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Bacteriostatic and Bactericidal Activities of *Aspilia mossambicensis*, *Ocimum gratissimum* and *Toddalia asiatica* Extracts on Selected Pathogenic Bacteria

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ABSTRACT

Aspilia mossambicensis (Oliv.) Wild, *Ocimum gratissimum* (L.) Labiatae. and *Toddalia asiatica* (L.) Lam. (Rutaceae) were investigated with an overall aim of identifying and evaluating the bioactive antibacterial agents. Hexane, ethyl acetate and methanol realized yields of the range 0.5% *Ocimum gratissimum* stem bark ethyl acetate extract to 2.7% for *Toddalia asiatica* root bark methanol extract. The extracts were assayed for *in vitro* activity against gram positive; Methicillin Resistant *Staphylococcus aureus* and the gram negative; *Pseudomonas aeruginosa,* using disc diffusion method. The highest activity was with *T. asiatica* stem bark methanol extract (15 mm diameter zone of inhibition) against Methicillin Resistant *Staphylococcus aureus*. Bioactivity-guided fractionation of the *T. asiatica* (stem bark methanol extract) yielded up to four isolates labeled F1-C, F2-C, F3-C and F4-C. Preliminary phytochemical analysis gave positive results for mostly alkaloids, flavonoids, steroids and amines. By bioautographic selection the isolates showed antibacterial activity against Methicillin Resistant *Staphylococcus aureus* (0.3125 mg mL^{-1}) which compared well to that of the standard drug gentamycin (0.5 mg mL^{-1}).These results validate the ethno botanical use of *Toddalia asiatica,* a Kenyan medicinal plants for conditions that may be of bacterial etiology.

Key words: Antibacterial activity, *Aspilia mossambicensis, Ocimum gratissimum, Toddalia asiatica,* bioautography, leave, stem-bark, root-bark extracts, efficacy

INTRODUCTION

The Acquired Immuno-Deficiency Syndrome (AIDS) caused by the Human Immunodeficiency Virus (HIV) is still an important public health problem. Among the various opportunistic infections, bacterial infections account for up to 70% of HIV/AIDS defining illness. These include diseases like bacterial pneumonia which is higher in the HIV infected patients than in the general population (Shailaja *et al.*, 2004). Antibacterial agents utilized in management of these opportunistic infections are either bacteriostatic or bactericidal. Therapeutic agents widely used here include the penicillins or glycopeptide antibiotics for example vancomycin and chloroamphenical. Nevertheless the draw back in their application is the emerging resistant strains and associated toxicity (Jabra-Rizk, 2006).

Bacterial pneumonia and other severe respiratory tract infections caused by one or several bacteria such as *Streptococcus pneumoniae, Haemophilus influenza, Escherichia coli, Salmonella* spp., *Shigella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common pathogens associated with infectious diseases. However, the role of *Legionella pneumoniae, Mycoplasma pneumoniae* and *Chlamydia pneumoniae* is still not clear in HIV/AIDS associated infections (Sharifi-Mood *et al.*, 2006).

Staphylococcus aureus, a gram positive bacterium is capable of developing resistance to commonly used antibiotics. For instance the Methicillin Resistant *Staphylococcus aureus* (M.R.S.a) is an emerging bacterial pathogen associated with significant morbidity and mortality. Hospital infections and community acquired M.R.S.a infection are on the increase causing more serious infections, such as abscesses, pneumonia and bone or bloodstream infections (Blasi, 2004). Similarly the *Pseudomonas aeruginosa* is also a significant opportunistic human pathogen and the most common gram-negative bacterium associated with nosocomial infections that are equally fetal (Cowan, 1999). This therefore makes antibacterial chemotherapeutic research to address the two a priority.

Antimicrobial agents used in the treatment of infectious diseases can be classified into two groups; antibiotics which are natural substances produced by certain groups of microorganisms and chemotherapeutic agents which are chemically synthesized (Todar, 2002). The most important property of an antibacterial agent, is its selective toxicity, that's, it kills bacterial pathogens but has little or no toxic effect on the host.

