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Biochemical Investigation of Oil of *Papaver somniferum*

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The oil of *Papaver somniferum* was extracted with *n*-hexane and acetone. The oil was subsequently investigated for its fundamental properties and antibacterial activity. The residue of *Papaver somniferum* left after extraction was checked for antibacterial activity. It was observed that the oil extracted with *n*-hexane was not active against *E. coli* but was active against *S. aureus*, while the residue left after extraction was active against both pathogens. The oil extracted with acetone was active against *E. coli* but was inactive against *S. aureus*. Its residue was found active against *E.coli* but was inactive against *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 its polar and non-polar lipid components.

Key Words: Oil, *Papaver somniferum*.

INTRODUCTION

The importance of fats and oils in human society is well known. They are source of a large number of nutrients for the human beings and other living organisms and are also a richer source of energy as compared to protein and carbohydrates. Oils are key components of all living cells. Certain unsaturated fatty acids contained in oils are essential for the growth and maintenance of skin, liver and kidneys. They also act as lubricants in the alimentary canal and other sites due to their greasy nature. The vegetable oils are used in large amounts in industries such as soaps, detergents, paints, varnishes *etc*. Some of the oils have been recently proved to exhibit antibacterial activity. An important example is the oil from *Carum copticum*[1]. Many studies on the seeds and their oils have been reported[2-4]. Here the choice of seeds in the said context was of *Papaver somniferum*, which was investigated by Benesova and Boda[5]. These workers reported thin layer chromatographic examination of this seed oil and determined lipids, fatty acids and lipoxygenase activity in developing poppy seeding *Papaver somniferum*. Some studies of similar nature have been carried in Pakistan[6-8] also.

The object of work here was to rationalize the use of *Papaver somniferum* as a cure of infectious diseases and improve its medicinal value on sound analytical basis.

EXPERIMENTAL

Seeds of *Papaver somniferum* were obtained from a Hakeem shop in Baghbanpura Bazar, Lahore.

Determination of moisture in seeds: A weighed quantity of seeds taken in a china dish 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. Moisture percentage 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 was extracted by solvent extraction method. 100 g dry seeds were ground in a grinder and 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: *n*-hexane and acetone. The percentage of oil extracted was calculated as below.

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

Determination of antibacterial activity

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

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 to be the best medium for the reproduction of pathogenic microorganisms. Its composition was as under:

		g (L)
i	Beef heat infusion	2.00
ii	Peptone 220* yeast casein polypeptone)	10.00
iii	Peptone 140 (pancreatic digest of casein)	6.00
iv	Sodium chloride	5.00
v	Yeast extract	2.00
vi	Agar	15.00
pH 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 121 ºC and 15 psi for 20 min. 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 petridishes. The medium was then allowed to set. In comparison, control petridishes were also prepared following the same course but without test samples. Both sample and control pertridishes were inoculated with bacterial cultures and incubated in a Gallenkamp incubator at 37 ºC for 24-48 h and the growth was compared.

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

Determination of chemical characteristics of oils: 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, to it was added 30 mL to 10 % KI 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.

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

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.

To determine acid value, to 1 g of oil sample was added 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:

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

The free fatty acid contents of oil were 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 (0.1 N), W = Weight of oil, M = 282 (molecular weight of oleic acid).

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 half an hour and subsequently titrated against 0.5 N HCl using phenolphthalein as indicator. The saponification value was calculated as under:

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

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

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

Ester value = Saponification value – Acid value

The thin layer chromatographic 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 one hour and later used for the separation of neutral and polar lipids by 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 *Papaver somniferum* seed was 3.4 %. The percentage of oil extracted from the seeds with *n*-hexane was 21 % while that with acetone was 46 %.

General characteristics of the oil extracted with polar acetone and non polar (*n*-hexane): Physical and chemical characteristics of oil extracted with two solvents acetone and *n*-hexane are consolidated in Table-1.

TABLE-1
GENERAL CHARACTERISTICS OF THE OIL EXTRACTED FROM *Papaver somniferum* SEEDS

Characteristics	Results with acetone	Results with *n*-hexane
Color	Yellow red	Light yellow
m.p. (ºC)	-1	-3
sp. gr.	0.9183	0.9114
Refractive index	1.46	1.431
Viscosity	42.58	41.98
Iodine value	145.93	136.41
Acid value	45.36	25.56
Free fatty acid (%)	22.80	14.38
Saponification value	196.35	188.9
Ester value	150.99	160.39

Antibacterial activity: The antibacterial activity of homogenized *Papaver somniferum* seeds, oil extracted from *Papaver somniferum* seeds and the residue left after the extraction of oil is determined against some standard bacterial species, commonly used by the clinical and research laboratories such as *E. coli* and *S. aureus*, for both routine tests and research work'.

Antibacterial activity of homogenized seeds of *Papaver somniferum*: The antibacterial activity of homogenized seeds

TABLE-2
ANTIBACTERIAL ACTIVITY OF HOMOGENIZED SEEDS OF *Papaver somniferum* 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
5 g	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve

TABLE-3
ANTIBACTERIAL ACTIVITY OF SOILS EXTRACTED FROM SEEDS OF *Papaver somniferum* USING DIFFERENT SOLVENTS

Solvent	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
n-Hexane	1 g	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve
acetone	1 g	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve

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

Residues	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
n-Hexane residue	5 g	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
Acetone residue	5 g	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve

of *Papaver somniferum* against *E. coli* and *S. aureus* is given in the Table-2. Results indicate that the seeds *Papaver somniferum* contain antibacterial activity 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 *Papaver somniferum* using different solvents: Antibacterial activity of oils extracted from seeds of *Papaver somniferous* using *n*-hexane and acetone as solvent is recorded in Table-3. Results show that oil extracted from the seeds of *Papaver somniferum* with *n*-hexane exhibits the antibacterial activity against *S. aureus* while that extracted with acetone exhibits against *E. coli*.

Antibacterial activity of residues left after extraction of oils: Antibacterial activity of residues left after extraction of oils is recorded in Table-4. Results show that the residue left after extraction exhibits antibacterial activity. Not a single colony of *E. coli* grew on the agar surface in the presence of residue, which was left after extraction by acetone as solvent.

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

TABLE-5
DIFFERENT LIPID CONTENTS OF *Papaver somniferum* OIL AS DETERMINED BY TLC

Neutral lipids	Polar lipids
Wax ester	Phosphatidlyl ethanolamine
Sterol ester	Phosphatidly1 inositol
Triglyceride Free fatty acid Diglyceride Unknown Glucoside Monoglyceride	Trilactoside diglyceride
Neutral lipids	Polar lipids

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