ANTIBACTERIAL EVALUATION OF SOME INDIGENOUS MEDICINAL VOLATILE OILS

A. KAR & S. R. JAIN

Department of Pharmaceutical Sciences, University of Saugar, Sagar, M. P., India

ABSTRACT

The *in vitro* antibacterial property of six essential oils namely: *Piper cubeba, Acorus calamus, Litsea chinensis, Colubrina asiatica, Hyptis suaveolens* and *Blumea laciniata* was carried out using the filter paper disk method. The oils and oil combinations (1:1) were screened against fifteen pathogenic and non-pathogenic bacteriae. The essential oil of *B. laciniata* showed maximum activity against *C. diphtheriae, V. cholerae, B. subtilis* and *S. aureus* and *P. cubeba* against *S. faecalis, B. pumilus* and *P. solanacearum*. The combinations of *L. chinensis* with *P. cubeba* and *C. asiatica* displayed the maximum inhibitory response and the rest failed to show any synergistic or potentiating effect.

The striking feature in most of the aromatic plants enlisted in the indigenous system of medicine is attributed to their essential oil contents in them which exert their marked therapeutic potency. In general, these essential oils containing terpenes and other unsaturated compounds are solely responsible for inhibiting the growth of a number of pathogenic microbes. Since ages the crude herbal extracts of these aromatic plants are in use as baths and local applicants for curing infectious diseases. The large volume of work accumulated so far, obviously justifies the importance of medicinal activity of the aromatic plants; the antimicrobial activity being credited to their essential oil fraction only.

Studies of Lucas (1-3), Gottshall (4-6) and Frisbey (7, 8) are typical surveys of plants for their antimicrobial property. The studies made by above said workers led to the isolation of many active principles (9, 10, 11).

The present investigations deal with the antibacterial activity exhibited by the essential oils of *Piper cubeba* Linn. (Piperaceae); *Acorus calamus* Linn. *(Azaceae); Litsea chinensis* Lam. *(Lauraceae); Colubrina asiatica* Brongn. *(Rhamnaceae); Hyptis suaveolens* Poit. *(Labiatae); Blumea laciniata* D.C. *(Compositae)* and their combinations in the ratio (1:1).

MATERIALS AND METHODS

All the oils and their combinations were subjected to test their antibacterial activity adopting the „filter paper disk diffusion plate" method (12). Whatman No. 1 sterilised filter paper disks of 6 mm diameter were dipped into the samples, drained and were subsequently placed on the seeded agar plates aseptically. A control record of the experiment was obtained by placing a blank disk at the centre of each plate simultaneously. In all, fifteen test bacteriae belonging to the pathogenic and non-pathogenic

origin were selected to indicate the effectiveness of the essential oils and their combinations under investigations. The microbes were kept active by subculturing them on fresh nutrient broth media after every seven days.

The responses of oils against individual microbe were obtained by recording the size of resulting zones of inhibition after 24 and 48 hours respectively while maintaining the plates at 37° C in an incubator.

A comparison between the antibacterial activity of oils and their combinations was made under identical conditions with eight known substances viz: salicylic acid, benzoic acid, chlorobenzoic acid, benzyl penicillin, streptomycin sulphate, hexachlorophene, hamycin and chlorobutol using them in their known concentrations as standards.

All the testings were repeated three times applying four disks on each plate of 100 mm diameter. The average results of all these observations are shown in Tables – I and II. All the zones of inhibition of less than 10 mm diameter were considered to be negligible in response.

The zonal contents were subjected separately to fresh broths to observe the survival of microbes in them under the effect of oils. An appearance of turbidity in broth tubes signified the bacteriostatic effect of the oil whereas a clear broth indicated a bactericidal response.

RESULTS AND DISCUSSION

From the six essential oils studied, the essential oil of *P. cubeba* showed maximum antibacterial activity, whereas *L. chinensis, B. laciniata, and A. calamus* exhibited inhibitory response in a decreasing order. All the oils displayed pronounced antibacterial activity against most of the test microbes, however, *P. solanacearum* and *C. Diphtheriae* were found to exhibit the highest activity.

Table-I records the activity of the essential oils and that of standard substances employed. The essential oil of *B. laciniata* exhibited maximum activity against *C. diphtheriae, V. cholerae, B. subtilis and S. aureus: P. cubeba* against *S. faecalis, B. pumilus and P. solanacearum; A. calamus* against *S. albus and S. typhi; L. chinensis* against *S. faecalis, Sh. dysenteriae and B. subtilis*; whereas *C. asiatica and H. suaveolens* did not show marked activity.

