

# Biological Sciences Section

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## ESSENTIAL OILS OF THE SPECIES OF LABIATAE

### Part III. Studies on the Essential Oil of *Zataria multiflora*

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Chemical composition and physico-chemical values of the essential oil of *Zataria multiflora* (N.O. Labiatae), grown in Pakistan, have been investigated. The essential oil (0.69%) consists of  $\alpha$ -phalendrene (2.40%), *p*-cymene (7.10%),  $\gamma$ -terpinene (0.35%), caryophyllene (8.30%), borneol (1.72%), unknown alcohol (1.80%), thymol (15.60%), carvacrol (57.40%) eugenol (1.69%), methyl eugenol (1.54%) and an unidentified phenol (2.10%).

**Key words:** Essential oil; *Zataria multiflora*; Labiatae; Saponification.

#### INTRODUCTION

*Zataria multiflora* (N.O. Labiatae) is a small aromatic shrub found in Pakistan, Afghanistan and Iran. Locally known as 'Saatar' it has a fragrant odour like lemon and thyme. The odoriferous plant material consists of small ovate or nearly round, dotted, leathery leaves ( $\frac{1}{2}$  inch long) mixed with numerous minute flowers. The leaves when magnified present a mossy surface which is thickly pitted and containing a reddish yellow essential oil in each pit [1]. Many medicinal properties, similar to lemon and mint, are attributed to the plant and it is used extensively in preparations as a remedy for numerous diseases [2]. Its infusion is valued as an aromatic stimulant, diaphoretic and a good cure for stomachache [3].

An earlier study, describing the chemical composition of the essential oil of *Zataria multiflora* from India, is rather old [4]. It was carried out when the use of modern instruments was not common. It was, therefore, necessary to evaluate the essential oil for its quality and accurate chemical composition. In continuation of the previous work for developing new sources of such oils, the present study on the evaluation of *Zataria multiflora* of Pakistani origin, was therefore, carried out [5,6]. The results of the present and earlier reported studies are compared in Table 3. The results indicate that the essential oil of *Zataria multiflora* is rich with regard to phenolic contents (81.75%). The medicinal attributes of 'Saatar' may thus be due to these oxygenated compounds.

#### MATERIALS AND METHODS

The plant material, comprising dry leaves, wood, stems and numerous minute flowers was collected from the local

market. The essential oil from this material was recovered by steam distillation. Standard methods, usually employed for the evaluation of the recovered oil were followed [7,8]. The percentage yield and the various physico-chemical properties of the essential oil are given in Table 1.

**Chromatographic analysis of the oil.** The essential oil (5g), recovered by steam distillation, was resolved into hydrocarbon fraction and oxygenated components by column chromatography using a glass column (100 cm x 3.5cm) packed with silica gel (60-80 mesh; 200g). The column was first eluted with hexane to recover the hydrocarbon fraction. The oxygenated components were then eluted with increasing proportions of diethyl ether in hexane. The column was finally washed with neat diethyl ether to obtain more polar components.

The hydrocarbon fraction was resolved into individual components by gas chromatography using a column of 15% OV-275 on chromosorb PAW-DMCS. Nitrogen was used as the carrier gas and the temperature of the column

Table 1. Physico-chemical properties of the essential oil of *Zataria multiflora*.

S. No.	Constants	Values
1.	Yield	0.69%
2.	Distillation time	6 hours
3.	Colour	Reddish yellow
4.	Odour	Aromatic
5.	Specific gravity	0.946
6.	Refractive index 30°	1.573
7.	Acid value	3.46



Table 2. Chemical constituents of the essential oil of *Zataria multiflora*.

Components	Percentage
$\alpha$ -Phallendrene	2.40
<i>p</i> -Cymene	7.10
$\gamma$ -Terpinene	0.35
Caryophyllene	8.30
Borneol	1.72
Unidentified	1.80
Thymol	15.50
Carvacrol	57.40
Eugenol	1.69
Methyl eugenol	1.54
Unknown phenol	2.10

Table 3. Comparative statement of the results of the previous and the present studies of the essential oil of *Zataria multiflora*

Components	Studies by Farooq & Gupta %	Present studies %
$\alpha$ -Phallendrene	—	2.40
<i>p</i> -Cymene	17.00	7.10
$\gamma$ -Terpinene	—	0.35
Caryophyllene	—	8.30
Borneol	3.00	1.72
Zatarinol	2.00	1.80
		(unidentified)
Thymol	Traces	15.50
Eugenol	—	1.69
Methyl eugenol	—	1.54
Zatarol	0.50	2.50
		(unidentified)

was kept at 120<sup>o</sup>, while the detector temperature was maintained at 200<sup>o</sup>. The identity of the components was varified by co-injecting with the authentic samples and observing identical retention times.

The oxygenated components, recovered by column chromatography, were resolved and identified by GLC comparison techniques with the authentic samples using SE-30 WCOT column maintained at 180<sup>o</sup>. Detector temperature was kept at 250<sup>o</sup>. The chemical composition of the essential oil thus determined is recorded in Table 2.

## DISCUSSION

The essential oil distilled from the whole plant of *Zataria multiflora* possesses a sweet fragrance. The oil was resolved into hydrocarbons and oxygenated fractions by column chromatography on silica gel using hexane and a mixture of hexane with different proportions of diethyl ether as an eluent.

