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BIOLOGICAL ACTIVITY OF SOME STEAM DISTILLATES FROM LEAVES OF TEN SPECIES OF RUTACEOUS PLANTS

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Abstract : Some steam distillates collected from leaves of ten Rutaceous plants by Simple Steam Distillation method have been investigated for antimicrobial and insecticidal activity. *Murraya paniculata, Toddalia asiatica, Limonia acidissima* and *Glycosmis pentaphylla* have shown significant antifungal activity against *Cladosporium cladosporioides.* High antibacterial activity was displayed by *L. acidissima* and *M. paniculata* against *Staphylococcus aureus. Atalantia monophylla* and *Acronychia pedunculata* caused significant mortality of the aphid, *Aphis craccivora.*

1. Introduction

Rutaceae is a large family comprising of about 150 genera. Twenty genera have been recorded in Sri Lanka of which the majority are indigenous.[7] The family is best known for the citrus fruits. Several other species, for example *Murraya koenigii* (Karapincha or Curry leaf), are grown for their aromatic leaves used in cooking and popular medicine. Some species provide edible fruits, also reputed to have medicinal value, such as the wood apple (*Limonia acidissima*) and beli fruit (*Aegle marmelos*).

Compounds having antifungal properties have been isolated from Rutaceae, for example in *L. acidissima* where the unripe fruit shell, stem and root-bark contain at least four compounds, *viz* 2-6-dimethoxybenzoquinone, psoralen, xanthotoxin and osthenol, inhibitory to a range of fungi.[1,16] *Euodia lunu-ankenda* is one of the several plants in this family showing

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antibacterial activity.[1 3] The insecticidal component, 'neoherculin', has been isolated from *Zanthoxylum clava–hercules.*[5] The present study describes the antimicrobial and insecticidal properties of some steam distillates collected by Simple Steam Distillation method[9] from leaves of ten species belonging to the family Rutaceae.

2. Experimental

2.1 Plant Material and Separation of Steam Distillates

Plant material was collected at Belihuloya in the Central Province of Sri Lanka and identified by comparison with specimens deposited at the National Herbarium, Peradeniya. Fresh leaves (500g) were chopped and loosely packed in 5 litre round bottommed flasks and the flasks were kept in boxes containing saw dust. Steam was passed into the flasks continuously for about 12 h. and the steam coming out from an outlet was allowed to pass into a separate receiving flask through a condensor unit at room temperature. The resultant aqeous solution was extracted with dichloromethane (1.5–2 litres), dried with Na_2SO_4 and the solvent was evaporated *in vacuo* (below 40^0C).

2.2 Antifungal Activity (*Cladosporium* TLC–bioassay)

Steam distillates (2mg) were spotted on TLC plates (silica gel 60 $PF_{254-366}$, 0.50mm X 20cm X 20cm) and the plates were developed in dichloromethane : light petroleum (2:3) . After air–drying the plates inside the laboratory at ambient temperature (28^0C) they were sprayed with a suspension of conidia of *Cladosporium cladosporioides* in Czapek–Dox nutrient solution. Plates were then incubated in a moist chamber at 25 ± 2^0C for 48 h. Inhibition areas appeared white against a background of green mycelia. The diameters of zones in which the growth was inhibited, which were approximately circular, were measured (mm).

2.3 Antibacterial Activity

Tests for antibacterial activity[6] were carried out using type strains of *Staphylococcus aureas* (NCTC 6571). *Escherichia coli* (NCTC 10418) was used as the control organism in antibiotic activity testing. The essential oils (16mg each)were dissolved separately in 4ml aliquots of ethanol and ten fold dilutions were prepared from each with nutrient broth. A series of doubling dilutions were then dispensed in 2ml aliquots in tubes, with concentrations ranging from 1/10 to 1/5120 and inoculated with a fixed bacterial inoculum. The bacterial inoculum was 0.1 ml of a 10^{-4} dilution of bacterial suspension with a density equal to that of a 2% aqueous suspension of barium sulphate. At the same time the bacterial inoculum was also dispensed into an identical set of tubes of dilutions made from ethanol alone, and a tube each of nutrient broth, these being used as controls.

