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THE ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OILS OF THE PAKISTANI *ACORUS CALAMUS, CALLISTEMON LANCEOLATUS* AND *LAURUS NOBILIS*

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The essential oils of *Acorus calamus, Callistemon lanceolatus*, and *Laurus nobilis* have been tested against *Staphylococcus aureus, Escherichia coli, Shigella flexneri*, and *Salmonella typhi, para-A*, spectrophotometrically in emmulsified broth. All the essential oils were found to be active against the pathogens of intestinal tract whereas the oil of *Callistemon lanceolatus* enhanced the growth of *S.aureus* in the medium. The essential oils from different parts of the same plant, did not differ in their activity.

Key words: Antibacterial activity, Essentials oils, Pathogens of intestinal tract.

Introduction

The herbs and plants, since antiquity have been investigated for their medicinal effects. The discoveries about active constituents from many plant have led to new formulations in allopathic drugs. Three of such plants having medicinal activities are: *Acorus calamus* or Sweat flag (family: Araceae), *Callistemon lanceolatus* or Bottle brush (family: Myrtaceae), and *Laurus nobilis*, or Bay (family: Lauraceae), which we have tested against the pathogenic bacteria of *Staphylococcus aureus, Escherichia coli, Shigella flexneri,* and *Salmonella typhi, para-A.*

Acorus calamus or Sweat flag is found in the marshy areas of Malay, and Hamalay velleys, and also in Africa and Europe. The rhizomes of this plant are successfully used in diseases of stomach, bronchi, kidney, liver, skin and hair [1]. Products from this plant have been added in hair, body and brain tonics [2,3] and are known to have well defined spasmolytic [4] and anti- inflamatory [5] activities. These effects might be attributed to the presence of amino acids [6], and the vitamins of B-compound [7] in this plant, and to the plant's ability to inhibit prostaglandin synthesis [5]. However the antimycotic, and antibacterial effects are wholy due to the components present in its essential oil, [8,9] i.e. asarone-isomers, thymol, asarone- aldehyd, cadinene, myristic acid [1] acorone, shyabunone, α-pinene, β-pinene, and camphene [11]. The active principles, α-asarone, and β-asarone have LD_{50} of 226 mg, and 184 mg/kg in mice, respectively, whereas the LD_{50} of both isomers of asarone in chick embryo is 0.04 mg/egg [12].

The red flowered ornamental trees of *Callistemon lanceolatus* (Curtis, Bottle brush) are cultivated by road sides. The flavonoids in the essential oil of this plant have been identified [13]. The essential oil from the flowers contains α-pinene, and cineol, whereas the major constituents of the essential oil from the leaves are cineol and α-terpineol. The percentage of these components with the oil varies with the age of the plant [14]. The essential oil of *C. lanceolatus* has been found to have a broad- spectrum fungicidal activity, which withstands high temperature, autoclaving and long storage time. The oil was nontoxic to the plant tissues tested upon [15]. The antibacterial effect of the plant have not been studied yet.

Laurus nobilis (Bay) is another medicinal plant, and occurs as wild, and cultivated species in Asia and Europe. The main constituents of its essential oil are hydrocarbon terpenes [16]. Young leaves contain 1, 8-cineol, α-terpinyl acetate, terpinene, linalool, Me-eugenol and monoterpene hydrocarbons [17]. The essential oil of the plant has insect repellent properties [18], and is highly active against *Trichophytum rubrum*, the causative agent of Athletes foot disease [19]. Not only it inhibits *Aspergillus parasiticus*, but also controls from it the production of aflatoxin [20]. This oil is also highly active against *Clostridium botulinum* [21] and some other bacteria [22].

We have tested the essential oil of *Acorus calamus, Callistemon lanceolatus,* and *laurus nobilis* of local origin in emulsified broth, by spectrophotometric method [23], against *S.aureus, E. coli, S. flaxneri,* and *S. typhi, para-A.*

Materials and Method

1. *Standard culture of bacteria. (i). Staphylococcus aureus* (From Nuclear Institute of Agr. Biology, (NIAB), Faisalabad.) *(ii). Escherichia coli* (From Food Division, PCSIR Laboratoris Lahore. *(iii). Shigella flexneri, (iv). Salmonella typhi, para-A.* (From National Inst. Health, Islamabad.

2. *Essential oils.* by steam distillation of different parts of plant. *(i). Acorus calamus* (Main root and aerial root) Swat. *(ii). Callistemon lanceolatus* (Flowers and leaves), Lahore. *(iii). Laurus nobilis* (Leaves), Lahore.

3. *Media. (i).* -Merck's Agar Medium. (For culture slants to keep as stock). *(ii).* Oxoid's antibiotic medium No. 3. (For Broth test).

4. *Emulsifier., (i).* Tween -20 (Polysorbate-20).

TABLE 1. ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *ACORUS CALAMUS*.

Amt. of oil in ppm	% growth *S. aureus*	*E. coli*	*S. flexneri*	*S. typhi* Para-A
Main root				
0	100	100	100	100
400	89	87	79	86
800	84	65	59	78
1200	83	49	47	63
1600	80	46	41	59
2000	78	35	34	57
2400	76	24	31	50
Aerial root				
0	100	100	100	100
400	97	95	80	80
800	95	83	61	69
1200	95	71	55	56
1600	94	47	37	53
2000	89	40	33	52
2400	88	34	26	49

TABLE 2. ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *CALLISTEMON LANCEOLATUS*.

