A study of the antimicrobial activity of oil of Eucalyptus

Sir,

The emergence of resistance to conventional antimicrobials is a serious problem that physicians face. This necessitates constant development of newer agents, which can inhibit the growth of or kill resistant organisms.

Eucalyptus is a tall, evergreen tree, native to Australia and Tasmania, successfully introduced worldwide, now extensively cultivated in many other countries including India.[1-4] The genus name Eucalyptus comes from the Greek word eucalyptos, meaning "well-covered," and refers to its flowers that, in bud, are covered with a cup-like membrane.[2] Though native to Australia, its therapeutic uses have been introduced and integrated into traditional medicine systems, including Chinese, Indian Ayurvedic, and Greco-European. Its volatile oil is obtained by steam distillation and rectification from the fresh leaves or the fresh terminal branches.[5] Eucalyptol (1, 8-cineole) is the active ingredient of the eucalyptus oil, responsible for its various pharmacological actions. Pharmacopoeial grade dried eucalyptus leaf must contain at least 2.0% (v/m*)* volatile oil, composed mainly of 1, 8-cineole.[3] The *Indian Pharmacopoeia* requires not less than 60% w/w of cineole.[5]

Oil of eucalyptus has been traditionally used in Ayurveda as an antiseptic and for respiratory tract infections. Moreover, Nadkarni has reported its antibacterial action.[1] However, much scientific data is still not available regarding its antibacterial action.

Oil of eucalyptus (containing 63% of eucalyptol) was obtained from the local market and used for the study. Organisms were isolated from the pus samples of patients from the surgery department. The organisms (isolated and identified by standard methods) were Klebsiella spp., Proteus spp., Pseudomonas spp., *E. coli*, and *S. aureus*. Only those organisms resistant to conventional antimicrobials (tobramycin, gentamicin, amikacin, ciprofloxacin, chloramphenicol and cefotaxime) were used for the study. The resistance of the isolate to the conventional antimicrobials was confirmed using disc diffusion as well as MIC testing.[6] The sample size for each organism was twenty five.

The samples were inoculated on Mac Conkey and Blood agar and incubated at 37^{0}C for 24 h. Three to four colonies of organisms from the culture media were inoculated into 4 ml nutrient broth and incubated for 2 h at 37^{0}C. The inoculum was standardized by matching its turbidity with Mc Farland no. 0.5 standard.

With a sterile cotton swab the test culture was spread evenly on the surface of a petri dish containing solidified Mueller-Hinton (M.H.) agar (pH 7.2 to 7.4). Five wells were made in the M.H agar plate using a sterile cork borer (0.5 cm). Different doses of oil of eucalyptus, *i.e.,* 5, 10, 25, 50, 100, 150 and 200 micro liters were poured in the wells with the help of a sterile micropipette. The plates were incubated at 37^{0}C for 24 h and at the end of 24 h the diameter of the resulting zone of inhibition was measured and the average values were recorded.

The data show that, *E. coli* and Klebsiella spp. were sensitive to 5 µl; *S. aureus* to 25 µl while *Pseudomonas* and Proteus spp. required 50 ml of eucalyptus oil (Table 1). With an increasing dose of oil of eucalyptus, the resulting diameter of the zone of inhibition increased for all the organisms. The results of the study revealed that oil of eucalyptus has antibacterial activity against Gram-positive as well as Gram-negative bacteria resistant to commonly used antimicrobial agents.

However, toxicological data on the systemic use of eucalyptus oil are lacking. Currently, it is focused towards local use as an antiseptic. A study done by Sherry *et al*[7] reveals that topical application of eucalyptus oil is effective against methicillin resistant *S. aureus* infection. Moreover, Kumar[8] and Ahmad[9] also report the antibacterial action of oil of eucalyptus on local application.

However, further studies are still required regarding its effectiveness in the presence of pus and its safety report when applied on infected skin or mucous membrane.

