COMPOSITION OF NON VOLATILE OILS AND ANTIMICROBIAL ACTIVITIES OF EXTRACTS FROM *MONANTHOTAXIS DISCOLOR*, AND AN UNDESCRIBED *UVARIONDEDRON* SPECIES

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ABSTRACT

The chemical compositions of non volatile oil extracts from two Annoneceous plant species Monanthotaxis discolor *and an undescribed* Uvariodendron *species which are endemic to Eastern Arc Mountains, Tanzania were determined by GC-MS. The biological activities of extracts and fractionated samples were also determined. Both methanol and dichloromethane extracts of the root bark of M. discolor showed mild antimicrobial activity and had positive brine shrimp test (BST). The BST test for dichloromethane extract of M. discolor root bark were LC_{50} 41.794 μg/ml and that of methanol extract showed LC_{50} 13.560 μg/ml. The petroleum ether and dichloromethane extracts of root bark of Uvariodendron species showed cytotoxicity of LC_{50} 33.06 μg/ml and LC_{50} 35.35 μg/ml, respectively. Twelve major constituents were identified from the dichloromethane extract of M. discolor root bark of which the following compounds were in high composition; α-cadinol (42.85%), (-)-alloaromadendrene (11.7%) aristolone (10.57 %), γ-cadinene (8.72%), δ-cadinene (3.44%) and cubenene (2.28%). The Second fraction of the third repeated column chromatography from the VLC fraction of dichloromethane extract and the first fraction of the VLC of methanol extract root bark revealed, among others 23 components of which the most abundant were; (-)-alloaromadendrene (15.1%), T-cadinol (8.08%), chamigren (5.3%) and γ-Cadinene (5.1%). Other components were also identified from other methanol fractions; (+)-aromadendrene, (18.2%), (-)-alloaromadendrene (12.8%), 4,9-muurodiene (5.3%), T-cadinol (83%), ς-himachelene (0.63%) and ledol (0.3%). The ethanol:dichloromethane (1:1) extract of the leaves of M. discolor showed four different components from those identified from root bark extracts among which heptacosane and tributylamine had percentage composition of (3.42%) and (0.34%),.respectively. The petroleum ether extract of the root bark of the undescribed Uvariodendron species revealed seven components of which the most abundant were methyl eugenol (38.7%) and elemicin (18.2%). For the ethanol extract of stem bark oil mixtures the most abundant components were δ-cadinol (0.25%), methyl eugenol (0.12%), isoelemecene (0.04%), and diisooctyphthalate (0.02%).*

KEYWORDS: Annoneceous, *Monanthotaxis discolor*, undescribed *Uvariodendron species*, Antimicrobial, cytotoxicity, α-cadinol, (-)-alloaromadendrene, T-cadinol, methyl eugenol, elemicin, aromadendrene.

INTRODUCTION

Plants like other living organisms such as fungi, microbes, marine organisms and others produce secondary metabolites the majority of which do not appear to participate directly in growth and development but have shown high potency as antimicrobial agents. Classes of compounds which are found from the family Annonaceae include polyphenols, chalcones,

dihydrochalcones, flavonoid derivatives, acetogenins, and isoquinoline alkaloids.

Compounds from this family have been shown to possess unique structures and various biological activities. The essential oils from these plants have also shown broad antimicrobial activities. The family Annonaceae is known to be rich in essential oils, hence most of these plants are used for various purposes including medicinal, fragrance and food spices. The overuse of these species places them in danger of extinction.

Some species of the genus *Monanthotaxis* are known to be used in folklore medicine. For example in Ghana, leaves of *M. declina* are used as foot ointment (Mevy *et al.* 2004) whereas in Madagascar the boiled leaves of *M. heterantha* are used as medicine against malaria (Fourinier *et al.* 1997).