Table I. *Inhibitory Activity of Essential Oils and Standards employed on Microbes*

ORGANISMS	Diam. of the zones of inhibition in mm													
	ESSENTIAL OILS						STANDARDS							
	P. cubeba	*A. calamus*	*L. chinensis*	*C. asiatica*	*H. suaveolens*	*B.laciniata*	Salicylic acid 1%	Benzoic acid 1%	Penicillin/G 1000 U/ml	Streptomycin 2 mg base/ml	Chloroben-zoic acid 1%	Hexachloro-phene 1%	Hamycin 0.5%	Chlorobutol 1%
1. *Escherichia coli*	9	7	10	–	7	8	8	19	17	22	20	–	–	8
2. *Bacillus subtilis*	11	9	12	9	8	12	8	–	14	26	–	11	8	–
3. *Vibrio cholerae*	11	8	11	–	9	11	7	–	14	26	8	12	7	8
4. *Staphylococcus aureus*	9	9	8	–	–	10	8	–	16	19	9	13	–	–
5. *Staphylococcus albus*	10	12	9	–	–	9	8	14	50	36	13	14	–	20
6. *Shigella dysenteriae*	10	9	16	–	9	9	8	–	14	30	8	12	–	–
7. *Corynebacterium diphtheriae*	13	10	14	13	10	18	9	15	42	18	18	16	9	18
8. *Salmonella typhi*	12	14	12	10	9	8	–	16	44	38	14	18	10	13
9. *Sarcina lutea*	9	7	7	7	–	10	–	25	50	20	15	17	9	10
10. *Streptococcus faecalis*	11	14	18	8	9	11	7	8	14	18	8	14	7	7
11. *Bacillus pumilus*	16	10	8	7	9	8	–	12	21	35	8	12	–	8
12. *Pseudomonas pyogenes*	–	–	9	–	–	–	7	7	7	8	7	8	7	7
13. *Streptococcus pyogenes*	14	*13*	*19*	8	8	10	–	9	21	33	–	12	–	8
14. *Micrococcus*	8	–	9	–	8	–	8	8	7	26	–	8	8	9
15. *Pseudomonas solanacearum*	30	20	15	18	10	13	14	13	24	41	15	17	12	14

– Zone of inhibition absent.

Table-II represents the effect of the combinations of five essential oils in their 1:1 ratio. As the essential oil of *B. laciniata* was insufficient in quantity, it could not be tested in the form of its combinations. Among the various combinations which were subjected to test their antibacterial activity, maximum inhibitory response was exhibited by *P. cubeba* together with *L. chinensis* A little lesser degree of rsponse was shown by the combination made from *C. asiatica* together with *L. chinensis.* The effects of combinations in general, were not found to be pronounced as to when compared with those of individual oils.

While confirming the bactericidal and bacteriostatic activity of the test oil samples, it was noticed that mostly the action exhibited, was bacterio-

Table II. *Antibacterial activity of the various combinations (1:1) of essential oils under study*

	Diam. of the zones of inhibition in mm									
	I	II	III	IV	V	VI	VII	VIII	IX	X
ORGANISMS	*P. cubeba* + *A. calamus*	*P. cubeba* + *L. chinensis*	*P. cubeba* + *C. asiatica*	*P. cubeba* + *H. suaveolens*	*H. suaveolens* + *C. asiatica*	*H. suaveolens* + *L. chinensis*	*H. suaveolens* + *A. calamus*	*C. asiatica* + *L. chinensis*	*C. asiatica* + *A. calamus*	*L. chinensis* +
1. *Escherichia coli*	9	8	10	8	–	9	9	9	8	7
2. *Bacillus subtilis*	9	11	10	10	8	10	8	10	8	9
3. *Vibro cholerae*	11	13	10	10	11	9	9	10	10	10
4. *Staphylococcus aureus*	11	10	9	9	–	8	8	8	11	11
5. *Staphylococcus albus*	10	9	9	8	–	9	11	14	9	10
6. *Shigella dysenteriae*	7	11	9	8	7	9	9	9	7	8
7. *Corynebacterium diphtheriae*	10	12	9	8	7	10	8	8	7	8
8. *Salmonella typhi*	9	12	10	9	11	8	9	10	10	9
9. *Sarcina lutea*	9	10	8	9	8	8	8	9	9	9
10. *Streptococcus faecalis*	9	10	9	10	8	9	10	9	9	9
11. *Bacillus pumilus*	14	15	8	9	7	10	9	11	8	8
12. *Pseudomonas pyogenes*	8	8	7	7	–	–	7	7	–	7
13. *Streptococcus pyogenes*	9	10	9	8	10	11	11	10	8	8
14. *Micrococcus*	7	7	–	7	–	8	8	–	–	–
15. *Pseudomonas solanacearum*	7	9	8	8	7	10	9	10	8	9

– Zone of inhibition absent.

static in nature. Only *S. pyogenes* was found to respond to bactericidal effect under the influence of the essential oils obtained from *A. calamus* and *L. chinensis.*

In short, the present study reveals the utilisation of these essential oils in such preparations where favourable effect is either desired against skin manifestations or against systemic infections.

