Chemical characterization and antimicrobial activity of *Sesamum indicum* (Sesame oil)

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Abstract- It has been noted from the past few decades, researchers are striving for the development of natural alternate to antibiotic due to their resistance problems. Bacterial infection and its resistance is a major health issue that affects millions of people throughout the world. *Sesamum indicum* L. is one of the important seed crops worldwide to get edible seed and oil. In the present study we evaluate the chemical characteristics including refractive index, wt/ml, pH, viscosity, acid value, iodine value and peroxide value and found that the obtained Results are under the specification. The antimicrobial activity of oil shows that the minimum inhibitory concentration (MIC) is in the range of 17 µl to 460 µl. the best MIC is 17 µl against *Salmonella species.* The zone of inhibition of sesame oil against the tested microorganism is in the range of 18 mm to 25 mm. the best zone of inhibition is 25 mm against *Salmonella species.* So we can say that the antimicrobial activity of sesame oil is best against *Salmonella species*out of the microorganism which we have taken for activity.

Introduction[1-2]

Sesamum indicum L. is one of the important seed crops worldwide to get edible seed and oil. This crop is cultivated since ancient times. Various species of sesame were found in Africa and generally believed that it is originated in Africa while a smaller number in India. Sesame seed is used wholly in cooking because of its rich nutty flavor and oil. Commonly the paler varieties of sesame seem to be more valued in the West and Middle East, while the black varieties are highly valued in the East. It is used as a cooking oil, as a soap fat, in pharmaceuticals and as a synergist for insecticides. In many countries sesame oil is very popular as cooking oil and is more expensive than other vegetable oils. Sesame oil apply for darker hair and also for loss of hair. Refined sesame oil is used in herbal drugs manufacturing company. Sesame oil is a source of anti-oxidant as it contains vitamin E and its anti-oxidant activity has been reported. Sesame oil is also used in lowering cholesterol levels. Sesame oil contains magnesium, copper, calcium, iron, zinc and vitamin B6. Copper present in sesame oil can provide relief in rheumatoid arthritis. Magnesium of sesame oil supports vascular and respiratory health systems while Calcium helps to prevent colon cancer, osteoporosis, and migraine. The aim of the present study is to chemically characterize

as well as evaluate the antimicrobial activity of sesame oil.

Material and Methods

Sesame oil is purchased from local supplier of Delhi (Shiv Sales Corporation). All chemical and reagent are HPLC or Analytical grade.

Chemical Characterization[3]

Refractive index

The refractive index (η) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. The Abbe's refractometer is used for measurements of refractive index. To achieve accuracy, the apparatus should be calibrated against distilled water which has a refractive index of 1.3325 at 25^0.
Apparatus is used per the manufacturer instruction or as per the SOP.

Weight per ml

The weight per millilitre of a liquid is the weight in g of 1 ml of a liquid when weighed in air at 25 ^{0}C. Calibrate the pycnometer by filling it with recently boiled and cooled water at 25 ^{0}C and weighing the contents. Assuming that the weight of 1 ml of water at 25 ^{0}C when weighed in air of density 0.0012 g per ml, is 0.99602 g. Calculate the capacity of the pycnometer. (Ordinary deviations in the density of air from the value given do not affect the Results of a determination significantly). Adjust the temperature of the substance to be examined, to about 20 ^{0}C and fill the pycnometer with it. Adjust the temperature of the filled pycnometer to 25 ^{0}C, remove any excess of the substance and weigh. Subtract the tare weight of the pycnometer from the filled weight of the pycnometer. Determine the weight per milliliter dividing the weight in air, expressed in g, of the quantity of liquid which fills the pycnometer at the specified temperature, by the capacity expressed in ml, of the pycnometer at the same temperature.

pH

The pH value of a liquid can be determined potentiometrically by means of the glass electrode, a reference electrode and a pH meter. Operate the instrument as per the manufacturer instruction or as per the SOP.

Viscosity

Viscosity is a property of a liquid, which is closely related to the resistance to flow. Viscosity is measured by the help of Brookfield Viscometer using spindle no. 3 and speed of 50 RPM.

Acid Value

The acid value is the number of mg of potassium hydroxide required to neutralize the free acids in Weigh accurately about 10 g of the substance into a 250 ml flask and add 50 ml of a mixture of equal volumes of alcohol and solvent ether, which has been neutralized after the addition of 1 ml of solution of phenolphthalein. Heat gently on a water-bath, if necessary until the substance has completely melted, titrate with 0.1 N potassium hydroxide, shaking constantly until a pink color which persists for fifteen seconds is obtained. Note the number of ml required.

$$\text{Acid Value} = \frac{a \times 0.00561 \times 1000}{W}$$

where 'a' is the number of ml of 0.1 N potassium hydroxide required and 'W' is

the weight in g of the substance taken.

