Short Communication

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Biochemical and Antibacterial Studies of Ajowan (*Carum copticum*) Oil

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Carum copticum is used for the treatment of gastric and kidney trouble, eye sore, fever, diarrhea, etc (Umar Skii and Krutic 1945). The antibacterial activity of *Carum copticum* essential oil and fruit was investigated by Kasymova *et al* (1957). Wahid and Ikram (1961) determined that thymol was the active constituent present in *Carum copticum*. Singh *et al* (1980) tested its essential oil for fungicidal activity and reported that these oils were also toxic to some human pathogens. Amira and Khan (1990) studied the antibacterial activity of *Carum copticum* oil extracted with ethanol and acetone. Ijaz *et al* (1993) studied the triglycerides of its seeds oil extracted with chloroform at room temperature.

The major purpose of the present work was to rationalize the use of *Carum copticum* as cure of infectious diseases and prove its medicinal value on sound analytical basis.

Seeds of *Carum copticum* (ajowan) were obtained from a Hakeem's shop in Missri Shah Lahore. The moisture was determined by heating a weighed sample at 110°C. The loss in weight per 100g was reported as percentage of moisture. Oil was extracted separately with *n*-hexane and ethanol by Solvent Extraction Method.

Determination of antibacterial activity.Culture. The antibacterial activity was determined against two types of pathogenic bacteria, *Escherichia coli* and *Staphylococcus aureus* provided by Labex Clinic, Jail Road, Lahore. The medium used for the growth of both pathogenic bacteria was blood-agar base. Its composition was as follows:

	$g\ l^{-1}$
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
Agar	15.0
	pH 6.8 at 25°C

Adequate quantities of seed powder, seed residue, extracted oil, etc. were added to the media.

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

*Analysis of oil.*The colour of oil was determined with the help of Lovi Bond Tintometer. Melting point was determined by capillary tube method. Specific gravity of the oil was determined at room temperature and the refractive index of the oil was determined by means of Abbe's refractometer.

Iodine value: Iodine value of the oil was determined by Wiji's method. 0.5 g of oil was dissolved in 5ml CCl_4 solution in a flask and 25ml of Wiji's solution (8g ICl_3 in 200ml glacial acetic acid + 9g iodine in 500 ml glacial acetic acid) was added. After placing the flask in dark for 30 min 30ml of 10% KI solution was added and liberated iodine was titrated against $Na_2S_2O_3$ (0.1N) solution using starch solution as an indicator. A blank as above without sample was also run side by side. Iodine value of oil was calculated from the relationship.

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

B = Vol. of (0.1N) $Na_2S_2O_3$ used for blank.

A = Vol. of (0.1N) $Na_2S_2O_3$ used for sample.

Acid value: To determine the acid value, to 1g of oil sample,

Table 1

General characteristics of the oil extracted from *Carum copticum*

S No	Characteristics	Extracted with Ethyl aclohol	Extracted with *n*-hexane
1	Colour	Dark green	Green
2	Melting point	-1°C	-1°C
3	Specific gravity	0.944	0.961
4	Refractive index	1.47	1.463
5	Iodine value	108.00	109.134
6	Acid value	7.30	7.28
7	Free fatty acid	3.90	3.66
8	Saponification value	170	168
9	Ester value	162.70	160.72

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Table 2

Antibacterial activity of powder seed, oil extracted from seed using different solvents and residues left after extraction

Solvents/ samples	Wt of samples	Growth in control against *S.aureus* after		Growth in control against *E.coli* after		Growth in samples against *S.aureus* after		Growth in samples against *E. coli* after	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Seed powder	5g	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve
Oil									
n-hexane	1 g	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
ethanol	1 g	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Residue									
n-hexane	5 g	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
ethanol	5 g	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve

25ml acid free alcohol was added in a flask. The flask was heated for 10 min at 80°C and the contents were titrated against 0.1N KOH solution using phenophthalein as indicator. The acid value was calculated as follows:-

$$\text{Acid value} = \frac{\text{Vol. of KOH (.1N) used x 0.0056x1000}}{\text{Weight of oil}}$$

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

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

V = Volume of KOH used (0.1N)
W = Weight of oil
M = 232 (molecular weight of oleic acid)

Saponification value of oil. One gram oil was dissolved in 50ml of (0.5N) alcoholic KOH in a flask. The contents of the flask were refluxed for half an hour and subsequently titrated against 0.5N HCl using phenolphthalein as indicator.

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

B = Vol. of 0.5 N HCl used for blank.
A = Vol. of 0.5 N HCl used for sample.

Ester value. The ester value was calculated as:
Saponification value - Acid value

Determination of lipid components of oil by TLC. The thin layer plates of 0.25 mm thickness were prepared by using 30g of silica jel and 60ml water (for five plates). These were activated at 105°C for one hour and later used for separation of neutral and polar lipids by using hexane: ether: acetic acid (80:20:2 v/v) and chloroform: methanol: 30% NH_4OH: water (60:35:5:2.5 v/v) solvent system respectively.

Moisture and oil yield: The moisture content in the seeds was 1.7%. The percentage of seed oil extracted with *n*-hexane was 31.80% while that with ethanol was 28.0%.

General characteristics of oil with polar (ethanol) and non polar (n-hexane) solvents. The physical and chemical characteristics of the oils extracted with the two solvents indicate slight difference due to polar and non-polar nature of solvents (Table 1).

Neutral lipid components of the oil included hydrocarbons, wax esters, sterol esters, triglycerides, free fatty acids, diglycerides, sterols and monoglycerides whereas the Polar lipid components were phosphatidyl ethanolamines, phosphatidyl cholines.

Antibacterial activity. The antibacterial activity of homogenized seeds of *Carum copticum* seed oil and residues (Table 2) indicates that the powdered seeds of *Carum copticum* exhibit antibacterial activity only against *S.aureus*. The oil extracted with *n*-hexane exhibit the antibacterial activity against both the organisms while the oil extracted with ethanol and *n*-hexane residue did not exhibit antibacterial activity against both the organisms. The residue left after ethanol extraction exhibits antibacterial activity only against *E.coli*.

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