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Biological Activities of Essential Oils from Plants Growing in Tanzania

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Essential oils from eleven plant species belonging to the Asteraceae, Lamiaceae, Lauraceae and Myrothamnaceae families growing in Tanzania were screened for mosquito larvicidal and anti-candida activities, and were subjected to the brine shrimp lethality test. In the larvicidal and brine shrimp tests, the organisms were exposed to varying oil concentrations for 24 h, after which mortality was assessed. The anti-candida activity was determined using the bioautography agar overlay method. All oils showed larvicidal activity with two *Ocimum suave* oil samples being the most active with LC_{50} values of 169.8 and 151.3 ppm. The same *Ocimum suave* oils also exhibited the highest brine shrimp mortality (LC_{50} 4.0 and 12.6 ppm). Most of the oils showed anti-candida activity, with oils from *Ocimum* species being the most active compared to the others. Thus, *Ocimum suave* oils merit further investigation towards the development of safe and biodegradable larvicides. Furthermore, oils from *Ocimum basilicum* and *Ocimum kilimandscharicum* could offer useful alternatives for combating candidiasis, a common opportunistic infection in HIV/AIDS patients.

Key words: *Ocimum* species, larvicides, brine shrimp, *Candida albicans*

INTRODUCTION

As part of a continued study on essential oils from plants growing in Tanzania, oils from 11 plant species belonging to four families, namely Asteraceae, Lamiaceae, Lauraceae and Myrothamnaceae, were tested for larvicidal and anticandida activities as well as for lethality to the brine shrimp. Brine shrimp lethality test is normally used as an indicator for presence of cytotoxic, antitumor, pesticidal and antitrypanosoma activities in natural products [1]. In addition, activities of a broad range of known active compounds and extracts are manifested as toxic to the brine shrimp [2].

Insect vectors, especially mosquitoes, are responsible for spreading serious diseases such as malaria, lymphatic filariasis, Japanese encephalitis, yellow fever and dengue fever which cause morbidity, mortality, economic loss and social disruption [3]. Synthetic insecticides used to control these vectors are associated with a number of problems such as the development of resistant insect strains, ecological imbalance and general harm to mammals [4, 5]. Since mosquitoes can effectively be controlled by targeting the larvae rather than the adult insect [6] and also due to the setbacks encountered with synthetic insecticides, there is a need to develop larvicides from plants which are associated with fewer hazards to man and the environment [7].

Previous studies involving essential oils obtained from various plants such as *Ipomoea cairica* [8], *Blumea mollis* [6], *Cymbopogon citratus* [9], *Ocimum americanum* and *O. gratissimum* [10] yielded good LC_{50} values against larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. The current study included determination of the larvicidal activity of essential oils against *Culex quinquefasciatus* mosquito larvae.

Candidiasis is one of the major opportunistic infections in individuals living with HIV infection especially those with severe

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immunosupression [11]. The control of candidiasis suffers from a number of problems including the limited number of effective drugs, the slow rate at which new drugs are being developed and the side effects and cost associated with these drugs [12]. Furthermore relapse of candidiasis and resistance of *Candida* species to commonly used drugs major drawbacks to the management of candidiasis [13]. These difficulties associated with the management of *Candida* infections necessitate the development of new anti-fungal agents that are active against *Candida*.

Several essential oils have previously been investigated for their antimicrobial activity including anticandida activity. Some of the essential oils with promising results include those from *Cinnamomum cassia* [14], *Zingiber nimmonii* [15], *Satureja biflora*, *S. masukensis*, *S. pseudosimensis*, *Plectranthus laxiflorus* and *Vernonia smithiana* [16, 17].

EXPERIMENTAL

Plant Materials

Plant materials were collected from Dar es Salaam, Iringa, Mbeya and Tanga regions as shown in Table 1**.** They were identified at the Botany Department, University of Dar es Salaam and voucher specimens were deposited in the herbarium of the Department of Pharmacognosy, School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS). Prior to extraction of the essential oils, the materials were air dried at ambient temperatures, in the shade, except for the wood of *Ocotea usambarensis* which was dried in the sun, and *Pluchea dioscoridis* leaves which were extracted while still fresh.

Extraction of essential oils

Essential oils were extracted by hydro-distillation using a Clevenger-like apparatus. The oils were collected over distilled water, separated and dried over anhydrous sodium sulphate. They were stored in air-tight screw-cap vials in a refrigerator until use.

