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Biochemical Investigation of Oil of *Elettaria cardamomum*

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The oil of cardamom was extracted with petroleum ether and ethyl alcohol. The oil was subsequently investigated for its fundamental properties and antibacterial activity. The residue of cardamomum left after extraction was checked for only antibacterial activity. It was observed that the oil extracted with petroleum ether was not active against *E. coli* and *S. auerus*, while the residue left after extraction was also inactive against *E. coli* and *S. aureus*. The oil extracted with ethyl alcohol and residue left after the extraction was also found inactive against *E. coli* and *S. aureus*. 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: Oil, *Elettaria cardamomum*.

INTRODUCTION

The importance of fats and oils in human society is well known. They are source of large number of nutrients for human beings and other living organisms and are also a richer source of energy as compared to proteins and carbohydrates. Oils are very important constituents of all living cells and tissues. Some unsaturated fatty acids contained in oils are essential for growth and maintenance of skin, liver and kidneys. Due to being greasy materials, they also act as lubricants in the alimentary canal and other sites. Both oils and fats have extensive applications as raw materials in chemical industry. The vegetable oils are used in large amounts in industry for production of soaps, detergents, paints, varnishes, *etc*. Different Hakeems, Vaidyas and herbal specialists extensively use a number of plant seeds to treat various infectious diseases as the seeds carry significant antimicrobial activity. The activity is due to the presence of natural products, which have been extracted from the plant seeds and their medicinal value has been established. The medicinal plants, herbs, seeds and their oils have been in use as cures of a large number of diseases in Indo-Pak sub-continent since ages without any scientific basis. Moreover, the synthetic drugs such as antibiotics, sulfa drugs, *etc*., are now expensive and beyond the reach of common man particularly in the developing countries. That is why the plants and seeds were considered worth investigating in medicinal context.

Some studies have been recently undertaken in Pakistan to rationalize their use as medicines. Important examples are *Nigella sativa* (Kalvangi) and *Carum copticum* (Ajowain), which have been reported to exhibit antibacterial activity and thus there is the likelihood of their use as substitutes of antibiotics[1]. Here, the investigation was focused on *Elettaria cardamomum*. Its characteristics were studied by Muller in 1950[2] and by Layer in 1955[3]. Shankaracharya and Natarajan[4] studied the chemistry and uses of *Elettaria cardamomum*. Zacharia and Gopalan[5] studied the composition and properties of its oil. Ramadan *et al.*[6] determined the percentage yield, specific gravity, refractive index and antimicrobial activities of ten essential oils including *Elettaria cardamom*. Huang *et al.*[7] showed that the oil of cardamom could be used as an enhancer of skin permeation. It could also be used as substitute for indomethacin, piroxican and diclofenac.

The work reported here was carried out to determine the antibacterial activity of *Elettaria cardamomum* against *S. aureus* and *E. coli*, the common human pathogens. Of course, both chemical and biological investigations of the oil of *Elettaria cardamom* were carried to determine its status for use in industry and rationalize its use as medicinal cure of various diseases.

EXPERIMENTAL

The seeds of *Elettaria cardamomum* (Ilachi) were purchased from the local market of Lahore.

Determination of moisture in seeds: A weighed quantity of ground seeds was placed in an oven at 105 ºC and after heating for 1 h it was cooled and re-weighed. The heating and

cooling was repeated till the weight was constant. From the loss of weight, the percentage of moisture was calculated.

Oil was extracted by solvent extraction method. 100 g dry ground seeds were filled in a filter paper thimble, which was subsequently placed in 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 remained in the distillation flask. The extraction was carried out independently using two solvents ethyl alcohol and petroleum ether. The oil extracted was weighed and its yield calculated.

For the determination of the antibacterial activity, two types of pathogenic bacteria *E. coli* and *S. auerus* were selected because of their quick reproduction, short life span, adequate resistance to environmental conditions and easy availability. Labex Clinic, Jail Road Lahore, provided cultures of both bacteria.

