STUDIES ON THE ANTIMICROBIAL ACTIVITY AND PHARMACOLOGICAL PROPERTIES OF SOME ESSENTIAL OILS, EXTRACTED FROM LOCALLY CULTIVATED PLANTS

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Summary

The essential oils from some plants of medicinal value are investigated for their tuberculostatic properties and antibacterial action.

The toxicity against a ciliated protozoa and the degree of irritation produced on rabbit's skin is determined.

The essential oil from *Ocimum gratissimum* which exhibited marked antitubercular and antibacterial activity with a low degree of irritability has been subjected for further pharmacological study.

This oil in a concentration of 10γ/c.c. is found to inhibit the movements and relax the intestinal and uterine musculature; the oil antagonises the activity exhibited by barium chloride on intestinal movements.

The empirical use of this oil in conditions as ear-ache, tooth-ache, and abdominal colic in children can probably be explained by the local anæsthetic action and the relaxation of the musculature produced by the essential oil.

The therapeutic value of many indigenous plants is attributed to their essential oil contents.[1] Of particular interest are the plant juices of ocimum species, which are used as household remedies for various common ailments of children, like stomach-ache, ear-ache and tooth-ache. The application of the leaf juice is claimed to cure septic wounds and even tubercular affections of the skin.

Some essential oils are more or less powerful protoplasmic poisons.[2] The inhibiting and damaging effect of the oils on many life-processes has been turned to advantage in the use of these compounds as bactericidal and fungicidal agents. They are used as antiseptics both locally and internally. The deep irritation and with minimum tissue destruction produced by some

essential oils has been the pharmacological basis for their medicinal use in chronic inflammatory conditions.[3]

Some essential oils possess an analgesic action; some others act as sedatives, stimulants and stomachics. Pharmacological basis for the medicinal use of these oils is very confusing, contradictory and require further elucidation.

The following essential oils, some from plants of known medicinal value and others from sources uninvestigated so far, have been tested for their tuberculostatic properties, the other antibacterial activities, toxicity against a ciliated protozoa and irritant effect on the rabbit skin. *Ocimum gratissimum* which showed a high antibacterial activity and low toxicity, has been subjected to further pharmaco-dynamic studies.

TABLE I

The Essential Oil-bearing Plants Examined and the Main Chemical Constituents

Sl. No.	Plant	Seeds obtained from	Chemical constituents
1	*Ocimum killimanjaricum* N.O. Libiatæ	Africa	70% d. camphor 10% L. pinene 6% Limonene 6% Limonene and 5% Terpinolene
2	*Ocimum gratissimum* N.O. Labiatæ (Syn. Ram Tulasi)	U.S.S.R.	61·8% Eugenol 15% Ocimene
3	*Ocimum canum* N.O. Libiatæ (Syn. Nayi Tulasi)	Indigenous	87% Methyl cinnamate 19–20% Camphor
4	*Pogostemon patchouli*[4] N.O. Libiatæ	Malaya	Benzaldehyde Cinnamic aldehyde Eugenol Patchouli alcohol Sequiterpene Patchoulene
5	*Atlantia monophylla* N.O. Rutacea (Syn. Adavinimbe; wild lime)	Indigenous	l. Sabine l. Linolol Linolyl acetate
6	*Hymenantherum tentifolium* N.O. Compositæ (Syn. Vanamuli)	Introduced Plant from Mexico	Hydrocarbon fraction 60% mainly acyclic monoterpene-hymenantherene.

The seeds of the plants obtained from various sources were cultivated locally. The oils were extracted and the chemical analysis conducted at the Organic Chemistry Department of the Indian Institute of Science, Bangalore. Table I mentions plants examined, source of the seeds and the main chemical constituents.

Antitubercular Activity

In rural parts of India, the external application of the fresh juice from the leaves of ocimum species is a common remedy for various affections of the skin including lupus and leprosy. The ulcers are claimed to get healed and even the discoloration to be much reduced. The following experiment deals with the *in vitro* tuberculostatic effect of various essential oils.

$H_{37}Rv$ strain of Myco-tuberculosis was the test organism. 1/10 dilution of the oils were prepared in propylene glycol and further dilutions were taken in Youman's media. The incubation was at 37° C. and the results recorded at the end of 21 days. The procedural details have already been described.[5] Shark liver oil, though not an essential oil was included for a comparative study, because of its well-known therapeutic value in tuberculosis.

TABLE II

In vitro *Tuberculostatic Activity of Essential Oils on $H_{37}Rv$ Strain of* Myco. tuberculosis *at the End of* 21 *Days*

Essential Oil* Serial No.	Dilutions					
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	C
1	—	—	+	2+	2+	2+
2	—	—	—	—	—	2+
3	—	—	—	+	2+	2+
4	—	—	±	+	2+	2+
5	—	—	±	+	2+	2+
6	—	—	—	—	+	2+
Shark liver oil ..	—	—	+	2+	2+	2+

* Serial number of oils same as in Table I

Legend :— — No growth

± to 2+ various grades of growth.