In one of the previous studies, the root bark and fruits of *Monanthotaxis diclina* yielded 0.3% of essential oil with complex chemical compositions, the oil being pale yellow with a spicy smell. The main constituents of these essential oils were β-caryophyllene and α-humulene from the fruits, and spathulenol and caryophyllene oxide from the root bark (Fourinier *et al.* 1997). The essential oils components with percentage composition greater than 5 % were *trans*-pinocarveol (22.4%), spathulenol (19.9%) and cedrol (7.1%) from root bark; verbenone (17.5%), caryophyllene oxide (8.9%), β-humulene (8.4%), α-humulene (6.9%) and β–caryophyllene (6.1%) from the fruits. It was also reported that 90% of the essential oils from *M. capea* consisted of phenylbutane derivatives including several sesquiterpene hydrocarbons, and α-copaene (0.4–5%), α-humulene (2–6%) and β-caryophyllene (1.4–3%), which are commonly found in plant oils. Some uncommon components, such as ishwarane (0.2–2%) and drima-7,9(11)-diene (0.6–2.5%), were also reported to be present in significant amounts (Mevy *et al.* 2004).

Bayom *et al.* (2005) reported the study on *Uvariodendron calophyllum R. E. Fries* which was shown to contain 0.39%, 0.04% and 0.52% oils form roots, wood and stem bark, respectively. An α-santalene along with β-caryophyllene consituted more than 50% of the total oil mixture. Other constituents included bisabolene, santalene and bergamotene. It was also noted that caryophyllene, humulene and their oxygenated derivatives were abundant in wood and stem bark oils

Juma 2008 conducted investigations on Tanzanian *Uvariodendron* species in different seasons and geographical locations and reported varying essential oil percentage composition. The studied species include *U. kirkii, U. oligocarpum, U. picnophyllum, U. gorgonis* and one undescribed *Uvariodendron* species. The undescribed *Uvariodendron* species from different localities and seasons yielded oils with ranging percentage composition of 2.07 to 3.85 % for the root bark and 0.52 to 0.71 % for the stem bark. *U. pycnophyllum, U. kirkii* and *U. gorgonis* yielded amounts varying from 1.5 to 2.6 % of the oil composition. The chemical constituents of oil from the undescribed *Uvariodendron* species were reported to constitute *ca*.90 % and *ca*.97 % of linalool from leaves and stem barks respectively. Other major constituents that were reported in this plant had percentage composition between 20 and 41 percent from the stem bark consisting mainly of methyl eugenol, *trans*-methyl isoeugenol, elemicin, and 2-butylnaphthalene.

In this work, both crude extracts and fractionated samples were tested for anti-bacterial activity and anti-fungal activities, whereas the oil extracts were analyzed for their constituent compounds by GC-MS..

MATERIALS AND METHODS

Plant materials

All plant materials were collected from Tanzania. Whereas *Monanthotaxis discolor* was collected from Chimala Scarp Forest Reserve, Mbeya region, *Uvariodendron sp.* was collected from Chalinze, Coast region. The plant materials were identified at the herbarium, Department of Botany, University of Dar es Salaam, where voucher specimens were deposited.

Extraction and fractionation

The air-dried and pulverized plant material was soaked consecutively in petroleum ether, dichloromethane, ethano:dichloromethane (1:1) and methanol for 48 hrs at room temperature. The extracts, were concentrated using a rotary evaporator. The crude extracts were fractionated by VLC starting with non polar solvents and gradually increasing polarity. The solvent were evaporated by using rotary evaporator (some fractions were left on the bench to allow solvent evaporation) where oil fractions were then eluted under gravity column chromatography using non-polar solvents with gradual increased polarity.

Brine Shrimp Test

The brine shrimp test (BST) was carried out in the Department of Chemistry, University of Dar es Salaam. Shrimps (*Artemia salina)* were used as preliminary test for cytotoxicity of the crude extracts and VLC fractions by using standard procedures as described by Moshi *et al.* (2005).

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The LC_{50} values (µg/ml) were obtained using a PoloPlus computer program by using concentration of the sample and the percentage mortality rate. The confidence interval (CI) was also determined simultaneously by the same computer program.