Table 1: Plants under study and locations where they were collected

Plant Names	Family	Plant part used	Where Collected District (Region)
Artemisia afra Willd.	Asteraceae	Leaves & flowering tops	Rungwe (Mbeya)
Helichrysum cymosum (L.)	Asteraceae	Leaves	Rungwe (Mbeya)
Leucas glabrata (R.Br.)	Lamiaceae	Leaves & flowering tops	Rungwe (Mbeya)
Myrothamnus flabellifolius (Sond)	Myrothamnaceae	Leaves	Kilolo (Iringa)
Ocimum basilicum L.	Lamiaceae	Leaves & flowering tops	Rungwe (Mbeya) & Mbeya Rural (Mbeya)
Ocimum kilimandscharicum Guerke	Lamiaceae	Leaves & flowering tops	Mbeya Urban (Mbeya)
Ocimum lamiifolium Benth.	Lamiaceae	Leaves & flowering tops	Rungwe (Mbeya)
Ocimum suave Willd.	Lamiaceae	Leaves & flowering tops	Rungwe (Mbeya) & Mbeya Urban (Mbeya)
Ocotea usambarensis Engl.	Lauraceae	Steam & Bark	Lushoto (Tanga)
Pluchea dioscoridis D.C.	Asteraceae	Leaves	Ilala (Dar es salaam)
Tagetes minuta L.	Asteraceae	Leaves, Flowers &Stem	Lushoto (Tanga)

Mosquito larvicidal tests

Culex quinquefasciatus larvae were collected from the stagnant drains within MUHAS. They were identified at the Department of Parasitology and Entomology, MUHAS and kept under laboratory conditions for one day before performing the tests.

Stock solutions of the oils in methanol were prepared at a concentration of 50 mg/ml, and then further serially diluted with methanol to six different concentrations. An appropriate amount of each of the stock solutions contained in shallow plastic cups was further diluted with distilled water containing about 15 larvae, to make 10 ml of the final test solutions with concentrations ranging from 50-600 ppm and a methanol content not exceeding 1% v/v. A 1% v/v aqueous methanol solution containing about 15 larvae, served as a control. The tests were performed in duplicate and mortality was assessed after 24 h. The procedure was repeated three times for each of the oils under test. The larvae which died were counted and the average percentage mortality was calculated after correcting for control mortality using Abbott's formula [18]. The LC_{50} values were determined by linear regression analysis.

Brine shrimp lethality test

Brine shrimp eggs were a kind donation by Prof. Robin Marles of Brandon University, Vancouver, Canada. The eggs were hatched in a shallow glass container, containing artificial brine containing 9.5 g artificial sea salt in 250 ml distilled water. The pH of the solution was adjusted to 7. The container was covered with aluminium foil (80%) and partially illuminated with a lamp. Hatching took about 72 h, after which the shrimps were ready for tests.

Stock solutions of oils were prepared by dissolving 100 mg of oil in 1 ml of dimethylsulfoxide (DMSO), except for *Artemisia afra* oil, which was dissolved in methanol. Tenfold dilutions were prepared by diluting the stock solutions with 10% DMSO or methanol, to obtain three different concentrations. Further ten-fold dilutions were prepared in test tubes by using artificial sea water, containing 15 actively swimming shrimps. The final test concentrations were 10, 100 and 1000 ppm. The test tubes used as controls contained 0.5% DMSO or 0.5% methanol in artificial sea water, containing 15 actively swimming shrimps. The tubes were loosely covered with aluminum foil and kept at room temperature (29^{o}C) for 24 h. The number of dead shrimps was determined by counting completely motionless shrimps found at the bottom of the tubes. Tests were performed in duplicate and the procedure was repeated twice for all the oils under investigation. The average percent mortality was determined, after correcting for control mortality by Abbott's formula [18]. The LC_{50} values were determined by linear regression analysis.

Screening for anticandida activity

The essential oils were screened for anticandida activity by using a slightly modified bioautography agar overlay method [19]. Five (5) µl of the various dilutions of the essential oils ranging from 25 - 200 µg/µl in methanol, as well as 5 µl of methanol and amphotericin B (0.002 µg/µl), were applied onto the heated and cooled TLC plates and kept in a laminar flow chamber. The TLC plates used were of dimensions 20 x 20cm, 250 µm, and were from Merck Darmstadt, Germany. Methanol was allowed to evaporate from the plates which were then over-layered with heated (45^{o}C) *Candida albicans* ATCC 90028 inoculum (2 x 10^{7}cells/ml) prepared in Sabouraud dextrose agar (Biotec Laboratories Ltd, Suffolk, UK.) using a sterile Pasteur pipette. The plates were placed in polythene containers lined with moist cotton wool and incubated at 31^{o}C. After an overnight incubation the plates were removed and sprayed with an aqueous solution (2.5 mg/ml) of thiazolyl blue (3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) (BDH, Poole, England). They were incubated for a further 4 h, after which the inhibition zones appeared colorless against a purple background. The diameters of the inhibition zones were measured. For oils which showed inhibition zones at 125 µg/ml, the test was repeated using 62.5 µg/µl and 31.25 µg/µl.

