} for was acetone crude 263 nm. Catechin of ethyl acetate extract of *C. mellei* and *C. quadrifidus* gave maximum absorbance at 218 nm and 282 nm [43]. Generally, the maximum absorbance of catechin ranges from 220 nm to 280 nm. The λ_{max} for ethyl acetate crude and acetone crude also falls under this range. So the compounds isolated by this two fraction might be catechin like flavonoid.

The ethyl acetate crude showed a single chromatogram in HPLC analysis. This indicated the presence of a single compound. The retention time for ethyl acetate fraction chromatogram was 3.770 min [33].

DPPH test was carried out with standard solution of ascorbic acid, sample solutions of ethyl acetate crude and acetone crude. There was a good linear relationship between the absorbance of DPPH and its concentration. The linear regression equations for ascorbic acid, ethyl acetate crude and acetone were $y = 0.257x + 40.65$, $R^2 = 0.988$; $y = 0.372x + 37.35$, $R^2 = 0.995$; $y = 0.422x - 3.021$, $R^2 = 0.955$ respectively in this study, where y is the absorbance, x is the concentration.

The both samples could react completely with DPPH within 30 min and be stable for more than 30 min. Ttudies showed that sesame and its components serve as viable natural sources of antioxidants for food and non-food applications. Feeding sesame lignans to rats have shown to reduce Fe 2+ induced oxidative stress [6]. In a study, the ethyl acetate fraction of ethanol extract of tea flowers showed highest DPPH-scavenging activity at a concentration of 1 mg/ml and the activity was related to the polyphenol and flavone content [44]. Flavonoids, including flavonols, flavanols, flavones and isoflavones have capacity to act as antioxidants [45]. In our study, the IC_{50} values of ascorbic acid, ethyl acetate fraction of W.E. and acetone fraction of W.E. were 36.38µg/ml, 34µg/ml and 125.64µg/ml respectively [Table 2]. The ethyl acetate fraction potentially scavenged DPPH up to 74% but the acetone fraction showed low scavenging activity (42%) than the ethyl acetate fraction. Since sesame seed compounds and flavonoids are potential antioxidant, the desired antioxidant activity was found in case of ethyl acetate fraction. So the isolated compound could be a future drug interest for antioxidant activity.

A study reported that the total polyphenolic content found in ethanolic extract of black and white sesame seed were 19.8 mg/g GAE and 78.4 mg/g GAE (gallic acid equivalent) [46]. In our study, the phenolic content of ethyl acetate fraction of aqueous extract of black sesame seed was 40.67 mg/g eq. of salicylic acid which was higher than the phenolic content acetone fraction of aqueous extract of (38.17 mg/g eq. of salicylic acid) and also higher than reported the phenolic content of ethanolic extract of black sesame seed.

In antimicrobial screening the results showed that the ethyl acetate fraction of water extract isolated from the seeds of *S.indicum* had moderate to good antimicrobial effect against the tested strains of bacteria and fungi whereas the negative control (pure ethyl acetate) showed no antimicrobial effects. This can be explained by the flavonoid nature of the isolated compound because flavonoids are a family of plant-derived compounds with potential antibacterial activities. In addition to direct and synergistic antibacterial activities activities, flavonoids inhibit a number of bacterial virulence factors, including quorum-sensing signal receptors, enzymes and toxins [47]. Studies conducted over the last 20 years have shown that the green tea polyphenolic catechins can inhibit the growth of a wide range of Gram-positive and Gram-negative bacterial species with moderate potency [48]. The acetone fraction exhibited mild antimicrobial activity against some strains of microbes. Here also negative control had no effect. In this case, the ethyl acetate fraction showed better activity than acetone fraction.

In MBC test, ethyl acetate fraction showed 50% bactericidal concentration against *E.coli* at the concentration of 355.9µg/µl and showed 50% bactericidal concentration against *S.aureus* at the concentration of 228.9µg/µl. Ethyl acetate fraction exhibited better bactericidal activity against *S.aureus* in comparison with *E.coli*. This activity of ethyl acetate fraction might be due to presence of flavonoid compound because the MBC test uses colony forming units as a proxy measure of bacterial viability and it can be confounded by antibacterial agents that cause aggregation of bacterial cells like flavonoids [49]. Another explanation is, the ethyl acetate fraction might also contain catechin like flavonoid because in a study it was found that aqueous extracts of teas (*Camellia sinensis*) inhibited a wide range of pathogenic bacteria, including *methicillin-resistant Staphylococcus aureus* which was due to the presence of catechins in tea [50].

The brine shrimp lethality assay was used for the evaluation of cytotoxic effects of ethyl acetate and acetone fraction with a view to evaluate their possible anticancer effect. The LC_{50} values for ethyl acetate fraction and acetone fraction were 4.67 mg/ml and 4.85 mg/ml respectively. Sesamol from sesame seed showed a remarkable cytotoxic activity in the brine shrimp lethality assays [15]. Some other components of S.indicum seeds had anticancer properties [15]. Flavonoids also could be used in combination therapy with other chemotherapeutic agents in cancer [51]. So, the brine shrimp lethality values of ethyl acetate and acetone fractions might be of importance.

Hemagglutination inhibition assay was performed to investigate the receptor binding affinity of ethyl acetate and acetone fraction on human erythrocytes. It was observed in previous study that the aqueous crude of *S.indicum* seed has different binding affinity to the different receptors of erythrocytes and prevent agglutination [41]. The results showed that the both fractions isolated from aqueous extract of *S.indicum* seeds could have possible benefits as an antiviral therapeutics.

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