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# Antibacterial and Antifungal Activities of Essential Oils from Satureia biflora D. Don, Benth, Speng (Chepsagitiet). Lippia javanica Burm.F. (Labotuet) and Toddalia asiatica (L) Lam. Rutaceae (Chepindoruet)

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## I. Introduction

Plants have been used by human against bacterial and fungus there is and other related conditions *in vogue* –"time immemorial". Currently this is influx in nosocomial and opportunistic infections, therefore an urgent need for new antibiotics to tackle the epidermic. *Lippia javanica,* a Verbenaceae, are used to treat fresh wounds. The same species has been used as a mosquito repellent by populations in East Africa up to South Africa for a long time (Lukwa, 1996). Previous studies in Kenya have shown that oils from *L. javanica* have very strong and lasting repellent activity against Bruchids (Tarus, 2006). Other studies showed that extracts of *L. javanica* were not active against *E. coli* and other bacteria (McGraw *et al.*, 2000).Long before mankind discovered the existence of microbes, the idea that certain plants possess healing potential and indeed, that they contained what we could currently identify as antimicrobial principles, was well-accepted (*Rosa damscena* mill. L. *Camiphora* spp). The plant species used in the study are those that are used by the Ogiek community of Kenya who are forest dwellers as hunters and forest product users.

The three plants that are traditionally used by the community and were analysed are: 1. *Satureia biflora* D. Don. Benth. Lamiaceae. (Labiatae) (Chepsagitiet) (Voucher No. AO/08/2/12/2006) Plant description; an erect woody herb to 50 cm, hairless to hairy with elliptic to circular entire leaves 12 (-20) x 8 mm; inflorescence bracts leaf like flowers 2–20 in dense auxiliary clusters, pink, 8 mm long; sepal teeth all equal in lowland individuals but flower up to 2 x as long upper ones in alpine forms. Common in wastelands and roadsides.

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*Satureia biflora* (See arrow) Plate left, regenerating plant and right full grown plant Mariashoni (Dec, 2006).

*Usage and Dosage of the Medicine*

The preparations from the plant are used in the following manner: About 200 gm of the roots crushed and boiled in one litre of water, decanted and 200 ml of decoction drunk daily for 4 days against stomachache, gastroenteritis and pneumonia.

a) *Lippia javanicca* (Burm.F.) Spreng. Verbenaceae. (Mwokyot) (Voucher No. AO/015/NMK/06/12/2007)

i. *Plant Description*

Shrub 0.5-3 m leaves opposite (rarely in threes), aromatic ovate or elliptic, base cuneate,apex acute margin crenate, 2-8 by 0.6-3 cm, sandpapery abovepubscent beneath. Flowers white or cream with yellow throat, in short-peduncles (rarely long-stalked) crowded spikes 0.5-1cm long; corolla tube about 2 mm long. Locally abundant in secondary bushland or grassland; found abundantly in Mau wastelands.

*Lippia javanicca* Plants, Marioshoni, Dec, 2007

ii. *Usage and Dosage of the medicine*

About 50 grams of fresh leaves a bunch carefully wrapped around fresh wound to enhance healing.

b) *Toddalia asiatica* (L) Lam. Rutaceae (Chepindoruet) (Voucher No. AO/017/NMK/04/07/2007)

i. *Plant Description*

Semi-climber, spiny, stem green when young turning yellowish-brown, leaves green, palmately compound; leaves found in clusters. Flowers greenish yellow orange fruits when ripe in axillary and terminal panicles; petals 2-3 mm long. Commonly distributed in forest margin or secondary regrowth and grassland thickets, 450 m coast and 1200-3000 m has profile growth at high altitudes.

*Toddalia asiatica*: left, Thicket, right, fruiting branch; Kaptuiget Forest (July, 2007)

ii. *Usage and Dosage of the Medicine*

The roots chewed to cure various body ailments ranging from malaria, stomachache and sore throat. A handful of the roots boiled in 1 litre of water and the decoction taken as tonic against harsh environmental conditions.

## II. Materials and Methods

The three plant species that were used are part of the flora of Mau Forest complex and were collected from Mariashoni area in the Rift Valley of Kenya, identified at the National Museums in Nairobi and assigned the following: *S. biflora* Voucher No.AO/08/ 12/12/2006, *L. javanica* Voucher No. AO/015/NMK/06/ 12/2007 and *T. asiatica* Voucher No. AO/017/NMK/04/ 07/2007. Several studies were carried out as given in the text:

### a) *Collection and Extraction*

Fresh leaves of *L javanica* whole plant of *S. biflora* 2 kg of each of the plants were separately steam distilled through hydrodistillation using Cleveland apparatus until no more of the oil was recoverable from the samples. The oily substances obtained from each were put into separate vials, tightly corked, sealed with parafilm and frozen at 4$^0$C in a dark refrigerator for future use. The same process was done fresh leaves and ripening fruits of *T. asiatica* separately. The tests were then individually carried out.