GC-MS analysis of oil compositions

GC-MS analysis was performed on a GC Agilent 6890N Autosampler 7683B series injector interfaced with Water GCT Premier Mass Spectrometer. Electron ionization was done at 70eV with an ion source temperature at 240 ^{o}C. Oven temperature was initially programmed at 50 ^{o}C 6 minutes then ramped at 7 ^{o}C/min to 230 ^{o}C then gradually increased to 300 ^{o}C and held isothermally for 30 minutes. Instrument was set to automatic low to high 50-400 *m/z* with match factor of 60 and minimum identification factor of 70 with an HP-5 capillary column of 25 m 250 µm i.d., 0.25 µm film thicknesses. Other adjustments were made depending on the separation of peaks. The GC chromatograms were used for the determination of the abundance of the individual constituent while MS spectral data were used for the identification of the components through matching the mass spectra of the component to those recorded in the Wiley275 (Wiley, New York) and NIST 02L and AMDIS computer library with the associated data base and available literatures on reported compounds, through *m/z* for molecular ion, major peaks/fragments, base peaks and retention time comparison.

Determination of Anti-microbial Activity

Antimicrobial activity of the plant crude extracts and some VLC fractions were evaluated by the agar well method both in bacteria and fungi tests.

Anti-bacterial Tests. Antibacterial analyses were carried out using the agar well diffusion method as adopted from (Ndukwe 2007 *et al.* and Ferez *et al.* 1990). Fresh and pure isolates of *Escherichia coli, Streptococcus aureus* and *Pseudomonas aureginosa* collected from the Department of Molecular Biology and Biotechnology, University of Dar es Salaam were used for the test. Ampicilin was used as standard anti-bacterial drug.

Anti-fungal Tests. The agar well diffusion method (Parekh *et al.* 2007) was used to determine *in vitro* antifungal activity of crude extracts semi-purified fractions and pure compounds using *Candida albicans* and *Aspergillus niger*. Fluconazole was used as standard anti-fungal drug.

RESULTS AND DISCUSSION

GC-MS Analysis of oil composition

Oil content of M. discolor

Table 1 shows the chemical compositions of apolar oil from *M. discolor*, the dichloromethane extract of *M. discolor* root bark; (MDRD), repeated column chromatography from the VLC fraction of dichloromethane extract (MDRD 3.2). Methanol extract of the root bark (MDRM), repeated column chromatography of the first VLC fraction of methanol extract root bark (MDRM1), the third VLC fraction of dichloromethane extract of the leaves, (MDLD3.3). The dichloromethane extract of root bark (MDRD) of *M. discolor* was analyzed by GC-MS and found to have components that are typically found in most plant essential oils. The components were sesquiterpene and their derivatives α-cadinol(42.85%), (-)-alloaromadendrene (11.7%) aristolone (10.57 %), γ-cadinene (8.72%), δ-cadinene (3.44%) and cubenene (2.28%) (Table 1). Some compounds were also identified from less polar column chromatographed fraction of dichloromethane extract of the root bark (MDRD3.2). These were (-)-alloaromadendrene (15.1%), T-cadinol (8.08%), chamigren (5.3%) and γ-cadinene (5.1%) were identified. Some components found in chromatographic fractions were not detected in the crude dichomethane extract suggesting that they are minor components and hence their detection was suppressed by the major one. Other fractionated sample P11 obtained from methanol extract of the root bark of *M. discolor* was fractionated by column chromatography by eluting with less polar solvents revealing components with percentage composition ranging from 1-5 % except for (+)-aromadendrene, which was 18.2%. The identified compounds were T-cadinol, chamigren, α-muurolene, verticiol, (-)-spathulenol, γ-muurolene, and (+)-spathulenol. Other components identified from VLC fraction (MDRM) of the methanol extract were (-)-alloaromadendrene (12.8%), 4,9-muurodiene (5.3%) and ß-elemene (2.65%). (+)-cyclosativene and verticiol were present in less than 1% composition. Further fractionation gave fraction MDRM1.3.2. T-cadinol (83%), ς-himachelene (0.63%) and ledol (0.3%) were also identified. The lesser polar fractions of the methanol extract revealed few components that were differen from those diteeted before. Thus, six different constituents were identified from ethanol-dichloromethane extract of the leaves (MDLD3.3), heptacosane being the major component (3.42%) followed by tributylamine (0.34%).

