

# Biological Sciences Section

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## ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OILS OF UMBELLIFERAE FAMILY

### Part VIII. *Seseli libanotis*, *Ligusticum Stewartii* and *Pycnocyclus aucheriana* Oils

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The essential oils of the seeds of *Seseli libanotis*, *Ligusticum stewartii* and *Pycnocyclus aucheriana* have been tested against the pathogenic bacteria of *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysentery*, and *Vibrio cholera*. The tests were carried on in emulsified broth using spectrophotometric method. Of the oils tested, *Seseli libanotis* is most effective, especially against *Staph. aureus*. While at higher doses, the oil of *P. aucheriana* was also effective against pathogen like *S. typhi*.

**Key words:** *Seseli libanotis*, *Ligusticum stewartii*, and *Pycnocyclus aucheriana*.

#### INTRODUCTION

Umbellifers have a well recognized place in Hikmet, Tibbi and local remedies, throughout the world. *Seseli* is a genus of this family with 80 species distributed in Europe and Asia. The species *Seseli libanotis* (Chots eachga) grows wild in Pakistan (in Gilgit, Chitral, Ziarat, Kaghan and Swat). Its seeds have been used in local remedies for blood pressure control. The main constituents of pleasant smelling essential oil are, limonene,  $\beta$ -phellandrene, myrcene, pinene and 16-20% coumarins [1].

*Ligusticum* is a genus of 60 species grown in North temperate regions. Some species of this genus are known to be effective in cardiac diseases [2]. The species *Ligusticum stewartii* (Burha-eachga) grows wild in N.W.F.P. Alcohols and acids (48%), terpenes (30%), and coumarins with tarry material (17%) are the main constituents of this oil [3].

The genus *Pycnocyclus* includes some 10 species. The species *Pycnocyclus aucheriana* (Bibi buto) is a wild species endemic to Baluchistan region. Linalool constitutes the major portion of this oil [4].

The above mentioned species were tested against some pathogenic bacteria in continuation of our work on the antibacterial effect of essential oils specifically from the Umbelliferae family [5].

#### MATERIAL AND METHOD

1. **Cultures.** The standard cultures of *Staphylococcus aureus* ATCC-6538-P., *Escherichia coli* M/200, *Salmonella typhi*, *Shigella dysentery*, and *Vibrio cholera* were kindly provided from the National Institute of Health, Islamabad, and the Drug Testing Laboratories, Lahore.

2. **Media.** For the stock-culture-slants, Merck's medium was used, while for antibacterial assays, the oxoid antibiotic medium No. 3 was utilized.

3. **Essential oils.** The essential oils were obtained from the seeds of *Seseli libanotis*, *Ligusticum stewartii*, and *Pycnocyclus aucheriana* by steam distillation.

**Preparation of media and inoculum.** The media and inoculum were prepared using the methods described earlier [5]. The tubes were incubated at 35° for 20 hrs, and the optical density taken as index of bacterial growth, was measured spectrophotometrically, using Hitachi Model 100-200 UV-Vis spectrophotometer. The rate of growth was plotted against the quantity of essential oils in parts per million (ppm).

The bacteria wise results of the essential oils are reported herein.

#### DISCUSSION

1. ***Staphylococcus aureus.*** The essential oil of the seeds of *Seseli libanotis* at 400 ppm causes about 74% inhibition of *Staph. aureus*, which increases upto 94% at 800 ppm. The oil, at 1200 ppm causes 100% inhibition. The minimum inhibitory concentration (MIC) is found to be 600 ppm. The inhibitory effect of *S. libanotis* resembles with the activity of *Anethum graveolens* against *Staph aureus*.