### b) *Screening for Antimicrobial Activity*

Antimicrobial efficacies were tested using the filter paper disc diffusion method (Elgayyar *et al.*, 2000). 0.1ml of the extracted oils was individually loaded onto 6mm diameter paper disc. The Muller-Hinton and Potato Dextrose Agar (PDA) were used in the culture of bacteria and fungi, respectively. Each medium was prepared by weighing the quantities recommended by the manufacturer and dissolving in recommended quantities of distilled water. The media was then sterilized using an autoclave set at 121$^o$C for 15 min and poured onto sterile Petri dishes then allowed to cool on a clean bench. Each plate was seeded with 0.1 ml of bacterial and yeast culture directly from the 24 hr broth culture diluted to match 0.5 and 1.0 McFarland's standard, respectively ($10^8$ Colony Forming Units (CFU)/ ml) and fungi diluted to match 1.0 McFarland's standard ($10^8$ spores/ml). The discs loaded with the extracts were then placed onto the seeded plates. The bacterial and fungal cultures were incubated at 37$^o$C for 24 to 48 hrs, while fungi were incubated at 25$^o$C for 5 days. After the incubation period the zones of inhibition were measured and recorded in mm as described by (Elgayyar *et al.*, 2000). Negative control plates had discs with sterile methanol. The plants which were found to posses bioactivities were further subjected to screening for purposes of obtaining their inhibition abilities at different concentrations in milligram. *Trichophyton mentagrophytes* and *Microsporum gypseum* were incubated at 25$^o$C for a period of five days to ascertain their antimicrobial sensitivity and resistance were confirmed by use of standard discs containing ampicillin (10µg), chloramphenicol (30µg), erythromycin (15µg), gentamycin (10µg), ciprofloxacin (10µg), tetracycline (30µg), amikacin (30µg) and an additional oxycacilin (1µg) for *S. aureus* (oxoid, London). The standards for fungi were discs containing fluconazole.

### c) *Disc Diffusion and MIC ratings of the Extracts*

Disc diffusion and MIC of the seam extracts were carried out. The negative controls of the disc diffusion testing was done by use of methanol did not show any inhibition, The negative control was the paper disc dipped in methanol and showed no growth, while positive control was done by use of Standard antibiotic discs (Oxoid). The average zone of inhibition was calculated for the 3 replicates. A clearing zone of 9 mm for Gram-positive and Gram-negative bacteria and 10 mm for fungi or greater was used as the criterion for designating significant antibacterial and antifungal activity (Faizi *et al.*, 2003). The *in vitro* MIC results were classified as per Pessini *et al*. (2003). The extracts that displayed MIC lower than 100 μg/ml, the antimicrobial activity was considered very high; from 100-500 μg/ml, high; 500-1000 μg/ml, moderate; 1000-4000 μg/ml, low and anything above this, the extracts were considered inactive for both bacteria and fungi solvent had the least activity from the root bark extracts.

*d) Gas Chromatography (GC)/mass Spectrophotometer (MS)*

GC and MS co-injection were performed on capillary gas chromatograph Hewlett Packard (HP) 5890 A series II, equipped with a split- less capillary injector system, cross-linked Hewlett Packard ultra 1-methyl silicone (50m length, 0.22 m internal diameter, 0.33μm carbowax film thickness) capillary column and a flame ionization detector coupled to Hewlett Packard 3396 series II integrator. Hydrogen gas was used as source of fuel, while nitrogen gas flowing at a speed of 0.8ml/min was used as carrier gas. GC-MS analysis was carried out on a Hewlett Packard 5790A series II Gas Chromatograph coupled to a VG Analytical Organic Mass Spectrophotometer (Micromass, United Kingdom) formally known as VG Biotech. The mass spectrophotometer (MS) was operated in the electron ionization (EI) mode at 70 electron volts (eV) and an emission current of 200 micro amperes (μA). The temperature of the source was held at 180 °C and a multiplier voltage of 300 volts. The pressure of the ion source and MS detector were held at 9.4 x$10^{-6}$ and 1.4 x $10^{-5}$ milli-bar, respectively. The MS had a scan cycle of 1.5 seconds (scan duration of 1s and inter-scan delay of 0.5 s). The mass and scan ranges were set at mass charge ration (m/z) of 1-1400 and 38-650 respectively. The instrument was calibrated using heptacosafluoro-tributyl amine, $[CF_3\text{-}(CF_2)_3]_3N$. The column used for GC-MS was the same as the one described for GC above except that the film thickness was 0.5μm. The temperature programme was similar to the ones earlier described for GC. Helium was used as a carrier gas. In both GC and GC-MS, High Performance Liquid Chromatography (HPLC) grade dichloromethane (DCM) was used as a dilution solvent.