The tubes were incubated (37°C) overnight and observed the next day for turbidity, the highest dilution showing no turbidity being the minimal inhibitory concentration (MIC). A loopful of medium from each of the tubes was then inoculated on nutrient agar and again incubated overnight. The minimal bactericidal concentration (MBC) was considered to be that showing no growth or less than 20 colonies on semisolid medium. In the interpretation of results, the test solutions were compared with those of alcohol dilutions.

2.4 Insecticidal Activity

One day old opterous female aphids (*Aphis craccivora*) obtained from a laboratory culture maintained on cowpea plants (*Vigna unguiculata*) were used in these experiments.[14]

Each of the steam distillates of leaves were mixed with homogenate ($LF_{12}{}^{R}$, Ciba–Geigy, Bascle) (1:2 w/w), dissolved in analytical grade acetone and distilled water (1:9 v/v) to obtain a 1500 ppm emulsion.

Ten aphids were placed on the underside of a detached young cowpea leaf and placed in a glass Petri–dish (9cm diameter) lined with a moistened filter paper. When they were settled, the leaf was turned upside down and sprayed with 4.0ml of the 1500 ppm emulsion using the Potter's spray tower.[11] Then the leaf was turned right side up and the base of the petiole was wrapped with a moistened cotton plug to retard dessication. Each Petri-dish was covered with a plastic lid with a circular window of fine gauze. A mixture of distilled water, acetone and the homogenate was used as the standard control.

The experiments was conducted at a mean temperature 29.5 ± 4°C and humidity 80 ± 4% RH. Mortality counts were recorded 24 hours after treatment. A completely randomized design (CRD) with four replicates was used.

3. Results and Discussion

Plant essential oils belong to different classes of organic compounds having acyclic, cyclic, aromatic and heterocyclic structural features. These constituents include terpenes (mono–, sesqui– and diterpenes), aromatic compounds such as eugenol, nitrogen and sulphur containing compounds (allyl isothiocyanates) and miscellaneous compounds including unbranched long chain hydrocarbons.[15]

Most of the plants which were investigated are used in ethnomedical preparations[4] (Table 1). The therapeutic value of these plants is no doubt due to the presence of biologically active natural products. There are several reports on the isolation of chemical constituents from the plants that were studied: alkaloids from *M. koenigii*[3] and *G. pentaphylla*,[2] coumarins from

L. acidissima,[16] *T. asiatica*[17] and flavones and coumarins from *M. paniculata*[8] are some of them.

Table 1: The steam distillates of leaves of ten Rutaceous plants screened for antimicrobial and insecticidal activity.

Plant species[a] (Sinhala & Tamil names)	Habit[b]	Reported Medicinal Uses[c]	Part(s) Used Medicinally	Weight of Plant Material Used(g)	Weight of Essential oil (g) (%yield)
Acronychia pedunculata (L.) miq. (S:Ankenda)	ST	Sores and ulcers	Bk	1000	0.580 (0.058)
Atalantia ceylanica (Arn.) Oliv.(S:Yakinaran; T:Peykurutu)	Sh	Administration of pills, prevent ague	Lf	250	0.340 (0.136)
Atalantia monophylla (Roxb.) DC (S:Apassu; T:Perunkuruntu)	ST	Skin diseases rheumatism and paralysis	Lf,Ft	300	0.770 (0.257)
Clausena indica (Dalz.)Oliv. (S:Meegon Karapincha;T:Pannai)	ST	Not reported	–	990	4.240 (0.428)
Glycosmis pentaphylla (Retz.) A.DC. (S:Dodan pana; T: Kulapannai)	ST	Fever	Rt	800	2.090 (0.261)
Limonia acidissima L. (S:Diwul; T:Nila–vilam)	T	Astringent, Sore throat, dysentry and diarrhoea	Ft, St	1100	1.810 (0.163)
Murraya koenigii (L.) Spreng (S:Karapincha; T:Kuruvepillai)	Sh	Stomachic, purgative, vomiting, febrifuge	Rt, Lf	715	1.210 (0.169)
Murraya paniculata (L.) Jack. (S:Ettariya)	Sh	Stomachic, rheumatic fever, cough, giddiness, hysteria.	Wp	800	1.650 (0.206)
Toddalia asiatica (L.) Lamk. (S:Kudumiris; T:Milkaram)	Cl	Pains in the bowels, rheumatic swellings, fever	Lf, Rt	225	0.235 (0.104)
Zanthoxylum rhetsa (Roxb.) DC. (S : Katukeena; T : Rhetsamaram)	T	Not reported	–	200	0.080 (0.040)