The selection of an antibacterial to treat an infection depends on sensitivity of the causative agent of the infection (Campo *et al.*, 2007). Nevertheless Microorganisms have developed resistant to many antibiotics thereby creating immense clinical problems in the treatment of infectious diseases. This resistance has increased due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (Chanda *et al.*, 2011).

Major groups of antimicrobial compounds can be isolated from plants due to the fact that plants have an almost limitless ability to synthesize aromatic substances, most of which being phenols or their oxygen-substituted derivatives (Kristjansson *et al.*, 1994). Phenolic compounds such as flavonoids, phenolic acids and tannins are considered as the major contributors to the antioxidant capacity of plants (Coulidiati *et al.*, 2011). There are numerous plant derived drugs; the isoquinoline alkaloid emetine obtained from the underground part of *Cephaelis ipecacuanha* and related species. It has been used for many years as an amoebicidal drug and for treatment of abscesses (Iwu *et al.*, 1999).

In spite of the great advances observed in modern medicine therefore, plants still make an important contribution to health care. This is due in part to the recognition of the value of traditional medical systems and the identification of medicinal plants from indigenous pharmacopoeias which have significant healing power (Elvin-Lewis, 2001). Medicinal plants are distributed worldwide but they are most abundant in tropical countries and a wide range of plants in several families of the plant kingdom have been used for centuries in folk medicine (Calixto, 2000). Herbal medicines has become an ideal remedy for treatment of the diseases due to lesser amount of side effects, better compatibility and only accessible treatment for some diseases (Karim *et al.*, 2011).

Toddalia asiatica root extracts has been used medicinally as a traditional remedy for cough, indigestion, nasal and bronchial pains (Kokwaro, 1993). In the continuous search for phytochemicals active against pathogenic infections, *T. asiatica* has received considerable attention (Oketch-Rabah *et al.*, 2000). However, few studies have reported the antimicrobial activity of

T. asiatica. Across Africa, the medicinal plant *A. mossambicensis* is used in herbal medicine for various infections of bacterial origin such as gonorrhea, stomach trouble, cystitis and corneal opacity (Adeniyi and Odufowora, 2000). The plant has not exhaustively been screened for antimicrobial activity. *Ocimum gratissimum* leaves have been reported to show inhibitory effects on selected bacteria with a Minimum Inhibitory Concentration (MIC) range from 0.1% for *S. aureus* to 0.01% for *E. coli* and *S. typhimurium* and 0.001% for *S. typhi* (Adebolu and Oladimeji, 2005). These studies show the plant to be having compounds with promising antimicrobial activity, thus a basis for consideration of substantive research to isolate the bioactive compounds.

From literature search by online sources, databases and search engines, a number of plants used in traditional health systems in Kenya were identified for study, of which basing on ethno-pharmacotherapeutical applications and availability; *Aspilia mossambicensis* (Oliv.) Wild, *Ocimum gratissimum* (L.) Labiatae. and *Toddalia asiatica* (L.) Lam. (Rutaceae) were evaluated to ascertain their antibacterial potentials in management of opportunistic infection of bacterial origin.

MATERIALS AND METHODS

The chemicals; n-hexane, dichloromethane, ethyl acetate, chloroform (GPR), methanol, acetone, ammonia were sourced from Kobian Ltd, Nairobi, Kenya. Chloroform (Analar), methanol (Analar), vanillin, sulphuric acid, potassium hydroxide, anisaldehyde, acetic acid, acetic anhydride, antimony chloride, ninihydrine, bismuth subnitrite, potassium iodide, sodium nitrate all were obtained from Sigma chemical company, St. Louis, USA. Silica gel (70-230 mesh) (Macherey-Nagel GmbH and Co.).