Table III. *The Effectiveness of Essential Oils on individual Microbes**

E. coli
1. L. chinensis

B. subtilis
1. B. laciniata; L. chinensis
2. P. cubeba

V. cholerae
1. B. laciniata; L. chinensis; P. cubeba

S. aureus
1. B. laciniata

S. albus
1. A. calamus
2. P. cubeba

Sh. dysenteriae
1. P. cubeba

C. diphtheriae
1. B. laciniata
2. L. chinensis
3. P. cubeba C. asiatica
4. A. calamus; H. suaveolens

S. typhi
1. A. calamus
2. P. cubeba; L. chinensis
3. C. asiatica

S. lutea
1. B. laciniata

S. faecalis
1. L. chinensis
2. A. calamus
3. B. laciniata; P. cubeba

B. pumilus
1. P. cubeba
2. A. calamus

P. pyogenes
Nil

S. pyogenes
1. L. chinensis
2. P. cubeba
3. A. calamus
4. B. laciniata

M. coccus
Nil

P. solanacearum
1. P. cubeba
2. A. calamus
3. C. asiatica
4. L. chinensis
5. B. laciniata
6. H. suaveolens

* Data is taken from Table I and Oils are arranged in the order of decreasing activity.

Table IV. *The Effectiveness of the combination of Essential Oils on individual bacteria*

E. coli	*S. lutea*
1. III	1. II
B. subtilis	*S. faecalis*
1. II	1. II, IV, VII
2. III, IV, VI, VIII	
	B. pumilus
V. cholerae	1. II
1. II	2. I
2. I, V	3. VIII
3. III, IV, VIII, IX, X	4. VI
S. aureus	*P. pyogenes*
1. I, IX, X	Nil
2. VII	
3. I, X	*S. pyogenes*
	1. VI, VII
Sh. dysenteriae	2. II, V, VIII
1. II	
	M. coccus
C. diphtheriae	Nil
1. II	
2. I, VI	*P. solanacearum*
	1. VI, VIII
S. typhi	
1. II	
2. V	
3. III, VIII, IX	

* Data taken from Table II. Combinations of essential oils arranged in the order of decreasing activity.

ZUSAMMENFASSUNG

Die in vitro antibakterielle Wirkung von sechs ätherischen Ölen wurde mittels der Filtrierpapierscheiben-Methode geprüft: *Piper cubeba* L., *Acorus calamus* L., *Litsea chinensis* Lam., *Colubrina asiatica* Brongn., *Hyptis suaveolens* Poit. und *Blumea laciniata* DC.. Die ätherischen Öle und Öl-Gemische (1:1) wurden gegen fünfzehn pathogene und nichtpathogene Bakterien getestet.

Das ätherische Öl von *Blumea laciniata* zeigte maximale Wirksamkeit gegen *C. diphtheriae, V. cholerae, B. subtilis* und *S. aureus;* das ätherische Öl von *Piper cubeba* ergab maximale Wirksamkeit gegen *S. faecalis, B. pumilus* und *P. solanacearum.* Die Gemische der ätherischen Öle von *Litsea chinensis* mit *Piper cubeba* und mit *Colubrina asiatica* übten die stärkste Hemmung aus. Die übrigen Gemische zeigten weder synergistische noch verstärkte Wirkung.

REFERENCES

1. Lucas, E. H. et al (1951) *Bull. Torrev. Botan. Club,* 78: *310.*
2. Lucas, E. H. et al (1955) *Quart. Bull. Mich. Agr. Exptl. Sta.,* 37: *425.*
3. Lucas, E. H. et al (1955) *Quart. Bull. Mich. Agr. Exptl. Sta.,* 38: *60*
4. Gottshall, R. Y. et al (1949) *J. Clin. Invest.,*28: 920
5. Gottshall, R. Y. et al (1955) *Quart. Bull. Mich. Agr. Exptl. Sta.,* 38: *52.*
6. Gottshall, R. Y. et al (1950) *Ann. Rev. Tuberc.,* 62: *475.*
7. Frisbey, A. et al (1953) *Quart. Bull. Mich. Agr. Exptl. Sta.,* 35: *392.*
8. Frisbey, A. et al (1955) *Quart. Bull. Mich. Agr. Exptl. Sta.,* 38: *279.*
9. Weller, L. E. et al (1953) *Antibiot. Chemotherapy.,* 3: *603.*
10. Boll, P. M. et al (1955-56) *Antibiot. Ann., 255.*
11. Dull, G. G. et al (1956-57) *Antibiot. Ann., 682.*
12. Vincent, J. G., and Vincent, H. W. (1944) *Proce. Soc. Exptl. Biol. Med.,* 55: *162.*