a Nomenclature used follows Dassanayake and Fosberg[1]

b Sh – shrub; ST – small tree; Cl – climber; T – large tree

c According to Chandrasena[11]

d Lf – leaf; Ft – fruit; Bk – stem bark; Rt – root; Wp – whole plant

Steam distillates of ten plants were tested for antimicrobial activity against *Cladosporium cladosporioides, Staphylococcus aureus* and *Escherichia coli* and for insecticidal activity (Table 3) against *Aphis craccivora* koch. (Table 2).

Table 2 : Antimicrobial and insecticidal activity of the steam distillates of leaves of ten Rutaceous plants

Plant species	Antifungal activity (*C. cladosporioides*) Diameter of inhibition zone (mm)	Antibacterial activity (*S. aureus*) Minimal Bactiricidal Concentration (MBC) (μg/ml)	Insecticidal activity (*A. craccivora*) Mortality percentage 24** HAT
A. pedunculata	35 (0.20*)	50	60.0 b
A. ceylanica	12 (0.13)	50	40.0 cde
A. monophylla	16 (0.53)	25	82.5 a
C. indica	14 (0.20)	100	35.0 cdef
G. pentaphylla	30 (0.05)	100	30.0 cdefg
L. acidissima	36. (0.00)	6.25	27.5 cdefg
M. koenigii	23 (0.05)	100	45.0 bc
	8 (0.30)		
	9 (0.50)		
M. paniculata	40 (0.18)	6.25	17.5 g
T. asiatica	38 (0.20)	100	47.5 bc
Z. rhetsa	15 (0.20)	50	27.5 cdefg
Benlate (0.2 mg) Standard control	38		17.5 g

* Rf value (Dichloromethane : Light Petroleum – 2:3)

** HAT = Hours After Treatment

Arc sin$\sqrt{}$percentage transformation was used for insecticidal activity calculations.

$P < 0.05$ c.v = 17.8%. Means followed by the same letter are not significantly different ($P < 0.05$) by Duncan's multiple range test.[12]

Steam distillates of all ten plants showed antifungal activity against *C. cladosporioides* using the TLC–bioassay technique (Table 2). *M. paniculata, T. asiatica, L. acidissima, A. pedunculata* and *G. pentaphylla* showed high antifungal activity almost comparable with that of benlate. Interestingly, *M. koenigii* produced three inhibition areas, of which the highest polar constituent showed the greatest activity. The other steam distillates derived from *A. monophylla, Z. rhetsa, C. indica* and *A. ceylanica* showed comparatively low activity. There are no previous reports available on the antifungal activity of these plants other than *L. acidissima.*[1,16]

None of the steam distillates showed antibacterial activity against *E. coli*, the representative test organism of Gram negative bacteria. However, activity against *S. aureus*, the representative test organism of Gram positive bacteria, was shown by all steam distillates at different dilutions. Of the steam distillates the highest activity was shown by *L. acidissima* and *M. paniculata* (at 6ppm level of the crude extract).

The steam distillates from leaves of *A. monophylla, A. pedunculata, T. asiatica, M. koenigii* and *A. ceylanica* caused significant mortality of the aphid, *A. craccivora*, when compared with the untreated control (Table 2). To our knowledge this is the first report of such promising insecticidal activity in these plants.

This preliminary study indicates that the members of the family Rutaceae contain volatile substances having antimicrobial and insecticidal properties. Interestingly, the two plants for which there are no reported medicinal uses, *Z. rhetsa* and *C. indica* have shown only moderate or low activity compared to the other plants. *L. acidissima* and *M. paniculata* have shown significant antifungal and antibacterial activities while *A. monophylla* has shown the highest insecticidal activity. The probable role of at least some of these substances would be self–defence against invading pests. Further investigations into their biological importance and role in nature would therefore prove useful.

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