Table 1 Chemical constituent of *M. discolor* oil from root bark and **leaves**

Name of Compound	**% Composition**					
	MDRD	**MDRD3.3**	**MDRM1**	**MDRM**	**MDRM 1.3.2**	**MDLD**
(-)-Alloaromadendrene	11.70	15.1	-	12.8	-	-
Aristolone	10.57	-	-	-	-	-
Calarene	1.92	-	-	-	-	-
Cubinene	2.28	-	-	-	-	-
Germacrene B	1.93	-	-	-	-	-
Isolongifolene	0.79	-	-	-	-	-
ß-Elemene	1.34		5.02	2.65	-	-
α-Cadinol	42.85	-	-	-	-	-
α-Muurolene	1.81	-	4.69	-	-	-
γ-Cadinene	8.72	5.10	-	-	-	-
γ-Selinene	0.99	-	-	-	-	-
δ-Cadinene	3.44	-	-	-	-	-
1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalene	-	0.28	1.08	-	-	-
Verticiol	-	1.66	1.08	0.54	-	-
Elixene	-	0.84	-	-	-	-
(-)-Spathulenol	-	1.35	2.06	-	0.11	-
Cubenol	-	0.72	-	-	-	-
T-Cadinol	-	8.08	-	-	83	-
(+)-Cyclosativene	-	0.51	-	0.93	-	-
(+)-Epi-bicyclosesquiphellandrene	-	0.08	-	-	-	-
Caryophyllene	-	0.74	-	-	-	-
Caryophyllene oxide	-	0.15	0.53	-	-	-
Chamigren	-	5.3	-	-	-	-
Copaene	-	0.19	-	-	-	-
Cyclohexane,1,5-diethenyl-2,3-dimethyl-,(1alpha,2alpha,3alpha,5beta)	-	0.40	-	-	-	-
Cyclohexane,1-ethenyl-1-methyl-2-(1-methylethylidene)	-	0.80	-	-	-	-
Isoledene	-	0.50	-	-	-	-
Pentadecane	-	0.02	-	-	-	-
α-Calacorene	-	0.05	-	-	-	-

Name of Compound	% Composition					
	MDRD	**MDRD3.3**	**MDRM1**	**MDRM**	**MDRM 1.3.2**	**MDLD**
α-Cubebene	-	0.61	-	-	-	-
α-Gurjunene	-	0.49	-	-	-	-
α-Gurjunenepoxide	-	0.03	-	-	-	-
ς-Elemene	-	0.02	-	-	-	-
γ-Muurolene	-	-	1.08	-	-	-
(+)-spathulenol	-	-	2.06	-	-	-
(+)-Aromadendrene	-	-	18.20	-	-	-
Isologifolene	-	-	0.90	-	-	-
3,7-Cyclodecadiene-1-methanol,alpha,alpha,8-tetramethyl-,[s,(Z,Z)]	-	-	0.28	-	-	-
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	-	-	0.27	-	-	-
Aromadendrene oxide (I)	-	-	0.28	-	-	-
Germacrene D	-	-	0.45	-	-	-
Humulene oxide II	-	-	0.70	-	-	-
Ledene oxide	-	-	0.42	-	-	-
Longifolene	-	-	2.28	-	-	-
4,9-Muurodiene	-	-	-	5.3	-	-
Ledol	-	-	-	-	0.30	-
ς-Himachelene	-	-	-	-	0.63	-
Nerolidol	-	-	-	-	0.05	-
Tributylamine	-	-	-	-	-	0.34
Phytol						0.04
Heptacosane						3.45
1,2-Benzenedicarboxylic acid, diisooctyl ester	-	-	-	-	-	0.24

MDRD= Dichloromethane extract of *M. discolor* root bark; **MDRD3.2**= Second fraction of the third repeated column chromatography from the VLC fraction of dichloromethane extract *of M. discolor*; **MDRM1**= First fraction of the VLC of methanol extract from *M. discolor* root bark; **MDRM**= Methanol extract of *M. discolor* root bark; **MDRM1.3.2**= Second fraction of the third repeated column chromatography of the first VLC fraction of methanol extract of *M. discolor* root bark ; **MDLD3.3**= Third fractionated fraction of the third VLC fraction of DCM extract of *M. discolor* leaves.

Oil content of Uvariodendron sp.

