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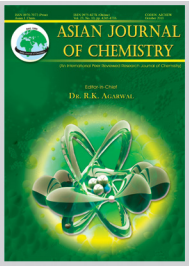

Biochemical Investigation of Oil of *Eugenia carophyllata*

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The oil of *Eugenia carophyllata* was extracted with petroleum ether and ethanol. The oil was subsequently investigated for its fundamental properties and antibacterial activity. The homogenized seeds and the residue of *Eugenia carophyllata* left after extraction were also checked only for antibacterial activity. It was observed that the homogenized seeds were active against *Escherichia coli* and *Staphylococcus aureus*. The oil extracted with petroleum ether and ethanol were active against both organisms, while the residue left after extraction with petroleum ether was active against *Escherichia coli* but was inactive against *Staphylococcus aureus*. The residue left after extraction with ethyl alcohol was active against both the organisms. The physical and chemical properties of oils were also determined and compared. The oils extracted were also subjected to thin layer chromatography to determine the polar and non-polar lipids.

Key Words: *Eugenia carophyllata*, Biochemical, Investigation, Oil.

INTRODUCTION

The medicinal plants, herbs, seeds and oils have been in use as cures of a large number of diseases, particularly, in Indo-Pak subcontinent since long without any scientific sanction behind this use. Important examples are studies by Khan and Amira[1], Khan and Saad[2], Khan and Javed[3], Ahmed[4] and Perveen[5] on different plant seeds that have been reported to exhibit antibacterial activity. Thus, there is the likelihood of their use as the substitutes of antibiotics.

Morris *et al.*[6] studied the antibacterial activity of this plant and its composition was reviewed by Chaieb *et al.*[7]. More recently, some researchers have actively worked on its different aspects *Eugenia carophyllata* in applied context with a special focus on its medicinal uses. These studies deal with isolation of virus-cell fusion inhibitory components[8], chemical constituents of its essential oil[9], antiplatelet activity of phenylpropanoids isolated from its leaf oil[10], its content of organochlorine pesticide[11], fumigant activity of its essential oils and components against the Japanese termite[12] and preservative action due to presence of sodium benzoate[13] and toxicology[14,15].

The central theme of the work being reported here is to rationalize the use of *Eugenia carophyllata* as cure of some infectious diseases and improve its value on sound analytical basis. Both chemical and biological characteristics of oil of *Eugenia carophyllata* were studied to determine its status for its use in industry and rationalize its application as medicinal cure of different diseases.

EXPERIMENTAL

The seeds of *Eugenia carophyllata* were obtained from a Hakeem shop in Akbari Mandi, Lahore. The dried seeds were ground in an electric grinder and the seed powder was subsequently preserved desiccated in a sample bottle.

Determination of moisture content of seeds: A weighed quantity of the ground seeds was placed in an oven at 105 ºC and heated for 1 h, cooled and weighed. The heating and cooling was repeated till the weight was constant. The percentage of moisture was calculated as follows:

$$\text{Moisture}\,(\%) = \frac{\text{Loss in weight of seeds}}{\text{Weight of seeds}} \times 100$$

Extraction of oil from the seeds: Oil of *Eugenia carophyllata* was extracted by solvent extraction method. 100 g dry seed powder was filled in a filter paper thimble, which was subsequently placed in a soxhlet apparatus. The oil extraction was switched on and after 48 h the extract was subjected to distillation. The solvent was recovered and the oil remaining in the distillation flask was weighed. The extraction was carried out independently using two solvents such as petroleum ether and ethanol. The percentage yield of oil extracted was calculated as below:

$$\text{Oil}\,(\%) = \frac{\text{Weight of oil}}{\text{Weight of seeds}} \times 100$$

Determination of antibacterial activity: In the determination of antibacterial activity, two types of pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* were selected because of their quick reproduction, short life spans, adequate resistance to environmental conditions and easy availability. Labex, Clinic Jail road, Lahore provided cultures of both bacteria.

Growth medium: Medium used for the growth of both pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* was blood-agar base. It was particularly designed and claimed in literature to be the best medium for the reproduction of pathogenic microorganisms. The growth medium contained G/L of beef heart infusion 2.0, Peptone 220 (yeast casein polypeptone) 10.0, peptone 140 (pancreatic digest of casein) 6.0, sodium chloride 5.0, yeast extract 2.0 and agar 15.0. The pH of the medium was adjusted to 6.8 ± 0.2 at 25 ºC.

This was then followed by the addition of adequate quantities of seed powder, seed residue, extracted oil, *etc*.

Method of determination: To determine antibacterial activity, 2 g of blood-agar base medium was dissolved in 50 mL of distilled water in 250 mL conical flask and sterilized by autoclaving at 121ºC and 15 psi. The contents of the flask were cooled to 60 ºC, sample and 2 mL of blood was added. It was then shaken well and poured into sterilized petri dishes and the medium was then allowed to set. For comparison, control petri dishes were also prepared following the same procedure but without test samples. Both sample and control pertri dishes were inoculated with bacterial cultures and incubated in a Gallenkamp incubator at 37 ºC for 24 to 48 h and the growth was compared.