The essential oil of the seeds of *L. stewartii* is not so effective against this organism. The effect however is more

Table 1. The activity of essential oils against *Staphylococcus aureus*.

| Amt. of oil in ppm. | Optical density (mean). |                             |                               |
|---------------------|-------------------------|-----------------------------|-------------------------------|
|                     | <i>Seseli libanotis</i> | <i>Ligusticum stewartii</i> | <i>Pycnocyclus aucheriana</i> |
| 0                   | 445                     | 459                         | 421                           |
| 400                 | 118                     | 325                         | 410                           |
| 800                 | 28                      | 268                         | 377                           |
| 1200                | 0                       | 248                         | 349                           |
| 1600                | 0                       | 208                         | 310                           |
| 2000                | 0                       | 204                         | 158                           |
| 2400                | 0                       | 112                         | 86                            |

than *Ferula copode* [3]. The oil at 400 ppm causes just 30% inhibition of *S. aureus*. The inhibition slowly rises to 56% at 2000 ppm concentration of oil. However at 2400 ppm the inhibition rises upto 76%.

The oil of *P. aucheriana* at initial concentration is even slower in its activity against this organism. Only 15% inhibition of the organism is observed at 400 ppm., which increases to 57% at 1200 ppm. At 1600 ppm., the inhibition becomes 70% and rises upto 80% at the maximum dose of 2400 ppm.

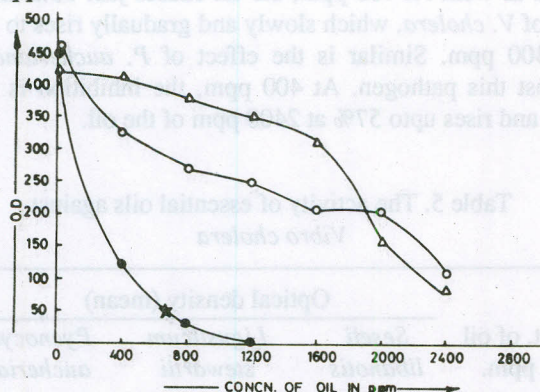


Fig. 1. Activity against *Staphylococcus aureus* of —●— Seseli libanotis; —○— Ligusticum stewartii; —△— Pyncocykla aucheriana, \* mic.

2. *Escherichia coli*. The oil of *S. libanotis* causes about 36% inhibition of *E. coli* at 400 ppm. which increases upto 60% at 800 ppm, while 1200 ppm. is its MIC. The activity then gradually rises upto 93% at 2400 ppm. The effect is very much similar to that of *Ferula ovina* [7].

The oil of *L. stewartii* is quite slow in activity at the initial doses, however 64% inhibition is caused by 1200 ppm, which gradually rises to 75% at the dose of 2400 ppm. The activity is somewhat like that of *Dorema ammoniacum* oil [8].

The activity of the oil of *P. aucheriana* against *E. coli* is slightly better than *L. stewartii*. At initial concentration of 400 ppm, the inhibition is 37% which gradually in-

Table 2. The activity of essential oils against *Escherichia coli*.

| Amt. of oil in ppm. | Optical density (mean)  |                             |                              |
|---------------------|-------------------------|-----------------------------|------------------------------|
|                     | <i>Seseli libanotis</i> | <i>Ligusticum stewartii</i> | <i>Pyncocykla aucheriana</i> |
| 0                   | 453                     | 452                         | 400                          |
| 400                 | 291                     | 376                         | 253                          |
| 800                 | 185                     | 280                         | 223                          |
| 1200                | 97                      | 162                         | 202                          |
| 1600                | 64                      | 132                         | 169                          |
| 2000                | 50                      | 117                         | 109                          |
| 2400                | 34                      | 113                         | 54                           |

creases to 58% at 1600 ppm. At 2000 ppm, the inhibition is 73% and at 2400 ppm, it is 87%.

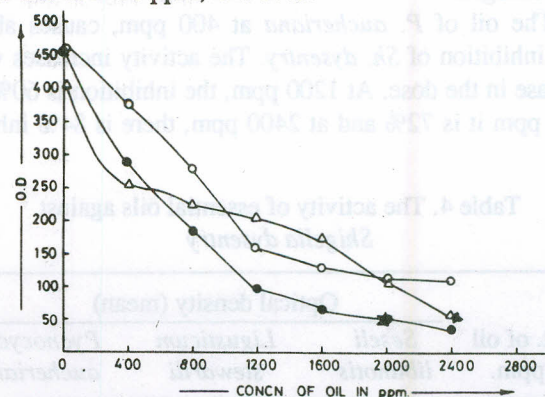


Fig. 2. Activiti against *Escherichia coli* of —●— Seseli libanotis; —○— Ligusticum stewartii, —△— Pyncocykla aucheriana; \* mic.