The petroleum ether extract of the root bark of the undescribed *Uvariodendron* species yielded 3.12 % of crude extracts (USpRP). The analysis of the oil contents revealed seven components including methyl eugenol (38.7%) followed by elemicin (18.2%). The rest had percentage compositions ranging from 0.24-0.72% (Table 2). The analysis of ethanol extract (oily crude) of the stem bark (USpSE) of this plant showed the presence of four major components namely; δ-cadinol (0.25%), methyl eugenol (0.12%), isoelemecene (0.04%), and diisooctyphthalate (0.02%).

Table 2 Chemical constituent of undescribed *Uvariodendron* species root bark and stem bark oil

	% Composition	
Name of compound	USpRP	USpSE
Methyl Eugenol	**38.7**	0.12
Copaene	0.44	-
ë-Selinene	0.25	-
Isocaryophillene	0.42	-
δ-Cadinene	0.23	-
Aciphyllene	0.74	-
Elemicin	**18.2**	-
Isoelemecene	-	0.04
δ-Cadinol	-	**0.25**
Diisooctylphthalate	-	0.02

USpRP = Petroleum ether extract of *Uvariodendron sp.* Root bark**,** USpSE = Ethanol extract of *Uvariodendron sp.* stem bark

These compounds (Table 1 and.2) were identified by the aid of the computer library search (www.chemindustry.com, and www.pubchem.com), comparison of mass spectra with literature data (Kirichenko *et al.* 2008 and Yasumoto *et al.* 2000) and by comparing their retention indices with those reported.

Biological Assays

Brine shrimp lethality test for crude extract and some VLC fractions

Results for brine shrimp cytotoxicity test (Table 3) for the leave extracts of the *M. discolor* and the ethanol extracts of leave, root and stem back of *Uvariodendron sp* showed very minimal toxicity levels of LC_{50} values. The methanol, dichloromethane and water extracts of the root bark of the *M. discolor* were the most toxic extracts with LC_{50} 13.5 6 µg/ml, 41.79 µg/ml and 90.10 µg/ml, respectively. The cytotoxicity may be contributed by components including (-)-alloaromadendrene that have been reported to show cytotoxicity on BST (Sudhakar *et al.* 2009)

Table 3 Brine shrimp lethality test for crude extract and some VLC fractions

sample	LC_{50} (µg/ml)	95% (CI)
1.	418.051	320.015 - 738.498
2.	90.108	53.492 - 195.361
3.	**41.794**	36.135 - 48.352
4.	**13.560**	12.176 - 15.009
5.	250.478	215.265 - 310.784
6.	316.446	269.069 - 419.465
7.	378.678	259.476 - 865.254
8.	**35.357**	31.324 -39.985
9.	418.051	320.015 - 738.498
10.	**33.068**	23.707 - 43.566
11.	204.748	174.562 -258.484

1.Third VLC fraction of ethanol:dichloromethane (1:1) extract of *M. discolor* leaves
2. Water extract of *M. discolor* leaves, **3.** Dichloromethane extract of *M. discolor* root bark, **4**. Methanol extract of *M. discolor* root bark, **5.** Ethanol extract of *M. discolor* stem bark, **6**.Dichloromethane extract of *Uvariodendron sp.* leaves, **7**. Methanol extract of *Uvariodendron sp.* Leaves, **8.** Dichloromethane extract of *Uvariodendron sp.* root bark, **9.** Ethanol extract of *Uvariodendron sp.* root bark, **10**. Petroleum ether extract of *Uvariodendron sp.* root bark,
11. Ethanol extract of *Uvariodendron sp.* stem bark

However, the root bark extracts of *Uvariodendron. sp* both petroleum ether and dichloromethane extracts were all active on BST with LC_{50} values of 33.06 µg/ml and 35.35 µg/ml, respectively. The activity of these extracts might be due to the presence of methyl eugenol which is known to have activity against *Aspergillus favus* (Shelly *et al.* 1996) also known to increase mating competence of male Diptera of *Bactrocera philippinesis* (Rossi *et al.* 2007). No study has been reported on the compound to be associated with carcinogenic properties (Directorate C, EU Health and Cosumer protection 2001). Other microbial activities revealed from some fractions of root bark extracts of *M. discolor* may be due to presence of some chemical constituents singly or synergistically which among the identified compounds; alloaromadendrene, cadinols, aristolone and γ-cadinene are known to posses activity to some bacteria (Deng *et al.* 2009).