Determination of physical characteristics of oil: Colour of the oil was determined with the help of Lovi brand tintometer and melting point by capillary tube method. Specific gravity of oil was determined at room temperature, using specific gravity bottle and refractive index of oil was determined by means of Abbe's refractometer.

Determination of chemical characteristics of oils: The chemical characteristics of oils were generally determined by applying the methods of AOAC[16].

Iodine value: Iodine value was determined by Wij's method. 2 g of oil was dissolved in 5 mL CCl_4 in a glass-stoppered bottle and 25 mL of Wij's solution was added. After placing it in dark for 0.5 h, 30 mL of 10 % KI solution was added and iodine liberated was titrated against $Na_2S_2O_3$ (0.1 N) solution using starch solution as an indicator. A blank as above without sample was run side-by-side. Iodine value of oil was calculated from the relationships.

$$\text{Iodine value} = \frac{(B - A) \times 12.69}{\text{Weight of oil}} \times \text{Normality } (0.1\text{N})$$

where, A = Volume of (0.1 N) $Na_2S_2O_3$ used for blank; B = Volume of (0.1 N) $Na_2S_2O_3$ used for sample.

Acid value: To determine acid value, to 1 g of oil sample was mix with 25 mL neutral alcohol in a flask. The contents were heated for 10 min and titrated against 0.1 N KOH solution using phenolphthalein as indicator. The acid value was calculated as follows:

$$\text{Acid value} = \frac{\text{Volume of KOH (0.1N) used} \times 0.0056}{\text{Weight of oil}} \times 1000$$

Free fatty acid: The free fatty acid content of oil was calculated from the data on acid value on the basis of the oleic acid and by applying the relationship.

$$\text{Free fatty acid} = \frac{V \times W}{W \times 100}$$

whereas V = volume of KOH (0.1 N) used, W = weight of oil and M = 282 (molecular weight of oleic acid).

Saponification value of oil: To determine saponification value, 1 g oil was dissolved in 50 mL of 0.5 N alcoholic KOH in a flask. The contents of the flask were refluxed for 0.5 h and subsequently titrated against 0.5 N HC1 using phenolphthalein as an indicator. The saponification value was calculated as:

$$\text{Saponification value} = \frac{(B - A) \times 0.028}{\text{Weight of oil}} \times 1000$$

where, B = volume of 0.5 N HC1 used for blank; A = volume of 0.5 N HC1 used for sample.

Ester value: The ester value was calculated from the relationship:

Ester value = Saponification value - Acid value

Thin layer-chromatography of oil: The thin layer chromatographic plates of 0.25 mm thickness were prepared using 30 g of silica gel and 60 mL water for preparation of five plates. These were activated by heating at 105 ºC for 1 h and later used for the separation of neutral and polar lipids using hexane: ether: acetic acid (60: 20:20:v/v) and chloroform: methanol: 30 % NH_4OH: water (60:35.2.5:2.5 v/v) solvent systems respectively.

RESULTS AND DISCUSSION

Moisture and oil yield: The percentage of moisture in *Eugenia carophyllata* seed was 6.7 %. The percentage of oil extracted from the seeds with petroleum ether was 16.80 % while that with ethanol was 46 %. The higher % age yield with alcohol indicates that the lipids present in the oil are dominantly polar.

General characteristics of the oil extracted with polar and non-polar solvents: Physical and chemical characteristics of oil extracted with two solvents ethanol and petroleum ether are consolidated in Table-1.

TABLE-1
GENERAL CHARACTERISTICS OF THE OIL EXTRACTED FROM *Eugenia carophyllata* SEEDS

Characteristics	Extraction with ethanol	Extraction with petroleum ether
Colour	Dark brown	Light brown
Melting point	-1 ºC	-3 ºC
Specific gravity	0.2430	0.3125
Refractive index	1.5268	1.5281
Viscosity	42.580	41.980
Iodine value	200.00	239.00
Acid value	142.80	153. 44
Free fatty acid (%)	33.840	39.760
Saponification value	250.50	256.00
Ester value	107.70	102.56

Eugenia carophyllata, being an expensive seed, the oils extracted may not be of industrial significance, yet, some points

about their physical characteristics are made below in the academic context.

The iodine value of the oil extracted with petroleum ether is 239, while that extracted with ethanol is 200 (Table-1). This means that the oil extracted with alcohol consists of lower degree unsaturated compounds and are more polar as compared to those present in the oil extracted with petroleum ether. This suggests that use of ethanol-extracted oil may be more suitable for the patients suffering from blood pressure and heart diseases.