Plant material were collected during the short rains period in the month of April 2008 from natural habitats; *Aspilia mossambicensis* (1°06'S 36°26'E), *Ocimum gratissimum* (1°06'S 36°27'E) and *Toddalia asiatica* (1°19'S 35°42'E) in the Mai Mahiu rain forest, Rift Valley, Kenya. Mr. G.M. Mungai from the East African Herbarium, Nairobi, Kenya confirmed the identity and voucher specimens (CM 144, 145 and 146) deposited in the East African Herbarium-National Museums of Kenya, Nairobi (EAH-NMK). On collection, the plant material was separated into parts as the root bark, stem bark and leaves. These were separately dried at room temperature and ground into fine powder using a Willy mill.

Initial sample processing: Extractions were carried out as per Harborne (1998) methods and similar to Olila *et al.* (2001) with little modification. Briefly 200 g plant powder of each part was extracted sequentially using n-hexane, the extraction was repeated with ethyl acetate and methanol. The extracts were then stored in sterile air-tight containers at 4°C awaiting evaluation of biological activity.

Bioassays: A representative of the gram positive bacteria Methicillin Resistant *Staphylococcus aureus* (clinical isolate) and gram negative *Pseudomonas aeruginosa* (ATCC 27853) were selected for antibacterial activity evaluation.

From the stocked isolates, the bacterial strains were sub-cultured onto Müller Hinton agar No. CM0337 (Oxoid Ltd, Basingstoke, Hampshire, England) and incubated at 37°C for 24 h to obtain freshly growing strains.

In the assays, negative controls were the solvents used in extraction (n-hexane, ethyl acetate and methanol) and for dissolution (Dimethyl sulfoxide). The standard drug was Gentamycin (5 μg mg^{-1} disc) sourced from Sigma-Aldrich Canada Ltd, Ontario Canada. A 0.5 McFarland

turbidity standard (10^8 colony forming units cfu mL^{-1}) was prepared in sterile distilled water and inoculated uniformly onto the Müller Hinton agar. A disc of 6mm diameter was impregnated with 10 µL of the test extract (100 mg mL^{-1}) and aseptically placed on the inoculated plates before incubating at 37°C for 24 h. The zones of inhibition were then measured and compared with the standard drug. All tests were performed in triplicate and antibacterial activity expressed as the mean of inhibition zones diameters (mm) produced by the plant extracts.

This was represented with an antimicrobial index (Rajakaruna *et al.*, 2002) ranging from 0 to 5+.

Minimum inhibitory concentrations: To determine the Minimum Inhibitory Concentrations (MIC) of the bioactive extracts, briefly 6 mm diameter paper discs were impregnated with 10 µL of the reconstituted samples at concentrations ranging from 0.156 to 10 mg mL^{-1}. The discs were then transferred aseptically onto Muller Hinton agar plates inoculated with the test bacterial strain. The MIC was regarded as the lowest concentration that produced a visible zone of inhibition.

Isolation of active chemical principles: Bioassay guided fractionation was adopted based on Minimum Inhibitory Concentrations (MIC). *Toddalia asiatica* stem bark methanol extract which exhibited the highest activity from preliminary bioassays was selected for isolation and screening of active chemical principles.

Crude methanol extract (5 g) was loaded onto the packed column and gradient elution carried out across a polarity gradient from n-hexane-chloroform-ethyl acetate and finally methanol. The eluants were collected manually, attaining 41 fractions that were analyzed by TLC before pooling according to content. Fractions of higher yields were further purified with preparative TLC (PTLC) and crystallized. These were washed with methanol and allowed to re-crystallize and stored in sealed vials awaiting confirmation of bactericidal activity.