The medium used for the growth of both pathogenic bacteria *E. coli* and *S. aureus* was blood-agar base. Its composition was as under:

	g (L)
Beef heart infusion	2.0
Peptone 220 (yeast casein polypepton)	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 ºC.

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 121 ºC and 15 psi. The contents of flask was cooled to 60 ºC, sample (1 g of oil or 5 g of powder seeds) and 2 mL blood was added. It was then shaken well and poured into sterilized petridishes. The medium was then allowed to set. In comparison, control petridishes were also prepared following the same course but without test samples. Both samples and control petridishes were inoculated with bacterial cultures and incubated in a Gallen Kamp incubator at 37 ºC for 24-48 h and the growth was compared.

Colour of oil was determined with Lovi bond tintometer using three sides: blue, yellow and red and refractive index of oil were determined by means of Aabbe's Refractometer. Iodine value, acid value, free fatty acids (%), saponification value and ester value was determined by standard methods of AOAC[8].

The thin layer chromatography plates of 0.25 mm thickness were prepared using 30 g of silica gel and 60 mL water (for five plates). These were activated at 105 ºC for 1 h and later used for the separation of neutral and polar lipids using hexane: ether: acetic acid (80:20:2 v/v) and chloroform: methanol: 30 % NH_2OH: water (60:35.5:2.5 v/v) solvent systems, respectively[9].

RESULTS AND DISCUSSION

The results reported after the biochemical investigation of *Elettaria cardamomum* oil are given below.

The percentage of moisture in *Elettaria cardamomum* seeds is found to be 8 %. The percentage of oil extracted from seeds of *Elettaria cardamomum* by different solvents is reported in Table-1.

TABLE-1
YIELD OF OIL FROM SEEDS OF *Elettaria cardamomum* USING DIFFERENT SOLVENTS

Solvent	Oil (%)
Pet. ether	6.4
Ethanol	7.3

The physical and chemical characteristics of oil extracted from seeds of *Elettaria cardamomum* using petroleum ether as solvent are given in Table-2.

TABLE-2
PHYSICAL AND CHEMICAL CHARACTERISTICS OF OIL EXTRACTED FROM SEEDS OF *Elettaria cardamomum* BY PETROLEUM ETHER

Colour	Pale yellow
Specific gravity	0.924
Refractive index	1.462
Iodine value	92.35
Acid value	20.16
Saponification value	192
Ester value	180.84
Free fatty acid (%)	10.15

The physical and chemical characteristics of oil extracted from *Elettaria cardamomum* using ethanol as solvents are given in Table-3.

TABLE-3
PHYSICAL AND CHEMICAL CHARACTERISTICS OF OIL EXTRACTED FROM SEEDS OF *Elettaria cardamomum* BY ETHANOL

Colour	Dark yellow
Specific gravity	0.927
Refractive index	1.467
Iodine value	97.67
Acid value	23.52
Saponification value	201
Ester value	168.48
Free fatty acid (%)	11.0

The antibacterial activity of homogenized seeds of *Elettaria cardamomum* against *E. coli* and *S. aureus* is shown in Table-4. Results indicate that the seeds of *Elettaria cardamomum* do not exhibit antibacterial activity because +ve sign indicates growth of bacteria in the sample. Antibacterial activity of oil extracted from seeds of *Elettaria cardamomum* using petroleum ether and ethanol as solvent is recorded in Table-5. Results show that oil extracted from the seeds of *Elettaria cardamomum* with pet. ether and ethanol does not exhibit antibacterial activity against *S. aureus* and *E. coli*. Antibacterial activity of residues left after extraction of oil is recorded in Table-6. Results show that the residue left after the extraction does not exhibit any antibacterial activity against *E. coli* and *S. aureus*. Different fatty components of oil extracted from seeds of *Elettaria cardamomum* are shown in Table-7.