Table 1

The mean zone of inhibition exhibited by various doses of oil of eucalyptus against various pathogens obtained from the pus sample of the patients showing resistance to commonly used antibacterials using well diffusion method

Bacterial species	*Dose of oil of Eucalyptus (µl)*						
	5	10	25	50	100	150	200
E coli spp.	+	+	++	+++	+++	+++	+++
Klebsiella	+	+	++	+++	+++	+++	+++
S. aureus	–	–	+	++	+++	+++	+++
Proteus spp.	–	–	–	+	++	+++	+++
Pseudomonas spp.	–	–	–	+	++	+++	+++

Zone of inhibition. – : < 5 mm, +: 5-9 mm, ++: 10-19 mm, +++: > 20 mm

Acknowledgements

The author is very thankful to Ms. Nishita and Dr. M. B. Shah, Head, Department of Microbiology for their help and cooperation.

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In vitro cytotoxic activity of *Lantana camara* Linn

Lantana camara Linn. is a large evergreen strong-smelling herb, native of tropical America, but now naturalized in many parts of India. All the parts of this plant have been used traditionally for several ailments throughout the world.[1] The leaves are used as a bechic, antitumoral, antibacterial and antihypertensive agent.[2] The root of this plant is used for the treatment of malaria, rheumatism and skin rashes.[3] Several tri-terpenoids, napthaquinones, flavonoids, alkaloids and glycosides isolated from this plant are known to exert diverse biological activities including cytotoxic and anticancer properties.[4] The crude methanolic extract of different parts of this plant was studied for its *in vitro* cytotoxic potential.

Natural products provide an inexhaustible source of anticancer drugs in terms of both variety and mechanism of action. Hence, in continuation of our studies to identify potent natural products for antitumor activities,[5] investigation on the *in vitro* cytotoxic properties of the crude extracts of different parts of *Lantana camara* was taken up against four cancerous cell lines viz. HEp-2, $B_{16}F_{10}$, A-549 and DLA and a normal NRK-49F cell line using standard procedures.

The fresh flowers (30 g), fruits (50 g), leaves (57 g), root (55 g) and stem (45 g) of *Lantana camara* were collected from the forests of The Nilgiris, Tamil Nadu, India, in the month of June 2001. Mr. Rajan, Survey of Medicinal Plants and Collection Unit, Government Arts College, Ootacamund, India authenticated the plant. The plant materials were coarsely powdered and extracted in a Soxhlet apparatus with methanol (1:5 w/v) for 18 h. The extracts were then concentrated to dryness under reduced pressure and controlled temperature. The yields obtained were 12.12, 8.64, 12.15, 9.67 and 12.75% respectively, for flowers, fruits, leaves, root and stem. Each extract was separately dissolved in distilled dimethyl sulphoxide (DMSO) and the volume was made up to 10 ml with Dulbecco's Modified Eagle's medium (DMEM), pH 7.4, supplemented with 2% inactivated newborn calf serum (Maintenance medium, PAA Laboratories, Austria), to obtain a stock solution of 1 mg/ml concentration, sterilized by filtration and stored at -20°C till use.

NRK-49F (normal rat kidney) and $B_{16}F_{10}$ (mouse melanoma) cell cultures were obtained from the National Centre for Cell Sciences, Pune, India. HEp-2 (caucasian male larynx epithelium carcinoma) cell line was obtained from the Pasteur Institute of India, Coonoor, India. A-549 (small cell lung carcinoma) cell line was obtained from the Christian Medical College, Vellore, India. Dalton's lymphoma ascites (DLA) cells were obtained from the Amala Cancer Institute, Trissur, India. The cultures of A-549, $B_{16}F_{10}$, HEp-2 and NRK-49F were propagated in DMEM, pH 7.4 supplemented with 10% inactivated newborn calf serum, penicillin (100 IU/ml), streptomycin (100 μg/ml) and amphotericin B (5 μg/ml), and maintained in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in phosphate buffer saline solution (TPVG). The stock culture was grown in 25 cm^2 tissue culture flasks (Tarsons India Pvt. Ltd., Kolkata, India) and all cytotoxicity experiments were carried out in 96 well microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). DLA cells used were propagated and maintained in the peritoneal cavity of Swiss albino mice.