Bioautographical selection: To confirm the bacteriostatic and bactericidal activity for the purified column chromatography fractions, bioautography was carried out. Aliquots (10 µL) of the purified column isolates coded F1-C, F2-C, F3-C and F4-C were spotted on silica gel Kieselgel DGF254 TLC plates and eluted with Chloroform: Methanol (98:2) with five drops of glacial acetic acid. After evaporation of the organic solvents the TLC plate was placed on sterile Petri dish (10×30 cm) and flooded with 100 mL of Müller Hinton (No. CM0337 OXOID LTD) (38 g) agar seeded in 1% aqueous Methicillin Resistant *Staphylococcus aureus* (clinical isolate), suspension (10^8 cells mL^{-1}). The TLC plate was incubated at 30°C for 48 h and then flooded with 50 mL of microbiological agar (10 g) containing 0.05% of MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (M-2128 lot. 40H5021 Sigma). Cell growth inhibition indicating bacteriostatic and bactericidal compounds was observed as yellowish TLC spots on a purple background, allowing the chromatographic retention to be observed. Measurement was done for the spots and R_f values recorded against a solvent front of 13 cm. The same was repeated with a separate TLC plate for gram negative bacteria while using agar seeded in 1% aqueous *Pseudomonas aeruginosa* (ATCC 27853) was also assayed.

MIC of bioautography selected spots: The bioautographically selected bactericidal active pure fractions; F2-C and F4-C were subjected to Preparative TLC, localized in regard to chromatographic R_f and recovered with Dimethyl Sulphoxide (DMSO). The recovered solution was kept in vial for

48 h to allow the solvent to evaporate. The residue was then suspended (10 mg mL^{-1}) in 1000 μL H_2O: DMSO (2: 8) prior to successive dilution on a 96-well microtiter plate. Finally an aliquot (100 μL) of culture medium (Mueller Hinton, 8 g) containing 0.5 McFarland of an aqueous microorganism suspension was added to a set of wells for each, to produce final concentrations of bioactive compound fractions of 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156 mg mL^{-1}. Controls were prepared by substituting test fraction with aqueous-DMSO plus inoculum. The plates were incubated at 30°C for 48 h. Aliquots (50 μL) of aqueous MMT (0.05%) were added to the wells and reduction of the tetrazolium salt (yellow) to formazan (purple) by living cells observed within 30 min. MIC was regarded as the lowest well concentration at which reduction of the tetrazolium salt occurred.

Phytochemical evaluation: Preliminary phytochemical analysis was done according to standard methods (Harborne, 1998). The plant extracts were subjected to qualitative chemical screening for identification of the various classes of active chemical constituents. TLC plates were developed with Chloroform: Methanol (98:2) with five drops of glacial acetic acid, before spraying with TLC visualization reagents.

Experimental design and statistical data analysis: Experimental design for bioassays involved varying treatments as follows:

- **Test group:** Consisted of the organisms (selected ATCC strains and clinical isolates) plus the extracts at different concentrations. This determined whether the extracts are effective antibacterial agents
- **Positive control:** This consisted of organisms plus a known antibiotic (gentamycin). This ensured that the utilized organisms were susceptible to therapeutics
- **Negative controls:** Organisms plus the pure solvents, this was necessary to confirm that the solvents used for extraction and dissolution had no inhibitory action on their own

All determinations involving quantitative data were carried out in triplicate.

The results were then subjected to statistical analysis for qualification of the variability. Statistical Package for Social Scientist (SPSS version 12.0) was utilized which enabled analysis of variance by one way-ANOVA to establish the significance of variability between and within different groups (plant parts, solvents, concentrations, or bacterial strain) with bioactivity as the dependent variable to establish the significance at 0.05 level of confidence.

RESULTS

Initial sample processing: For each extraction of 200 g powdered plant material, percentage yields were determined; *T. asiatica* root bark had the highest yield of 2.7% extracting with hexane while stem bark of *O. gratissimum* on extraction with hexane had the lowest (0.45%) (Table 1). Comparison of the average percentage yields for the specific solvents extractions of plant parts, it was revealed; Methanol as extraction solvent had the highest average yield of 1.62% followed by hexane extract with 1.52%. Lowest average yields were encountered with ethyl acetate where the average percentage yield was 1.18%.