Amt. of oil in ppm	% growth *S. aureus*	*E. coli*	*S. flexneri*	*S. typhi* Para-A
Leaf				
0	100	100	100	100
400	109	88	84	76
800	115	70	80	69
1200	118	52	76	60
1600	121	46	67	43
2000	122	42	22	37
2400	128	35	21	27
Flower				
0	100	100	100	100
400	101	60	80	76
800	117	52	56	65
1200	127	47	42	59
1600	134	38	13	39
2000	140	37	2	34
2400	142	36	0	25

TABLE 3. ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF *LAURUS NOBILIS* (LEAF).

Amt. of oil in ppm	% growth *S. aureus*	*E. coli*	*S. flexneri*	*S. typhi* Para-A
0	100	100	100	100
400	88	80	105	66
800	86	57	56	47
1200	83	45	52	27
1600	78	41	49	26
2000	71	38	32	23
2400	68	31	26	23

5. *Spectrophotometer.* LKB, Ultraspec-11, single unit, automatic, digital spectrophotometer.

6. *Test Tube -Mixer*

7. *Micro Syringes*

(To measure essential oil from 4µl–24 µl*).

Method. With 2% tween-20 emulsifier, 500ml of broth medium was sterilized in flask and after pouring in reference set, the rest was inoculated by 24 hrs old bacterial culture. For reference or blank set seven test tubes (plugged), each with 10 ml of sterilized medium were taken and doses of essential oil from 4 µl to 24 µl (400 to 2400 ppm), were added with the help of micro syringe, except one tube with zero dose. The cotent was mixed, using a tube-mixer. In similar way two sets of tubes were prepared with inocculated broth. After incubation at 35 - 37° for 20 hrs content in each tube were again mixed. The inhibition of bacterial growth, caused by different doses of essential oil was read spectro photometrically, using seperate reference tube with that dose of essential oil. The optical density of bacterial suspension with zero dose of oil was taken as 100% growth of bacteria and the inhibition caused by different doses was measured by the comparison of their optical density with that. The results are mentioned in Tables 1-3. (The method has been given in more detail in our previous wok with the plants of Umbellifereae family [23].

Results and Discussion

The essential oils both from the main root and aerial root of *Acorus calamus* (Sweat flag), when tested against different pathogenic bacteria, caused a gradual inhibition of all enteric Gram-negative bacilli, whereas both the oils did not show any marked activity against *S.aureus*. 1200 ppm of oil of main root of *A. calamus* caused about 50% inhibition of *E. coli* and *S. flexneri*, whereas one-fold inhibition of *S. typhi* was achieved by 2400 ppm of oil.

The oil from aerial roots of this plant when tested against these bacteria, one-fold inhibition of *E. coli* was caused by 1600 ppm of oil and that of *S. flexneri* and *S. typhi* by about 1200 ppm.

The reports of Vashi and Patel [9] differs with our results, which show the oil more active against *S. aureus* and *E. coli* and completely inactive against *S. typhi*, para-A. This difference might be attributed to the different strains of bacteria or the soil conditions in their region.

The active component of calamus oil is asarone, a hot topic of discussion and research these days, for its medicinal values and suspected mutagenic effects. The oil is classified into different groups, based upon content of asarone present in it. Though the permissible limit for β-asarone has been suggested to be 0.5% but in recent studies asarone has been re-

*µl:(micro litre)

ported to be non-mutagenic in Ames test [24]. The calamus from Pakistan contains 2.9% β-asarone in its aerial, and 7.4% in its main roots [11]. There was no marked difference in the activity of oil from its two types of roots, which reflects a considerable activity of the metabolic products of asarone as well.

The flowers of *Callistemon lanceolatus* (Bottle brush) plants have a penetrating smell like Eucalyptus plant. Its essential oil showed a significant activity against Enterobacters. One-fold inhibition of *E. coli* was achieved at 1200 ppm of oil and that of *S. flexneri* at about 800 ppm of oil. Almost complete inhibition of this pathogen attained at 2000 ppm of this essential oil. More than 1200 ppm of oil caused 50% inhibition of *S. typhi*. The oil from the leaves of *C. lanceolatus* exhibited similar activity both against *E. coli* and *S. typhi*, but less against *S. flexneri*.

The activity of essential oil of *C. lanceolatus* seems to be due to the alcoholic fractions present in it, i.e. cineol, and terpineol, In this work, the oil from three year old plants had been used. The total alcoholic fractions of cineol and terpineol were almost similar in flower oil, and leaf oil, i.e. 67% and 62% respectively [14]. The oils from both parts of the plant enhanced the growth of *S. aureus*, hence can be used in a selective medium for this organism, inhibiting the Gram-negative bacilli.

The essential oil of *Laurus nobilis* or Bay leaves contain the active fractions of cineol and Me-euginol. The oil causes 32% inhibition of *S. aureus* at its maximum dose, but was more active against all Gram-negative bacteria. The oil at dose between 800 to 1200 ppm caused one-fold inhibition of all enteric bacilli. The inhibition by this oil further enhanced to about 77% at 24 ppm of dose.

The essential oils reported here are though not as highly active, as the essential oils from some well known species, i.e. mint, eucalyptus, lemon grass, thyme and cumin [19,23] which cause about complete inhibition of many organisms at doses as low as 400 ppm or even lower, however have significant activity against some fatal Gram-negative bacteria like Shigella and Salmonella. These oils can be successfully used as synergists to enhance the activity of other essential oils and antibiotics.

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