## III. Results

The plants used posses' oils that have medicinal value. They were: *T. asiatica, S. biflora,* and *L. javanica.* Oils extracted from the leaves and fruits of *T. asiatica* showed high activities against both human pathogenic fungi bacteria. *S. biflora* whole plant distillate showed high activities against pathogens. This supports and justifies their continued use as sources of medicines by the community.

*Table 1 :* Screening for Activity

| Name of plants | *S.a.* | *P.a.* | *E.f.* | *K.p.* | *E.c.* | *S.t.* | *C.a.* | *C.b.* | *C.n.* | *T.m.* | *M.g.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *T. asiatica* | +++ | - | - | - | - | - | +++ | +++ | +++ | ++ | ++ |
| *L. javanica* | ++ | - | + | - | - | - | + | + | + | - | - |
| *S. biflora* | +++ | + | + | - | +++ | - | +++ | +++ | - | ++ | + |

Legend: *Staphylloccocus aureus (S.a.) Pseudomonas aeruginosa(P.a.) Klebsiella pneumoniae (K.p.) Escherichia coli (E.c.) Salmonella typhi (S.t.) Candida albicans (C.a.) Candida brusei C.b. Cryptococcus neoformas(C.n) Trichophyton mentagrophyte (T.m.) Microsporum gypseum(M.g.)>* 16 mm. high activity, 9-15 mm. moderate activity, 8-9 mm slight activity and <6mm no activity.

Extracts from *S. biflora*, *were* effective against most of the bacteria and *C. albicans*, *C. neoformas* and *T. mentagrophyte. S. biflora* were found to be slightly inhibiting, *S. aureus,* and *P. aeruginosa* and high inhibition of *E. coli.*. *S. biflora* was found to be effective on *K. pneumoniae, S. aureus P. aeruginosa* and *E. coli.*

*Table 2 :* MFC of fungi to various stem distillate of *T. asiatica* (μl/ml)

| Plant part used | Fungi/Conc. (μl/ml) | | | | |
|---|---|---|---|---|---|
| | *C.a* | *C.b* | *Cr.n* | *T.m* | *M.g* |
| Fruits | - | - | 4.1 | 1.2 | 1.0 |
| Leaves | - | - | - | 4.5. | - |

Distillate of the fruits of *T. asiatica* showed broad antifungal activities as compared to the leaves which had only an activity against *T. mentagrophyte* (MFC in μg/ml) of (*C.a*) *C. albicans* (clinical isolates), (*C.b*) *C. brusei*, (*Cr.n*) *C. neoformas,* (*T.m*) *T mentagrophytes* (clinical isolates*)* and (*M.g*) *M. gypseum* (clinical isolates) showed activities Essential oils *S. biflora* and *L. javannica* had similar activities (Table 3)

*Table 3 :* Zones of inhibition (mm) by *S. biflora* essential oils activity (100 μg/ml)

| Organism | Zones of inhibition (6 mm) |
|---|---|
| *S. aureus* (ORSA) | 19 |
| *S. aureus* | 10 |
| *K. pneumoniae* | 10 |
| *K. pneumoniae* (MDRS) | 11 |
| *E. coli* | 17 |
| Control | 6 |

*Table 4 :* Zones of inhibition (mm) by: *L. javanica* essential oils against *S. aureus* (100 µg/ml)

| Organism | Zones of inhibition(mm) |
|---|---|
| *S. aureus* (ORSA) | 13 |
| *S. aureus* | 13 |
| Control | 6 |

There were no activities against the organism by the extracts of *Satureia biflora* and *Toddalia asiatica* extracts (Table 4).

*Table 5 :* MIC/MBC of Various plants extracts in µg/ml or µl/ml

| Plant | Test Organism | MIC | MBC |
|---|---|---|---|
| *S. biflora* | *Pseudomonas spp* | 1000 | 2000 |
| *T. asiatica* | *E .coli* its strains | 20 | No reaction |
| | *S. aureus* and (ORSA) | 400 | No reaction |

There were also activities although not shown in Table 5 of the oil extracts (MFC in µg/ml (MFC) results in (µml) against: (*C.a*) *C. albicans* (clinical Isolates), (*C.b*) *C. brusei*, (*Cr.n*) *C. neoformas*, (*T.m*) *T. mentagrophytes* (clinical isolates) and (*M.g*) *M. gypseum* (clinical isolates).