Bioassays: Antibacterial activity of plant extracts is expressed in Table 2 represented in terms od 'Antimicrobial Index' (Rajakaruna *et al.*, 2002). The highest activity was realized with *T. asiatica*

Table 1: Percentage yields

Solvent	Plant	Plant part	Extract weight (g)	Percentage yield	Average percentage yield
Hexane	*A. mossambicensis*	Leaves	3.6	1.80	1.52
		Stem bark	3.2	1.60	
		Root	2.4	1.20	
	O. gratissimum	Leaves	2.3	1.15	
		Stem bark	0.9	0.45	
		Root	1.2	0.60	
	T. asiatica	Leaves	4.6	2.30	
		Stem bark	3.7	1.85	
		Root	5.6	2.70	
Ethyl acetate	*A. mossambicensis*	Leaves	3.8	1.90	1.18
		Stem bark	2.8	1.40	
		Root	2.5	1.25	
	O. gratissimum	Leaves	2.9	1.45	
		Stem bark	1.0	0.50	
		Root	1.5	0.75	
	T. asiatica	Leaves	1.2	0.60	
		Stem bark	2.7	1.35	
		Root	2.8	1.40	
Methanol	*A. Mossambicensis*	Leaves	4.0	2.00	1.62
		Stem bark	3.5	1.75	
		Root	3.5	1.75	
	O. Gratissimum	Leaves	5.5	2.75	
		Stem bark	1.3	0.65	
		Root	2.1	1.05	
	T. asiatica	Leaves	3.5	1.75	
		Stem bark	3.6	1.80	
		Root	4.1	2.05	

Percentage yield = extracted weight/200×100

stem bark methanol extract against M.R.*S. aureus,* expressed in terms of antimicrobial index to be 3+ (15 mm). On the other hand the least activity or no activity was evidenced in several extracts with no zone of inhibition being realized hence antimicrobial index of 0+ (6 mm).

Minimum inhibitory concentrations: Only extracts that showed significant activity were further subjected to bioassay to determine the minimum inhibitory concentration which was regarded as the lowest concentration that produced a visible zone of inhibition. *T. asiatica* stem bark methanol extract against M.R.*S. aureus* was confirmed to exhibit the highest antibacterial activity from its lower MIC value of 0.3125 mg mL^{-1} (Table 3).

Upon chromatographic isolation followed by bioautography of isolated fractions, the MIC of bioautography selected spots Rf 0.57 and 0.77 of isolates F2-C and F4-C, respectively as determined by the reduction of the tetrazolium salt to formazan by living cells was 0.625 mg mL^{-1} and 0.312 mg mL^{-1} against *P. aeruginosa* and M.R.*S.aures* respectively (Table 4). This was a further prove of the above results of bactericidal properties.

Phytochemical evaluation: TLC spray reagents used for preliminary screening of phytocompounds showed that alkaloids and flavonoids were present in all the studied plants,

Table 2: Antibacterial activity of plants' crude extracts

			Antibacterial activity index against	
Solvent	Plant	Plant part	M.R.*S. aureus*	*P. aeruginosa*
DMSO pure solvent - Control			0+	0+
Gentamycin standard drug (0.5 mg mL^{-1} disc)			4+	4+
Hexane	*A. mossambicensis*	Leaves	0+	0+
		Stem bark	2+	2+
		Root	0+	2+
	O. gratissimum	Leaves	0+	0+
		Stem bark	2+	0+
		Root	1+	2+
	T. asiatica	Leaves	0+	0+
		Stem bark	2+	0+
		Root	0+	0+
Ethyl acetate	*A. mossambicensis*	Leaves	0+	0+
		Stem bark	1+	0+
		Root	0+	0+
	O. gratissimum	Leaves	0+	0+
		Stem bark	2+	0+
		Root	0+	0+
	T. asiatica	Leaves	0+	0+
		Stem bark	0+	0+
		Root	0+	0+
Methanol	*A. mossambicensis*	Leaves	2+	2+
		Stem bark	2+	1+
		Root	1+	0+
	O. gratissimum	Leaves	0+	0+
		Stem bark	2+	1+
		Root	2+	2+
	T. asiatica	Leaves	0+	0+
		Stem bark	3+	2+
		Root	2+	0+

0: Zone of inhibition <8 mm (no inhibition), 1+: 8-9 mm, 2+: 10-14 mm, 3+: 15-19 mm, 4+: 20-24 mm and 5+: 25 mm and above

alkaloids occurred in all parts but for flavonoids there was no detection in the *A. mossambicensis* stem bark. Terpenoids, anthraquinones, phenolics, steroids, amines and cardiac glycosides were shown to be present in at least one part of each plant (Table 5).