TABLE-4
ANTIBACTERIAL ACTIVITY OF HOMOGENIZED SEEDS OF *Elettaria cardamomum* AGAINST *E. coli* AND *S. aureus*

Weight of powder 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	
5g	24	48	24	48	24	48	24	48
	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

TABLE-5
ANTIBACTERIAL ACTIVITY OF OILS EXTRACTED FROM SEEDS OF *Elettaria cardamomum* USING DIFFERENT SOLVENTS

Solvents	Weight 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
Pet. ether	1 g	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Ethanol	1 g	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

TABLE-6
ANTIBACTERIAL ACTIVITY OF RESIDUES LEFT AFTER EXTRACTION OF OILS

Solvents	Weight 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
Pet. ether	5 g	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Ethanol	5 g	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

TABLE-7
DIFFERENT LIPID CONTENTS OF *Elettaria cardamomum* OIL AS DETERMINED BY TLC

Neutral Lipids (from oil extracted from seeds by using pet. ether)	Polar lipids (from oil extracted from seeds by ethyl alcohol as solvent)
Hydrocarbon	Phosphatidyl ethanol amines
Wax ester	Phosphatidyl cholines
Sterol ester	Trigalactoside diglycerides
Triglyceride	Unknown
Free fatty acids	
Unknown	
1,3-diglyceride	
1,2-diglyceride	
Sterol	
2-Monoglyceride	
1-Monoglyceride	

The object of the work reported here was the determination of the characteristics of oil extracted from *Elettaria cardamomum* in industrial and medical contexts. From the percentage of oil extracted from the seeds shown in Table-1 from which it is clear that the percentage of oil extracted using ethanol as solvent is greater than that of percentage of oil extracted using petroleum ether as solvent. The comparison of the characteristics of oil extracted from seeds of *Elettaria cardamomum* with petroleum ether as solvent (Table-2) with the characteristics of the oil extracted with ethanol (Table-3) show that the oils differ in characteristics such as saponification value and ester value. The characteristics of the oils such as colour, specific gravity, refractive index, iodine value, acid value and free fatty acids (%) are almost similar. The differences observed in different values may be due to the differences in the composition of oil samples obtained by extraction with different solvents. From the physical and chemical values shown in Tables 2 and 3, it is clear that petroleum ether extract has iodine value 92.35 whereas for alcoholic extract it is 97.67. This indicates that the oil extracted with ethyl alcohol consists of slightly higher degree unsaturated compounds as compared to those present in the oil extracted with petroleum ether. The acid value of alcohol-extracted oil *i.e.*, 23.52 (Table-3) is greater than that of oil extracted with petroleum ether *i.e.*, 20.16 (Table-2). Thus petroleum ether extracted oil is more suitable for edible purpose than alcohol extracted oil. The iodine value of oil extracted from *Elettaria cardamomum i.e.*, 92.35-97.65 is less than that of cottonseed oil, 99-115 and sunflower seed oil, 122-136 and thus our results reported by previous workers[10]. The oil extracted from *Elatteria cardamomum* is less unsaturated than the oil extracted from cottonseed oil and sunflower seed oil. Saponofication value of the oil extracted from *Elettaria cardamomum*, 192-201 is greater than that of cottonseed oil, 189-198. The results are again in agreement with those already reported[10]. This means that chain lengths of the fatty acid components of oil of *Elatteria cardamomum* are less than those of the fatty acid of cottonseed oil. Out of the reported characteristics, different values may be considered in different contexts to examine the suitability of oil for different industrial uses particularly in the production of various oil based commercial products such as soaps, paints, commercial chemicals *etc*. For example, high saponification value 201 qualifies the oil for use in soap industry but *Elettaria cardamomum* being an expensive seed; oils extracted from it may not be of industrial significance. This does not mean that their physical characteristics may not be discussed. A few important characteristics may be discussed in academic context as done above. The oil extracted from the seeds with petroleum ether and ethyl alcohol as solvent is found inactive against both *E. coli* and *S. aureus* (Table-5). The residue left after the extraction of oil from the seeds is found inactive against both *E. coli* and *S. aureus* (Table-6). From the results shown in Table-7, it is clear that the oil contains both polar and neutral lipid components.

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