Lower acid value of alcohol extracted oil shows that it is more suitable for edible purposes as compared to the oil extracted with petroleum ether.

The saponification value of oil from *Eugenia carophyllata* (250 to 256) is greater than that of the cottonseed oil. This means that the chain lengths of the fatty acid components of the oil of *Eugenia carophyllata* are shorter than those of the fatty acid components of cotton seed oil. On the other hand, its oil will require more alkali for complete saponification than that required for the oil from cottonseeds.

Antibacterial activity of oil: The antibacterial activity of homogenized *Eugenia carophyllata* seeds, oil extracted from *Eugenia carophyllata* seeds and the residue left after the extraction of oil was determined against some standard bacterial species such as *E. coli* and *S. aureus*, commonly used by the clinical and research laboratories, for both routine tests and research work (Table-2).

TABLE-2
ANTIBACTERIAL ACTIVITY OF HOMOGENIZED SEEDS OF *Eugenia carophyllata* against *E. coli* AND *S. aurous*

Weight of powdered seeds in 50 mL of medium	Growth in control against *E. coli.* After hours		Growth in control against *S. aureus.* After hours		Growth in sample against *E. coli.* After Hours		Growth in sample against *S. aureus* After Hours	
	24	48	24	48	24	48	24	48
5g	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve

-ve = no growth, +ve = growth

Antibacterial activity of homogenized seeds of *Eugenia carophyllata*: The antibacterial activities of homogenized seeds of *Eugenia carophyllata* against *E. coli* and *S. aureus* are reported in the Table-2. Results indicate that the seeds of *Eugenia carophyllata* contain antibacterial activity against both the organisms because -ve sign indicates no growth of bacteria in the sample while the +ve sign in the control indicates growth of bacteria.

Antibacterial activity of oils extracted from seeds of using different solvents: Antibacterial activity of oils extracted from seeds of *Eugenia carophyllata* using petroleum ether and ethanol as solvent is recorded in Table-3. Data (Table-3) show that oils extracted from the seeds of *Eugenia carophyllata* with petroleum ether and ethanol exhibit antibacterial activity against both *E. coli* and *S. aureus.* Not a single colony of any of the organisms was observed on the surface of the agar in presence of these oils.

Antibacterial activity of residues left after extraction of oils: Antibacterial activity of residues left after extraction of oils is recorded in Table-4. Results (Table-4) show that the residue left after extraction of oil from *Eugenia carophyllata* with petroleum ether exhibits antibacterial activity against *E. coli* but does not exhibit any antibacterial activity against *S. aureus*. On the other hand, the residue left after extraction with ethanol exhibits antibacterial activity against both the organisms. Not a single colony of *E. coli* and *S. aureus* grew on the agar surface in presence of residue, which was left after extraction with ethanol as solvent.

The comparison of results computed in Tables 2-4 indicate that not all the antibacterial components are extracted by the neutral petroleum ether, while the polar ethanol is capable of extracting all antibacterial components. There is also the likelihood that some of the antibacterial components may be non-polar and may be extracted by petroleum ether.

Thin layer chromatography of extracted oil: Different components of oil extracted from seeds of *Eugenia carophyllata* are shown in Table-5.

TABLE-5
DIFFERENT LIPID CONTENTS OF *Eugenia carophyllata* OIL DETERMINED BY TLC

Neutral lipids	Polar lipids
Hydrocarbons (Traces)	Phosphatidlyl Ethanol Amine
Wax Ester	Phosphatidly1 Choline
Sterol Ester	Phosphatidyl Inositol
Triglycerides	Unknown
Free Fatty Acid	
Sterol	
Diglyceride	
Monoglyceride	

TABLE-3
ANTIBACTERIAL ACTIVITY OF OILS EXTRACTED FROM SEEDS OF *Eugenia carophyllata* USING DIFFERENT SOLVENTS

Solvent	Weight (g) of oil per 50 mL of medium	Growth in control against E. *coli* after hours		Growth in control against *S. aureus* after hours		Growth in sample against *E. coli* after hours		Growth in sample against *S. aureus* after hours	
		24	48	24	48	24	48	24	48
Petroleum ether	1	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Ethanol	1	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve

TABLE-4
ANTIBACTERIAL ACTIVITY OF RESIDUES LEFT AFTER EXTRACTION OF OILS

Residues	Weight (g) of oil per 50 mL of medium	Growth in control against *E. coli* after hours		Growth in control against *S. aureus* after hours		Growth in sample against *E. coli* after hours		Growth in sample against *S. aureus* after hours	
		24	48	24	48	24	48	24	48
Petroleum ether	5	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
Ethanol	5	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve

The results (Table-5) indicate that the oil extracted from *Eugenia carophyllata*, contains both neutral and polar components. After knowing the composition of *Eugenia carophyllata*, the project may be extended further to investigate, which components are responsible for the antibacterial activity.

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