3. *Salomonella typhi*. At 400 ppm, the essential oil of the seeds of *S. libanotis* causes 60% inhibition of *S. typhi*. The MIC is about 1000 ppm. The activity rises further with increase in the dose and at 2400 ppm, the inhibition of this organism is 93%. Against this pathogen the oil is more active than *Daucus carota* [6].

The oil of *L. stewartii* is less active against this pathogen. At 400 ppm the inhibition is 43% which increases to 70% at 1200 ppm. The activity then becomes almost static and at 2400 ppm the inhibition becomes 74%.

The oil of *P. aucheriana* at initial dose shows only 13% inhibition. At 1200 ppm, the inhibition becomes 77%. The MIC is about 1300 ppm. At 1600 ppm the inhibition rises upto 90% and becomes almost 100% at the maximum dose of 2400 ppm. The oil at higher doses is more active than the oil of *Stewartiella baluchistanica* [9].

4. *Shigella dysentry*. The oil of *S. libanotis* causes only 20% inhibition of *Sh. dysentry* bacteria. At 800 ppm, the inhibition becomes 43%, at 1200 ppm, the inhibition is 62% and rises upto 85% at 2400 ppm. The MIC is about 2200 ppm.

At initial concentration of 400 ppm, the oil of *L. stewartii* has virtually no effect against this organism. At the

Table 3. The activity of essential oils against *Salmonella typhi*

| Amt. of oil in ppm. | Optical density (mean)  |                             |                              |
|---------------------|-------------------------|-----------------------------|------------------------------|
|                     | <i>Seseli libanotis</i> | <i>Ligusticum stewartii</i> | <i>Pyncocykla aucheriana</i> |
| 0                   | 461                     | 462                         | 412                          |
| 400                 | 186                     | 262                         | 360                          |
| 800                 | 97                      | 169                         | 258                          |
| 1200                | 72                      | 136                         | 96                           |
| 1600                | 53                      | 126                         | 44                           |
| 2000                | 44                      | 122                         | 21                           |
| 2400                | 39                      | 121                         | 3                            |

maximum dose of 2400 ppm, there is just 27% inhibition of this pathogen.

The oil of *P. aucheriana* at 400 ppm, causes about 36% inhibition of *Sh. dysentru*. The activity increases with increase in the dose. At 1200 ppm, the inhibition is 60%, at 2000 ppm it is 72% and at 2400 ppm, there is 84% inhibi-

Table 4. The activity of essential oils against *Shigella dysentru*

| Amt. of oil in ppm. | Optical density (mean)  |                             |                              |
|---------------------|-------------------------|-----------------------------|------------------------------|
|                     | <i>Seseli libanotis</i> | <i>Ligusticum stewartii</i> | <i>Pycnocycla aucheriana</i> |
| 0                   | 578                     | 524                         | 665                          |
| 400                 | 467                     | 478                         | 428                          |
| 800                 | 331                     | 403                         | 384                          |
| 1200                | 225                     | 390                         | 306                          |
| 1600                | 179                     | 368                         | 260                          |
| 2000                | 126                     | 336                         | 189                          |
| 2400                | 89                      | 310                         | 111                          |

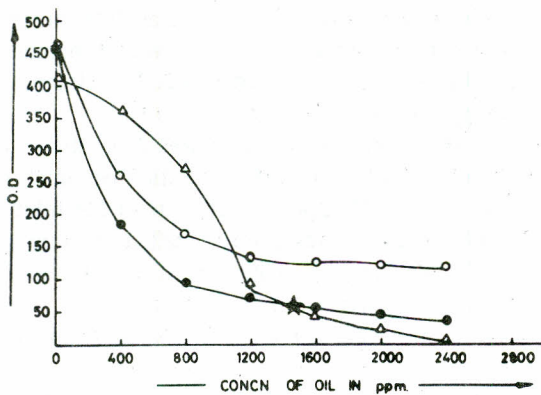


Fig. 3. Activity against *Salmonella typhi* of —●— *Seseli libanotis*; —○— *Ligusticum stewartii*; —△— *Pycnocycla aucheriana*; \* mic.