*Table 6 :* Essential oils from *T. asiatica, S. biflora* and *L. javanica* (GC)

| *T. asiatica* | *S. biflora* | *L. javanica* |
|---|---|---|
| β-phellandrene | Mycene | Limonene |
| 1,6-Octadien-3-ol | Linalool | β-Caryrophylene |
| 3,7-dimethyl-trans-sabinenehydrate | β- Caryophylene | Mycerene |
| 2-Cyclohexene-1-ol | Sabinene | Camphor |
| Methyl-4-(1-methyl)-1-octene, | Trans- sabinene | |
| 6-methyl-2-Cyclohexen-1-ol, | Humulene | |
| Bicyclo[3.1.1]hept-2-ene-2-methanol | Dihydro tagotene | |
| (S)-(+)-6-methyl-1-octenol | 3,9-Epoxy- p-menthal | |
| Caryophylene oxide | Cyclohexyl-1-pentyne | |

The GC indicate the classes of compounds found (Table 6)

## IV. Discussion

*Satureia biflora* oils had varied activities in that it was effective against both Gram negative and Gram positive bacteria. Its oil possessed activities against *S. aureus, K. pneumoniae* and *E. coli* at 12 µl/ml and its strains. Such activities may be attributed to different chemical composition of the oil as compared to *L. javanica* and *T. asiatica.* The activities confirm the use of the leaves and roots by the Ogiek community to combat upper respiratory tract ailments like colds, pneumonia, coughs and stomachache. This could be attributed to the fact that the drugs are able to penetrate the cell wall of the organisms. The inability may be, due to the fact that fungi being plants, though from the lower classes may have similar compounds as the extracts.

The mode of antimicrobial action of the oil may be due to the inhibition of respiration and disrupting the permeability of the cell wall structures. The other speculation on the enhanced efficacy of the oil is due to differential permeability as a result of molecular actions which have been prompted by adhesive activities of the oil molecules (Lukwa, 1994). Ultimately, even some Gram negative bacteria which possess complex cell wall structures that more often resist foreign molecules penetration will succumb to the oil.

Members of the Lamiaceae have been used in Asian medicine and involve several species in the family. Besides steroidal compounds which are found in the family, essential oils are of universal occurrence and have been used in medication in Asia and elsewhere successfully (Rajan *et al.*, 2002). The presence of these oils, steroidal compounds confirm the ability of *S. biflora* to combat pneumonia and stomachache. Furthermore, the plant is also used in cooking and warm drinks by the local community of the Ogiek. It is also often boiled and the decoction taken as a hot cup due to its strong aroma from the Artimesinin, terpenes and other aromatics. It is believed that the plant's strong aroma is able to alleviate common cold. The essential oils are found in the leaves which are freshly harvested. Some of these include *O. basilcum* (L), *O. sanctum, M. piperita* all of which yield eugenol and caryophyllene (Mehrotra and Rastogi, 1995). The same inferences conform to the other medicinal uses of *Dracocephalum moldavica* whose volatile oils are used in culinary, astringent and tonic. There are many plants like *Ocimum basilcum, Rosmarinus officinalis* and *Mentha piperita* whose extracts are used in perfumery and in the treatments of various antibacterial ailments (Willis, 1995) and are known to contain monoterpenes (Viyotch *et al.*, 2006).

Traditionally, amongst the Ogiek the infusion of the plant is used to treat fresh wounds, colds and stomach complaints. The variable antimicrobial activity might be due to chemotypes (Vijoen *et al.*, 2005). The oils found in freshly harvested and steam distilled leaves yielded limonene, camphor, ß- caryophyllene, and myrcene which give the same results as the leaf when tested against the test organisms. It has been observed that geographical location and time of plant collection has a lot of influence on the type and quantities of the oil collected through stream distillation of the plant leaves (Samie *et al.*, 2005).