Iodine Value

The Iodine value of a substance is the weight of iodine absorbed by 100 parts by weight of the substance, when determined by one of the following methods:-

Place the substance accurately weighed, in dry iodine flask, add 10 ml of carbon tetrachloride, and dissolve. Add 20 ml of iodine monochloride solution insert the stopper, previously moistened with solution of potassium iodine and allow to stand in a dark place at a temperature of about 25 ^{0}C for thirty minutes. Add 15 ml of solution of potassium iodine and 100 ml water; shake, and titrate with 0.1 N sodium thiosulphate, using solution of starch as indicator. Note the number of ml required (a). At the same time carry out the operation in exactly the same manner, but without the substance being tested, and note the number of ml of 0.1 N sodium thiosulphate required (b).

Calculate the iodine value from the formula:-

$$\text{Iodine value} = \frac{(b-a) \times 0.01269 \times 100}{W}$$

Where 'W' is the weight in g of the substance taken. The approximate weight, in g, of the substance to be taken may be calculated by dividing 20 by the highest expected iodine value. If more than half the available halogen is absorbed, the test must be repeated, a smaller quantity of the substance being used.

Peroxide Value

The peroxide value is the number of milliequivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance. Method Unless otherwise specified in the individual monograph, weigh 5 g of the substance being examined, accurately weighed, into a 250-ml glass-stoppered conical flask, add 30 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform, swirl until dissolved and add 0.5ml volumes of saturated potassium iodide solution. Allow to stand for exactly 1 minute, with occasional shaking, add 30 ml of water and titrate gradually, with continuous and vigorous shaking, with 0.01M sodium thiosulphate until the yellow color almost disappears. Add 0.5 ml of starch solution and continue the titration, shaking vigorously until the blue color just disappears (ml). Repeat the operation omitting the substance being examined (b ml). The volume of 0.01M sodium thiosulphate in the blank determination must not exceed 0.1 ml.

Calculate the peroxide value from the expression

Peroxide value = 10 (a – b)/W

Where W = weight, in g, of the substance.

Anti-Microbial Activity[4-12]

The following microorganisms were used for anti-microbial activity. Staphylococcus aureus, Escherichia coli, Salmonella species and Pseudomonas aeruginosa. All the microorganisms were maintained at 4°C on nutrient agar slants.

Determination of minimum inhibitory concentration (MIC) using micro dilution method:

The Minimum Inhibitory Concentrations (MICs) of sesame oil found to be active by the diffusion test were determined based on the macrodilution method (Berghe and Vlietinck, 1991) with some modifications as follows. The sesame oil was serially diluted (two-fold) in a

series of test tubes using nutrient broth supplemented with 10% glucose and 0.05% phenol red (colour indicator). These were later inoculated with 0.2ml suspension of the test organisms. The final concentrations were in the range 1000 to 10 µl/mL in the medium. Microbial growth was determined by observing for color change in the tube (red to yellow when there is growth). The lowest concentration that showed no change of color was considered as the MIC.

Anti-microbial activity

Cup plate method using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of sesame oil against different microbial strains. The agar medium was purchased from HI media Laboratories Ltd., Mumbai, India. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water was done as per the standard procedure. The cups each of 9mm diameter were made by scooping out medium with a sterilized corkborer in a petri dish which was streaked with the organisms. The sesame oil (50 µL) was added separatelyin thecups andpetri dishes were subsequently incubated. Kenamycin (30 µg) were used as standard reference drugs. Zone of inhibition produced by sesame oil was measured in mm.

Results and discussion

The chemical characterization of sesame oil shows that it has the quality required to use in the purpose of herbal product manufacturing.

The Results shows in the below table:

Sr. No	Parameters	Value ± SD (n=3)
01	Refractive index	1.471 ± 0.003
02	Weight/ml (g/ml)	0.914 ± 0.002
03	pH	4.40 ± 0.020
04	Viscosity (cp)	50 ± 1.000
05	Acid Value (%)	0.982 ± 0.007
06	Iodine Value	112.333 ± 0.007
07	Peroxide Value	3.354 ± 0.061

Antimicrobial activity

Minimum inhibitory concentration (MIC) of sesame oil

The lowest minimum inhibitory concentration of sesame oil is 17 µl against salmonella species. The MIC for other microorganism was found to be 330 µl for Pseudomonas aeruginosa, 380 µl for *Staphylococcus aureus*and 460 µl *Escherichia coli* **as shown in table below**:

Sr. No	Micro-organism	MIC Value of sesame oil (µl)
1	*Escherichia coli*	460
2	*Salmonella species*	17
3	*Pseudomonas aeruginosa*	330
4	*Staphylococcus aureus*	380

The sesame oil show antimicrobial activity against the tested microorganism with the inhibition zone ranging from 18 mm to 25 mm as shown in below table. Sesame oil show comparable Results with the kenamycin used as standard antibacterial drug.

Sr. No	Micro-organism	Zone of inhibition (mm)	
		Kenamycin	Sesame oil
1	*Escherichia coli*	20	18
2	*Salmonella species*	25	25
3	*Pseudomonas aeruginosa*	21	21
4	*Staphylococcus aureus*	20	23

Conclusion

The chemical characterization of oil justify that the quality is good and in the range of specification. The Results of the study shows that the MIC of oil is 17 µl against *Salmonella species* is the lowest against microorganism used in the test.Zone of inhibition for most of the microorganism is comparable with the standard drug kenamycin used. Now we can conclude that sesame oil have good chemical qualities and antimicrobial activity against the microorganism used in the test.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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