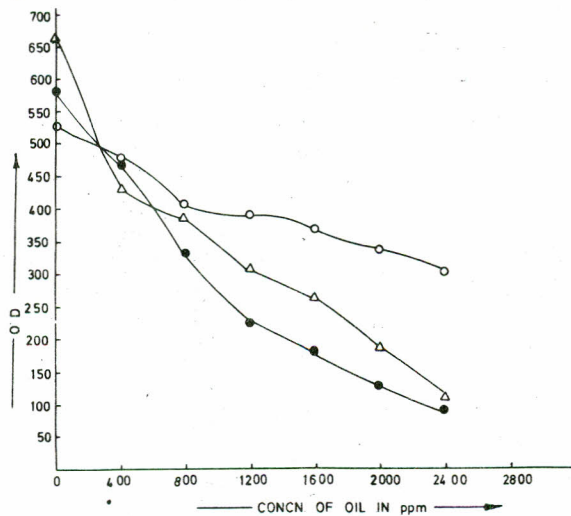


Fig. 4. Activity against *Shigella dysentru* of —●— *Seseli libanotis*; —○— *Ligusticum stewartii*; —△— *Pycnocycla aucheriana*; \* mic.

tion of this organism. This effect resembles with that of *S. libanotis* against this pathogen.

5. *Vibrio cholera*. At 400 ppm, there is only 13% inhibition of the pathogenic bacteria of *V. cholera* by the oil of *S. libanotis*. It slowly increases to 36% at 1200 ppm, but at 1600 ppm it suddenly rises upto 73% and then remains static even at higher doses.

The oil of *L. stewartii* at initial doses has similar effect but the inhibitory effect does not increase much at higher doses as well. At 400 ppm, the oil causes just 18% inhibition of *V. cholera*, which slowly and gradually rises to 50% at 2400 ppm. Similar is the effect of *P. aucheriana* oil against this pathogen. At 400 ppm, the inhibition is only 10% and rises upto 57% at 2400 ppm of the oil.

Table 5. The activity of essential oils against *Vibrio cholera*

| Amt. of oil in ppm. | Optical density (mean)  |                             |                              |
|---------------------|-------------------------|-----------------------------|------------------------------|
|                     | <i>Seseli libanotis</i> | <i>Ligusticum stewartii</i> | <i>Pycnocycla aucheriana</i> |
| 0                   | 928                     | 921                         | 910                          |
| 400                 | 810                     | 754                         | 815                          |
| 800                 | 641                     | 728                         | 740                          |
| 1200                | 561                     | 689                         | 622                          |
| 1600                | 258                     | 660                         | 541                          |
| 2000                | 258                     | 538                         | 460                          |
| 2400                | 258                     | 464                         | 395                          |