The combined hexane eluted fractions were hydrocarbons comprising 18.1% of the essential oil. These hydrocarbons were further resolved into four specific components by GLC using a 15% OV-275 column. The identity of these hydrocarbons was established from the retention time when compared with standard terpene hydrocarbons and using the co-injection technique. Thus *p*-cymene (7.10%) and caryophyllene (8.30%) as the major and  $\alpha$ -phallendrene (2.40%) and  $\gamma$ -terpinene (0.352%) as the minor constituents of the hydrocarbon fraction of *Zataria multiflora* essential oil were identified.  $\alpha$ -phallendrene, caryophyllene and  $\gamma$ -terpinene is being reported for the first time; as in the previous work on this essential oil, there has been no mention of these compounds [4].

Increasing quantity of diethyl ether in hexane further eluted the oxgenated substances which were found to be mixtures of alcohols, phenols and phenolic ethers.

The Rf values of these phenolic components on TLC were very close to each other ; they were, therefore, best resolved by GLC technique using SE-30 WCOT column and identified by coinjecting the column with standard samples of individual compounds. They were found to be composed of carvacrol (57.40%), thymol (15.50%), eugenol (1.69%), methyl eugenol (1.54%) and an unidentified phenol (2.10%), borneol (1.72%) and another unidentified compound (1.80%). The presence of zatarol and zatarinol has already been reported [4] but due to the non-availability of authentic samples of these compounds, their presence could not be confirmed.

The composition and percentage existence of these terpenes in the essential oil differred remarkably from the one reported earlier [4]. This difference could be due to many reasons such as varietal changes, climatic and soil conditions, analytical procedures or a combination of these factors.

It is thus concluded that because of a high phenolic content (81.75%) the essential oil of *Zataria multiflora* can find useful applications. Becuase of this reason a serious thought must be given for its wider distribution as a valuable industrial crop.

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INTRODUCTION

In recent years attention has been directed at control-  
 ing stored-grain pests with volatile oils [1-5]. Essential  
 oils [6-10] and mineral oils [Kishanrajah [11] and Abo-  
 lam et al. [12]] investigated the repellent and narcotic  
 properties of some plant extracts to *Sitona cerealis*  
 (Olivier). Kishanrajah et al. [10] studied the toxicity and  
 repellency of several plant products both singly and in  
 combination against major pest of stored paddy. Su [13]  
 has estimated the toxicity and repellency of cowardin weed  
 to four species of stored-product insects.

The present study summarizes the repellent activity of  
 some locally available plants against a stored grain pest in  
 the hope that some of them may be used to control the  
 infestation of grain stored for domestic use.

MATERIALS AND METHODS

Extraction method (i) Essential oils They were  
 obtained by hydro-distillation of the following plant  
 materials: (1) *Althoea officinalis*, (2) *Yucca baccata*,  
 (3) *Apium graveolens*, (4) *Cuminum cyminum*, (5) *Cine-  
 pylon citrius*, (6) *Convolvulus vulgaris* and (7) *Plantago*  
*major*.

(ii) Feed mix (insecticide). The seed-grains of the  
 following plants were crushed and extracted with hexane.  
 The solvent was removed under reduced pressure.

(1) *Althoea officinalis*, (2) *Yucca baccata*, (3) *Apium graveolens*, (4) *Cuminum cyminum*, (5) *Cinepylon citrius*, (6) *Convolvulus vulgaris*, (7) *Plantago major* and (8) *Plantago*

Culturing procedure. The culture of the test insect,  
*Tribolium castaneum* Halder, was maintained on wheat  
 flour with 2% yeast at 29 ± 1° and 60 ± 2% R.H. in glass  
 bottles.

Repellency method. The repellency of oils was evalu-  
 ated against two-to-three-week old adult beetles according to  
 the method described by Landman et al. [14] and Mc-  
 Donald et al. [15] with some modifications. Filter paper  
 strips (Whatman No. 1, 8x4 cm) were treated with 1 ml of  
 1% oil in acetone and dried at room temperature. The  
 treated paper strips were joined lengthwise edge-to-edge  
 to untreated paper strips (8x4 cm) with adhesive on the  
 underside of the strip. Two glass cages (4.5 cm in height  
 and 7 cm dia.) were placed over two matched strips in  
 such a way that the joined edges faced the ring providing  
 equal areas of treated and untreated papers. Ten adults  
 were released in each test arena and the number of insects  
 on treated and untreated halves was recorded twice daily  
 for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100 days. There were 8 replicates for  
 each treatment and tests were made at 1, 2, 4, and 8 weeks  
 after treatment of the paper strip. The average percent  
 repellency for each 2 days was calculated by doubling the  
 difference between the percent of insects on treated half  
 and the 50% distribution expected if only untreated papers  
 were used [16]. The mean repellency from exposure at  
 periods of 1 week, 2 weeks, 1 month and 2 months after  
 application was assigned a class by using the following  
 scale: Class I, 0.1 to 50%; Class II, 50.1 to 40%; Class III,  
 40.1 to 30%; Class IV, 30.1 to 20%; and Class V, 20.1  
 to 10%.