Antimicrobial activities of crude extracts

The crudes of dichloromethane, methanol, petroleum ether of root and stem bark of *M. discolor* and *Uvariodendron sp*. extracts exhibited higher antimicrobial activity. Only the petroleum ether extract of the root bark extract of *Uvariodendron sp* showed anti-fungal activity (Table 4). Basically, no activity was observed against *P. aeruginosa* and *Candida albicans*. The exhibited antimicrobial activity of the root bark extract of *M. discolor* which could be due to the presence of T-cadinol, and γ-cadinene, elemene, α-muurolene since these consituents have been reported to exhibit antibacterial activity against *Acetobacter calcoacetica, Bacillus subtillis, Clostridium sporogenes, Clostridium perfringens, Escherichia coli, Salmonella typhii, Staphylococcus aureus*, and *Yersinia enterocolitica* (Doughari 2006).

Table 4 Antimicrobial activities of crude extracts

Sample	Bacteria						Fungi			
	E. coli		*S. aureus*		*P. aeruginosa*		*C. albicans*		*A. niger*	
	IZ_{mean} (mm)	**AI**	**IZ_{mean} (mm)**	**AI**	**IZ_{mean} (mm)**	**AI**	**IZ_{mean} (mm)**	**AI**	**IZ_{mean} (mm)**	**AI**
3	8.0	0.33	5.0	0.21	-	-	-	-	-	-
4	-	-	5.0	0.21	-	-	-	-	-	-
5	-	-	14.0	0.58	-	-	-	-	-	-
8	8.0	0.33	2.5	0.10	-	-	-	-	-	-
10	6.0	0.25	5.0	0.21	-	-	-	-	3.0	0.17
11	-	-	-		-	-	-	-	-	-
Amp.	22.0	1.0	24.0	1.0	21.5	1.0	N/A		N/A	
Fluc	N/A		N/A		N/A		13.5	1.0	18.0	1.0

IZ_{mean}= Mean of inhibition zone, AI= activity Index (i.e IZ_{mean} of sample divided by IZ_{mean} of standard), N/A= not applicable, '-' = No activity; Amp.= Ampicillin, Fluc.= Fluconazole

The methanol extract of the root bark of *M. discolor* showed high level of cytotoxicity against brine shrimp larvae. Among the tested fungi for this extract mild activity exhibited on *S. aureus* whereas no activity was observed from any of the tested bacteria. The ethanol extracts of the *M. discolor* stem bark showed growth inhibition only to *S. aureus* at the mean inhibition zone of 14 mm, with the most active fraction exhibiting a mean inhibition zone ranging from 2.5 to 5.0 mm. Both methanol crude extract root bark (**4**) and ethanol crude extract stem bark (**5**) of *M. discolor* showed mild antimicrobial activity against *S. aureus* with IZ mean 5.0 mm and 14 mm, respectively. The petroleum ether extract of the root bark of *Uvariodendron* species was the only one that showed both antibacterial and antifungal activities. This can be attributed to the presence of methyl eugenol and elemicin which are found to be in higher percentage composition as they are known to show antimicrobial activities (Shelly *et al.* 1996 and Rossi *et al.* 2007).

However, the antimicrobial growth inhibition activity shown by most extracts were much lower when compare to selected standard drugs, this is suggesting that the active constituent in extract is of lower concentration.

CONCLUSION

Investigations on biological activity of dichloromethane and methanol extracts of *Monanthotaxis discolor* exhibited high cytotoxicity and mild antimicrobial activities. The dichloromethane root extracts of *M. Discolor* has been shown to constitute (-)-alloaromadendrene (15.1%), T-cadinol (8.08%), chamigren (5.3%). Also methanol extract have shown γ-Cadinene (5.1%), T-cadinol (83%), ς-himachelene (0.63%) and ledol (0.3%). The ethanol:dichloromethane (1:1) extract of the leaves of *M. discolor* showed very few but different components from those identified from root bark extracts. These were heptacosane (3.42%) and tributylamine (0.34%). The petroleum ether extract of the root bark of the undescribed *Uvariodendron* species revealed seven components of which the most abundant were methyl eugenol (38.7%) and elemicin (18.2%).

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