Cell lines in exponential growth phase were washed, trypsinized and resuspended in DMEM medium with 10% inactivated newborn calf serum. Cells were plated at 10,000 cells/well in 96 well microtitre plate and incubated for 24 h at 37°C, 5% CO_2 in a humidified atmosphere during which period a partial monolayer was formed. The cells were then exposed to different concentrations (1000 μg/ml to 15.6 μg/ml, prepared by serial two-fold dilution using maintenance medium from the stock solution) of the test extracts in quadruplicate. Control wells received only maintenance medium. The cells were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 72 h. Morphological changes of the cell cultures were examined using an inverted tissue culture microscope (Olympus, Japan, Model 1X70) at 24 h time intervals and compared with the control. At the end of 72 h, cellular viability was determined using standard 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT)[6] and Sulphorhodamine B (SRB)[7] assays. The short-term toxicity studies[5] were carried out on DLA cells employing Trypan blue dye exclusion technique[5] and the CTC_{50} value (concentration of the sample required to kill 50% of the cells) was calculated.

Of the five methanol extracts obtained from different parts of *Lantana camara,* the leaf extract exhibited comparatively more cytotoxic activity against all the five cell lines tested (Table 1). The human lung carcinoma cell line, A-549 was found to be more susceptible with a CTC_{50} value of 48.1 – 58.5 mg/ml extract. The other four extracts showed less activity as indicated by the relatively high CTC_{50} values. In the short-term toxicity studies, the methanol extracts of the root with 191.5 ± 5.1 μg/ml and leaf with 219.5 ± 8.4 μg/ml, showed moderate activity against DLA cells after 3 h of exposure. The extracts of the stem, fruit and flowers of *Lantana camara,* showed less activity with CTC_{50} values, 268.7 ± 10.2, 492.7 ± 14.4 and > 1000 μg/ml respectively.

The results obtained from the present study show that the extract of the leaf of *Lantana camara* is cytotoxic in nature and may possess antitumor activity. The study also supports

Table 1

Cytotoxic activity of *Lantana camara* on different cell lines by MTT and SRB assays

Extract no.	*CTC_{50} (µg/ml)**							
	NRK-49F		A-549		$B_{16}F_{10}$		Hep-2	
	MTT	SRB	MTT	SRB	MTT	SRB	MTT	SRB
Flower	360.32 ± 13.18	212.56 ± 5.49	446.91 ± 14.13	> 500	244.44 ± 4.91	452.41 ± 14.21	442.73 ± 11.2	436.18 ± 8.85
Fruit	494.17 ± 14.63	446.27 ± 11.42	> 500	> 500	432.84 ± 16.47	496.32 ± 15.21	312.49 ± 2.87	450.56 ± 2.67
Leaves	121.14 ± 6.42	128.51 ± 1.2	58.48 ± 2.83	48.14 ± 1.83	243.38 ± 8.46	127.58 ± 2.85	221.18 ± 3.94	184.57 ± 8.33
Root	465.62 ± 21.14	449.18 ± 9.14	179.25 ± 1.17	170.17 ± 4.32	445.57 ± 13.94	353.63 ± 10.72	248.27 ± 9.18	255.11 ± 10.41
Stem	175.63 ± 6.13	230.73 ± 5.86	448.28 ± 14.83	> 500	244.63 ± 4.98	452.35 ± 13.67	442.45 ± 14.48	436.87 ± 8.48

*Average of three independent determinations, 4 replicates, values are mean ± SEM

the ethnomedical data provided in an earlier study.[2] The cytotoxic activity may be due to the presence of toxic lantanoids and alkaloids from this plant.[4] The plant merits further investigation to identify the active principles and the nature of the antitumor activity in animal models.

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IJP gratefully acknowledges Prof Paul Flecknell and Dr. Kasim A. Mookhtiar, Vice President, Ranbaxy Research Laboratories Ltd., India who made the distribution of the CDs possible.