DISCUSSION

Initial processing of samples: Comparing the quantity of sequential extraction using the various solvents it was noted that the highest percentage yield was with the methanol and the lowest ethyl acetate. The plant materials studied therefore could be endowed with components that are mainly polar and non polar other than components of intermediate polarity as evidenced by the decreasing order of extract quantity per solvent (methanol>hexane>ethyl acetate). This difference could be ascribed to the fact that secondary metabolites are distributed differently from plant to plant.

Table 3: MICs of plant's crude extracts

Test organism	Solvent	Plant extract	MIC (mg mL^{-1})
	Gentamycin	Standard drug	*0.50
M.R. *S. aureus*	Hexane	*A. mossambicensis* stem bark	1.25
		O. gratissimum stem bark	2.50
	Ethyl acetate	*O. gratissimum* stem bark	1.25
	Methanol	*T. asiatica* stem bark	0.312
		T. asiatica root bark	1.25
P. aeruginosa	Hexane	*A. mossambicensis* root bark	1.25
		O. gratissimum root bark	1.25
	Methanol	*A. mossambicensis* leaves	2.50
		T. asiatica stem bark	0.625

*MIC for the standard drugs gentamicin was adapted from NCCL's Interpretative standards for dilution and disc diffusion susceptibility testing tables

Table 4: MIC of bioautographic selected spots

Test organism	Column isolate	Bioactive spot Rf value	MIC (mg mL^{-1})
	Gentamycin	Standard drug	0.50
M. R. *S. aureus*	F4-C	0.77	0.312
P. aeruginosa	F2-C	0.57	0.625

Bioactive spot Rf value is expression of the distance moved by isolate upon spotting on a TLC plate and developing expressed as a fraction of the solvent front

Table 5: Phytochemical profile of plant extracts

	Phytocompounds					
Plant/Part	Alkaloids	Flavonoids	Terpenoids	Steroids	Amines	Cardiac glycosides
A. mosambicensis						
Leaves	++	+	-	-	+	-
Stem bark	+	-	-	-	-	+
Root	++	+	-	+	-	+
O. gratissimum						
Leaves	++	++	++	++	++	+
Stem bark	+	++	-	+	+	-
Root	++	++	++	++	++	+
T. asiatica						
Leaves	++	+	+	+	+	+
Stem bark	++	+	+	+	+	-
Root	++	+	-	++	++	+

++: Abundant, + Trace, -: Not detected

Bacteriostatic and bactericidal activity: The results obtained in this study indicate a considerable difference in antibacterial activity across extracts obtained with different solvents (n-hexane, ethyl acetate and methanol) with methanol extracts being more active than the other extracts. The bactericidal activity was more pronounced against the gram-positive bacteria than gram-negative bacteria, with the plant extract exhibiting even higher activity (MIC = 0.312 mg mL^{-1}) than gentamycin (MIC = 0.5 mg mL^{-1}) the standard drug used.

The bacteriostatic and bactericidal activity could be ascribed to the presence of flavonoids and coumarin-related compounds. A probable degree of lipophilicity might be responsible for the extracts

being higher in activity than gentamycin. Lipophilicity toxicity is due to the interactions with the membrane constituents and their arrangement (Tomas-Barberan *et al.*, 1990). The reason for the different activity between gram-positive and gram-negative bacteria could be accounted for by the morphological differences between these micro-organisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic. The gram-positive bacteria become therefore more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Nostro *et al.*, 2000). Generally therefore Gram-negative bacteria are more resistant than Gram-positive bacteria because of the complexity of the cell wall of Gram negative bacteria (Vaghasiya and Chanda, 2010).