Table 2: Median lethal concentrations of tested essential oils against *Culex quinquefasciatus* larvae and brine shrimps

Plant species	Larvicidal activity	Brine Shrimp lethality
	LC_{50} (ppm)	LC_{50} (ppm)
A. afra	457.1	34.7
O. basilicum	269.2	> 100
O. kilimandscharicum	323.6	21.9
O. lamiifolium	229.1	Not tested
O. suave (**a**)	169.8	4.0
O. suave (**b**)	151.3	12.6

Table 3: Anticandida activity (inhibition zones in mm) of different amounts of the essential oils per spot

Amount in µg	1000	500	250	125	62.5	31.3
Species	**Inhibition zones in mm**					
A. afra	11.5	9.5	8.0	7.0	0	-
H. cymosum	8.5	7.5	7.0	6.0	0	-
L. glabrata	14.5	12.5	8.5	7.5	0	-
M. flabellifolius	14.0	11.5	10.0	7.0	0	
O. basilicum (**a**)	28.5	26.0	20.0	15.5	14	6.5
O. kilimandscharicum	21.0	20.0	16.5	12.5	9.0	6.5
O. lamiifolium	12.5	11.0	8.5	7.5	0	-
O. suave(**b**)	15.5	13.5	12.5	9.5	7.5	-
O. usambarensis	12.0	10.0	7.0	0	0	-
P. dioscoridis	0	0	0	0	0	-
T. minuta	12.5	10.5	8.5	7.5	7.5	-
Amphotericin B (0.01 µg)						14.2
Methanol (5 ul)						0.0

RESULTS AND DISCUSSION

Oils from *Artemisia afra*, *O. basilicum*, *O. kilimandscharicum* and *O. suave* were screened for larvicidal activity, toxicity against the brine shrimps and for anticandida activity. Oils from *Helichrysum cymosum*, *Leucas glabrata*, *Myrothamnus flabellifolius*, *Ocotea usambarensis*, *Pluchea discoridis* and *Tagetes minuta* were screened for anticandida activity only, while *O. lamiifolium* oil was screened for the larvicidal activity. Since the yield of some oils was low, it was not possible to subject all oils to all three tests. The results for the mosquito larvicidal activity and brine shrimp lethality test are as shown in Table 2 and the anticandida activity in Table 3.

All the tested essential oils showed varied activity against the *Cx. quinquefasciatus* mosquito larvae, these variations were also, observed for oils from the two populations of *O. suave* (**a** and **b**) which exhibited the highest larvicidal activity with LC_{50} of 169.8 and 151.3 ppm, respectively. *Artemisia afra* showed the least larvicidal activity with LC_{50} of 457.1. The observed differences for *O. suave* oils might be due to the variations seen in their chemical

composition. While the essential oil of *O. suave* (**a**) contains methyleugenol as the major component, germacrene B and D were found to be the main components of *O. suave* (**b**) oil [20]. Some essential oils from the genus *Ocimum* have been previously reported to have mosquito larvicidal activity with (LC_{50} < 70 ppm) [10].

Results for the brine shrimp lethality test showed that, with the exception of *O. basilicum* oil which was inactive (LC_{50} > 100ppm), the rest of the tested oils were toxic to the brine shrimps. Oils of the two populations of *Ocimum suave* **a** and **b** were highly toxic (LC_{50} < 20 ppm). Unlike in the larvicidal activity, *O. suave* (**a**) was more active in this bioassay system, with a LC_{50} of 4.0 ppm. The observed variations in activity might be due the variations in their chemical composition [20]. The essential oils of *O. kilimandscharicum* and *A. afra* also exhibited activity against brine shrimps with LC_{50} values of 21.8 ppm and 34.7 ppm, respectively. No mortality was observed in the control tubes indicating that the essential oils were responsible for the observed mortality.

Regarding the anticandida activity, it was found that with the exception of the essential oil of *Pluchea dioscoridis* (inactive), all the other essential oils exhibited varied activity against *Candida albicans* ATCC 90028 (Table 3). Essential oils from *Ocimum* species displayed the highest activity. The oil of *O. basilicum* was the most active followed by that of *O. kilimadscharicum.* The least active among the *Ocimum* oils was that from *O. lamiifolium*. However, in a previous study, none of the *Ocimum* oils showed activity on *C. albicans* when tested by the agar microdilution method at a maximum concentration of 20 mg/ml [20]. *P. dioscoridis* essential oil, showed no inhibition zone at 1000 µg. This oil has been reported to contain predominantly sesquiterpenes hydrocarbons [23]. Some of the studied essential oils obtained from plants growing in other countries have also been reported to have anticandida activity [24 - 27].

Oils from *Ocimum* species which displayed the highest anticandida activity have been previously reported to be very complex containing varied chemical compounds ranging from monoterpenoids; phenylpropane, sesquiterpenoids and their derivatives [20, 28, 29]. It is therefore difficult to predict the component responsible for the observed activity. The anticandida activity shown by oils from *O. basilicum* and *O. kilimandscharicum* merits further investigations in view of the fact that Candidiasis is among the important opportunistic infections in HIV and AIDs.

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