From the same genus in the same Verbenaceae, it has been reported that essential oil and selected terpenoid components of *Lippia scaberrima* posses fungastatic activities against *Bartyosphaeria parva* and Collectotricum gloespoiodes that are known to cause Mango spoilage (Sadeghi and Azish, 2013). However, during this study methanol extract and essential oils of this plant inhibited *S. aureus* at MIC of 140 µl/ml and 11µl/ml. traditionally, this plant is used by the community as mosquito repellant and fresh leaves rapped onto fresh wounds to assist in healing. Its action might be directed at the fresh wounds and thus combats the organism directly by preventing it from penetrating the host. Furthermore, sequential extractions of various portions of the plant had significant activities against selected human pathogenic fungi. When various extracts were set against the organisms with fluconazole as control, the ANOVA for all the extracts from both parts of the plant showed df=2 with leading results in the petroleum ether followed by methanol. Although ethyl acetate was not active against most of the test organisms, it was significantly active against *Microsporum gypseum* with a test of df=2 when compared to other extracts. Outstanding activities across the board were also reached when dealing with the extracts from the root bark. The phenomena are that most of the metabolites are transported from the sites of synthesis and ultimately stored in the roots in majority of the higher plants. This is perhaps why the activity is higher in root extracts.

Rutaceae are known to possess strong antimicrobial agents which have been tested (Quiroga, 2004). The genera *Pseudomonas, Enteroccocus* and *Salmonella* were all resistant to various levels of treatments of the root bark extracts *T. asiatica* root bark extracts of the plant had significant activity, $P< 0.05$ with a pooled StDev=0.725 and all the tested strains of *S. aureus* and *E. coli* with growth being inhibited significantly. Its inhibition increased proportionally with the increase in the drug concentration without any or much variation. Sensitivity of the organism to the plant extract justifies the fact that the community uses the plant against respiratory tract ailments such as cough, cold, stomachache and other chest related conditions. The plant was also mentioned to be used as tonic, possibly as an immune booster whereby, the roots are boiled decanted and the decoction taken by the Ogiek. However, members of the Studies of the plant extracts indicate that it can be used in abortion in India due to the presence of berberine, toddaline and toddanaline as selected chemical constituents (Mehrotra, 1993). The families are also known to contain triterpenoids as limonoids which are important in pharmaceutical chemistry due to their use in traditional medicine (Rajan *et al.,* 2002). The essential oils, like limnoides, were contained both in the leaves and in the fruits but with higher concentration in the fruits than in the leaves (Table: 6).

## V. Conclusions

This study set out with the objectives to: document the ethnomedicine of the Ogiek people, document the plant medicines used by the Ogiek. The community has empirical and vast knowledge in the use of the forests and forest products. Observations revealed that there are wanton and excessive environmental degradations which have led to drastic reduction in the biodiversity in the study area. Hence ascertain plant species are rare to find with the Mau forest complex and elsewhere. Such species are thus endangered.

## VI. Recommendations

The study has demonstrated that further studies can be carried concerning the medicinal plants used by Ogiek Community and the following recommendations came up. Documentation using other methods, other than ethnomedical approach should be employed to close the data gaps. Both *in situ* and *ex situ* conservation efforts are *introduced* in the area to counter the encroachment of fragile habitats by the fast increasing population. An exclusive reserve is created since it will allow natural regeneration of the ecosystem to take place thus the restoration of degraded areas. Failure to do so will lead to drastic reduction in the floral diversity which would otherwise be used in future studies. A comprehensive natural resource inventory, type on the plant species used by the people and the methods employed in their exploitation be made so that a holistic approach be employed for sustenance of the diversity.The plants which did not show any bio-activities against the test pathogens be further studied to ascertain their efficacies and the plants that showed bioactivities be studied *in vivo* using small laboratory animals to validate their efficacies. An interdisciplinary approach is employed in studying the community's ethnomedicine involving the pharmaceutical industry for purposes of new drug discovery and development. There should be a proper epidemiological record keeping so as establish the efficacy of the drugs used in traditional systems on endemic diseases and the newly emerging and reemerging ones.

The toxicity of plant medicines to human and livestock be carried out by laboratory cytological evaluations using small animal models. More organisms should be covered in future studies so as to leave no doubt on the validation of the efficacy of such plants. For purposes of scientific progress, phytochemists should be carried out comprehensive elucidation of the active principles. Thereafter, pharmacists may work on the several combinations and cocktails which could be effective and used in the manufacture of new drugs to combat the maladies currently afflicting humankind. Further assay these sequential extracts against some common human pathogenic fungi and profile the classes found in the biologically active plants. Several plants that were found to contain essential oils of reasonable efficacies against known human and animal pathogens, further researches be carried on their possible incorporation into toiletries, and other oral medicines for purposes of pharmaceutical uses. Post-harvest loses of perishable commodities like fruits are common in horticultural produce; it would be advisable to explore the possibility of incorporating extracts from botanicals in their spoilage reduction strategies as opposed synthetic pesticides which are often environmentally unfriendly.

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