In spite of this permeability difference, the methanol extracts also show inhibition of *P. aeruginosa* a gram negative bacteria hence they may be considered to have a broader spectrum of inhibitory activity than the other extracts. Intensive studies should be carried out to explain this. Both crude and purified extracts of *T. asiatica* had activity with MIC values that are comparable. However, for certain isolates (F1-C and F3-C) bioactivity was lost on purification which could be attributed to synergistic effects that are lost on separation and purification. Absence of *in-vitro* biological activity does not warranty disapprove of the ethnopharmacotherapeutic utilizations as this may suggest extracts acting in an indirect way where active ingredient exist as a precursor requiring activation *in-vivo*. It is also believed that crude extract from plants are more effective than isolated components due to their synergistic effect (Jana and Shekhawat, 2010). From this investigation it is also revealed that activity varies greatly from plant to plant in regard to part where by higher activity may be witnessed in the leaves of one plant while for the other activity is more pronounced in the root bark.

Bioautography results; isolate F4-C shows well-defined inhibition bands in assays with the gram positive bacteria Methicillin resistant *Staphylococcus aureus*. This band is in correspondence with coumarin and flavonoids bands, whose presence is demonstrated by TLC preliminary phytochemical screening and a confirmation from previous studies (Tsai *et al.*, 1998; Sharma *et al.*, 1981). Inhibition bands shown by F2-C in assays with gram negative *Pseudomonas aeruginosa* corresponds to terpenoids and amines bands. Other authors have shown the presence of terpenes and their bacteriostatic properties (Bourrel *et al.*, 1993). However, it is difficult to compare the data because of some variables like the choice of extraction method and antimicrobial tests. From broth dilution method used to determine the MIC of bioautography selected spots the quantitative data obtained can be sufficient for consequent studies aimed at identifying the single active principle(s) as there is close correlation to the positive control used.

Phytochemical evaluation: Phytochemical screening of the test plant extract using TLC visualization reagents showed that the plant extracts were positive for organic compounds mainly flavonoids, phenolics, terpenoids, steroids and amines while negative for alkaloids and cardiac glycosides. The presence of flavonoids, phenolic or terpenoids in plants has been shown to be responsible for antimicrobial activity in plants (Nostro *et al.*, 2000). Their role is to protect plants against microbial or insect damage (Cowan, 1999). Since medicinal plants contain pharmacologically active substances with antimycobacterial, antibacterial and antifungal properties (Mariita *et al.*, 2011), the antibacterial activity of the plants tested could therefore be attributed to the presence of these compounds.

Biological activity statistical data management: Analysis of variance using One Way- ANOVA to establish whether variation in biological activity was significant illustrated quite

significant variation in antibacterial activity; from plant to plant, among different plant parts, for extracts with different concentrations and amongst different test organisms was encountered with a calculated value of 0.00 and F-statistics values of 19.128, 19.566, 17.552 and 9.210, respectively. Nevertheless there was minimal significance in the variation of bioactivity of extracts of different solvents with a calculated value of 0.190 and an F value of 1.662. The statistical analysis was carried out at 95% level of confidence.

CONCLUSION

The plant extracts of *T. asiatica, O. gratissimum* and *A. mossambicensis* contain several antibacterial compounds that are identifiable. The highest antimicrobial activity is encountered in the stem bark of *Toddalia asiatica.* Bacteriostatic and bactericidal activity was more pronounced against Gram-positive bacteria than against Gram-negative bacteria. This difference in activity is ascribed to the morphological differences in terms of outer phospholipidic membrane (gram negative). Flavonoids and coumarins related compounds and the lipophilicity toxicity arising due to interactions with the membrane constituents and their arrangement also account for the activity exhibited. Loss of activity upon purification is attributed to synergistic effects that are lost on separation and purification. Absence of *in-vitro* activity does not warranty disapprove of the ethno botanical utilizations as this may suggest extracts acting in an indirect way where active Ingredients exist as a precursor requiring activation *in-vivo*. Elementary analysis of chemical principles reveals a coumarin-like phytochemical properties, indicating lipophilicity responsible for the toxicity and hence the bioactivity exhibited.

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