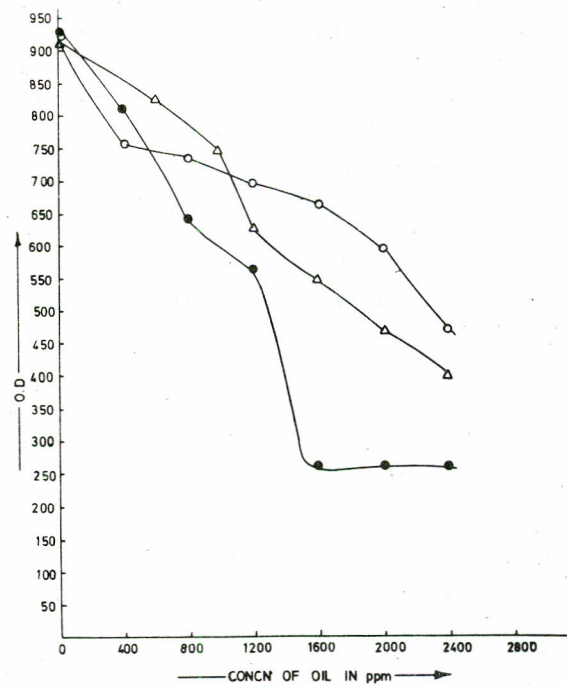


Fig. 5. Activity against *Vibrio cholera* of —●— *Seseli libanotis*; —○— *Ligusticum stewartii*; —△— *Pycnocycla aucheriana*; \* mic.

Of the three oils tested *S. libanotis* is most effective, especially against *Staph. aureus*. While at higher doses, the oil of *P. aucheriana* is also very effective against *S. typhi*.

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a shallow cavity slide and a glass cover slip was placed which was sealed using wax.

Egg deposition and embryogenesis were observed under high power of a light microscope at (28x) magnifications were made with the help of camera lucida.

## RESULTS

Egg. Eggs of *C. libanotis* are hyaline, colorless, and oblong in shape (Fig. 1). Fifteen eggs were measured (length 22.5-30.0 mic, width 12.0-16.0 mic) and the average egg size was 28 x 14 mic. The egg shell consists of two main layers, an inner thin and an outer thick layer. The egg membrane is transparent and such-like.

Egg deposition. *C. libanotis* has monoblastic gonads with ovary occupying about 2/3 of the body length. The ovary stretches towards the anterior and where it relaxes and stretches posteriorly well behind the vulva.

Eggs are deposited singly and it has been observed that 20-25 eggs are deposited in 10 mic. Vulva is a transverse slit like opening situated at the posterior region of the female. It has been observed that before egg-deposition, vulva like move rapidly and the egg comes down near the vulva opening. Finally the egg exits with a little jerk.

Embryogenesis. Different phases of embryogenesis drawn with the help of camera lucida are given in Fig. 1. First development phase. Frequently it has been noted that the first cleavage appears immediately after maturation of the egg. The single cell stage P<sub>1</sub> (Fig. 1-A) is usually occurred in the middle of ootid. The first cleavage is almost equatorial, dividing the egg axis transversely. This cleavage results into two blastomeres, an anterior blastomere (2) and a posterior blastomere (3). The P<sub>1</sub> is slightly larger than the 2. The first cleavage occurs in about 60-90 min (Fig. 1-B). The second cleavage is parallel to the first cleavage, thus dividing the anterior blastomere (2) into

## INTRODUCTION

Embryonic development of plant parasitic nematode has been studied due to their economic importance. However, in recent years, free living nematodes have produced great amount of interest on account of their large realized potential in many developmental studies (8).

The embryonic and post-embryonic (Thomas, 1937; Thomas, 1937; Rastbach, 1960; Schneider, 1960; Farnsworth, 1967; T. Goodby, 1967) have also been studied.

*Cephalobus libanotis* (Abbas, 1963; Anderson, 1984) is a free living nematode which reproduces parthenogenetically, however, males are also observed very rarely. *C. libanotis* has short life span i.e. 75-90 hr, prolific fecundity, and is incapable to a variety of laboratory purposes (5). It was for this reason that embryonic development of this nematode species was studied which forms the subject of this report.

The embryonic development of *Cephalobus libanotis* is similar to *Arotylus complexus* in most phases, but some aspects are variable.

## MATERIALS AND METHODS

Preparation of culture. Pure population of *Cephalobus libanotis* was raised from a single egg. Nematodes were cultured on Pea Meal Paste (PMP) as described by Saeed et al. (2). Large number of freshly deposited eggs in water and also from dissected gravid females were collected. They were washed in 0.02% sodium hypochlorite solution (containing bleach) for 5 min followed by rinsing with sterile distilled water to prevent micro-organisms during observation period.

Preparation of slides. A healthy looking single-celled egg was selected and placed in a drop of distilled water on