The *in vitro* activity indicates the high degree of inhibitory action of some of the essential oils, particularly of *Ocimum gratissimum* and probably explain the beneficial results obtained by the empirical use of some of these plant juices in tubercular affections of the skin.

Anti-Microbial Activity

The use of plant juices in intestinal disorders and urogenital affections besides the septic wounds, prompted us to test the antibacterial activity of these oils against some of the pathogenic organisms commonly responsible for above infections.

As the solvent has a relatively large effect on the antibacterial actions in the phenol coefficient technique, filter-paper disc method, on nutrient agar plates, seeded with test organisms and measuring the zone of inhibition as the criterion was the method adopted.

8 mm. filter-paper discs were placed in the four quadrants of a nutrient agar plate previously seeded with test organisms; 0·05 c.c. of a dilution of the substance in alcohol was placed on the disc and the zone of inhibition measured after incubation of 72 hrs. The production of a significantly large zone after 24 hrs. maintained after 72 hrs. was the criterion used for bacteriostatis. The test was repeated for several dilutions of each substance. Control tests omitting the oil and using only various dilutions of alcohol did not exhibit appreciable inhibition.

A comparative value of the essential oils in a 1/10 dilution is summarised in Table III.

TABLE III

Antibacterial Activity of Essential Oils in 1/10 *Dilution*

Sl. No. of oil*	Organisms			
	Staphylo aureus	*Strepto pyogenes*	*Bact. coli*	*Bact. typhosum*
1 ..	15	12	14	15
2 ..	25	20	25	25
3 ..	12	12	15	20
4 ..	15	15	12	12
5 ..	15	12	20	15
6 ..	12	11	12	15
Thymol	25	20	20	25

Ref.—Serial No. Same as Table I.

Figures indicate the zone of inhibition in mm.

All the essential oils exhibit antibacterial activity of some degree. The oil of *Ocimum gratissimum* is the most potent and simulates thymol in its antibacterial activity.

Toxic Effect

The degree of toxic effect of these oils on cells, was estimated by determining the time required for complete immobilisation of *Epistylis*, sp., an actively motile ciliated protozoa commonly found in activated sludge.

TABLE IV

The Toxic Effect of Essential Oils on Epistylis

Dilution	Serial No. of Oil					
	1	2	3	4	5	6
1/5,000 ..	Ins.	20	10	6	20	30
1/10,000 ..	6	30	30	15	30	30

Ins.: Instantaneous.

Figures indicates time in minutes required for complete immobilisation.

Oil No. 1 is the most toxic and No. 6 the least, of the series.

Irritant Action

The irritant effect of the oils was determined by the surface application method on the rabbits skin. A known quantity of the oil was applied to the skin and the reactions noted, as in Table V.

TABLE V

Essential Oil* Serial No.			Reaction
1	..	..	++
2	..	..	++
3	..	..	+
4	..	..	++
5	..	..	+
6	..	..	−
7	Turpentine	..	+++

Legend:— − No reaction
+ Instantaneous dilation of capallaries
++ Erythema persisting for 48 hrs. No vesication.
+++ Vesication and desquamation.

The essential oil from *Ocimum gratissimum*, which exhibited the maximum tuberculostatic property, a mild degree of toxicity and moderate degree of irritability was taken up for further pharmacological study.

Local Anæsthetic activity of the Essential Oil of Ocimum gratissimum.—As the juice of this plant is used to relieve ear-ache and tooth-ache, an attempt was made to determine the local anæsthetic activity of this essential oil.

Different dilutions of the aqueous suspensions of the oil were instilled into and allowed to remain in the conjunctial sac of the guinea pig eye for one minute, the other eye serving as an untreated control. Persistence of the lid reflex following the mechanical stimulation of the cornea is indicated by zero. Anæsthesia is indicated by + sign and figures in brackets indicate the duration of anæsthesia in minutes.

TABLE VI

Local Anæsthetic Activity of the Essential Oil from Ocimum gratissimum

Dilution of the Oil	Anæsthetic activity	Reaction
1/10,000 ..	—	
1/1,000 ..	+ (2)	No irritant effect on the cornea
1/100 ..	+ (10)	Slight circum-corneal injection; disappeared at the end of 10 minutes

It is thus seen that a 1/100 dilution of the oil gives a local anæsthetic action without much irritation. The slight irritation may, in fact, be helpful in resorbing the exudates from an inflamed area, while the anæsthesia produced gives relief from pain, and hence this may be found useful as an external application for inflamed joints and other chronic inflammatory conditions also.

Action on Smooth Musculature

A concentration of 10 γ/c.c. of the essential oil of *O. gratissimum* decreased the rate and extent of the movement and caused relaxation of the musculature of both the rat intestines and the guinea-pig uterus. The stimulant action of the barium chloride on the intestinal musculature could be neutralised and immediate relaxation obtained by the addition of the oil of *O. gratissimum*.

This direct action on the muscle probably explain the relief obtained in conditions of abdominal colic.

Other pharmaco-dynamical properties and the chemical constituents of the oil responsible for the pharmacological activities are under investigation.

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