Journal of Medicinal Plants Research Vol. 3(4), pp. 231-240, April, 2009
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2009 Academic Journals

Full Length Research Paper

Furans and Furanones with antimycotoxigenic activity isolated from *Warburgia salutaris* (Canellaceae)

Viresh Mohanlall and Bharti Odhav*

Department of Biotechnology and Food Technology, Faculty of Applied Science, Durban University of Technology, P. O. Box 1334, Durban 4001, South Africa.

Accepted 21 March, 2009

***Warburgia* is also known as Pepperbark, Isibhaha and Peperbasboom. This tree is one of most valuable of the natural African antimicrobials and the stem and leaves have been used to treat yeast, fungal, bacterial and protozoal infections for centuries. The bark of *Warburgia salutaris* is used in traditional medicine as an expectorant and smoked for coughs and colds. It is also used as a topical application for sores and inflammation. Previous screening of *W. salutaris* showed that this plant had promising antibacterial activity. Subsequently, this endangered tree species was selected for bioassay-guided purification in order to identify the active principles. Fractionation of the toluene: ethyl acetate (7:93 v/v) extract of the stem bark by chromatographic techniques yielded a sesquiterpenoid which exhibited antimicrobial activity against Gram-positive bacteria. The compound, muzigadial, has previously been reported in two other *Warburgia* species, this being the first time (5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-Octahydro-6, 6, 9A-trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin) and 5, 10-Dihydro-6, 7-dimethyl-4H-benzo [5, 6] cyclohepta [1, 2-b]-furan has been reported from *W. salutaris*.**

Key words: *Warburgia salutaris*, plant extract, antibacterial activity, furans, furanones.

INTRODUCTION

A fungal spore landing on the surface of the plant has to combat a complex series of defense barriers set up by the plant before it can germinate, grow into the plant tissues and survive. The arsenal of weapons against the fungus includes physical barriers and chemical ones. The chemical compounds can be preformed in the plant, the so-called “constitutive antifungal substance” (Bruno and Sparapano, 2007). Many antifungal compounds from plants have been reported (Grayer and Harborne, 1974). These are mainly flavonoids and isoflavonoids. The flavonones of *Humulus lupulus* (hops) have shown high antifungal activity against *Trichophyton mentagrophytes* and *Trichophyton rubrum.*

Plants need to protect themselves against predators. This is often done by producing repellents, antifeedants and toxins. An antifeedant has been defined as a compound that inhibits feeding but does not kill the predator directly, although it may die by starvation (Munakata, 1975). For instance, the neem tree, *Melia azedarach* is never attacked by desert locusts, because it contains the powerful antifeedant terpene, azadirachtin (Nakanishi, 1975).

The sesquiterpene and warburganal which has been isolated from the East African tree *Warburgia stuhlmannii*, is a specific antifeedant against larvae of the african army worm. However, it does not have any repellent effect on locusts (Braulio, 2007). Warburganal interferes with the stimulus transduction process in the chemoreceptor cells of the army worm (Kubo et al., 1977). Warburganal contains an α,β-unsaturated 1,4 –dialdehyde moiety in conformity with several other repellent or antifeedant terpenoids. Their repellency implies a role in the natural defense systems of the host organisms (Cimino et al., 1983).

In some cases, the predator does not even have to chew the plant. For instance, aphids have been shown to detect polygodial, another plant metabolite containing unsaturated dialdehyde functionality, with sensilla located on the antennal tips (Nakanishi, 1975). Until now, 81 terpenoid unsaturated dialdehydes have been isolated from

*Corresponding author. E-mail: odhavb@dut.ac.za. Tel.: +2731 373 533. Fax: +2731 373 5351.

most kingdoms in nature (Al-Said et al., 1990).

Most of them are sesqui-, di-, or sesterterpenes, while a few are monoterpenes. Several unsaturated dialdehydes with a drimane skeleton have been isolated from *Canellaceae,* a small family of plants consisting of nine species, grouped into four genera. Of these, *Winterana* and *Cinnamodendron* are endemic to South America, *Warburgia* to East Africa and *Cinnamosma* to Madagascar. Five unsaturated dialdehydes have been isolated (Kubo et al., 1977) from the stem bark of *Canella winterana,* a tree that grows in the subtropical areas of the Florida keys and throughout the Caribbean. The East African genus *Warburgia* consists of two species, *W. stuhlmannii* and *W. ugandensis*. The barks of these are employed widely in folk medicine and as spices in food. The bark extract, containing some unsaturated dialdehydes, possesses antifeedant activity against African armyworms *Spodoptera littoralis* and Spodoptera exempta, widely occurring African crop pests.

Another important field of research involving *Warburgia* spp. is its application as plant molluscicides. In rural communities the cost of synthetic molluscicides or chemotherapy prohibits their use thus plant molluscicides provide a cheap, effective and enviromentally acceptable alternative. Studies carried out by Clarke and Appleton (1997) show that *W. salutaris* had the greatest potential for cultivation and also showed the highest molluscicidal activity.

Although a lot of work has been conducted on the attractant/repellent properties and molluscicidal activity of medicinal plants, very few studies have focused on the effect of medicinal plants or plant extracts on mycotoxigenic fungi. This type of research has only recently come into focus. Thus, this study concentrated on the effect of aromatic compounds, sesquiterpenoid dialedhydes (isoprene compounds) on mycotoxigenic fungi.

MATERIALS AND METHODS

Plant materials

The plant materials were collected from the University of Zululand herbarium, Kwa-Zulu Natal, South Africa in September, 1998. These plants include *Bridelia micarantha*, (Hochst.) Bail., *W. salutaris*, (Bertol.f.) Chiov., *Bulbine fructesce*ns (L.) Willd., *Crysanthermoides monilifera* (L.) T. Norl., *Crocosmia aurea* (Pappe ex Hook.) Planch., *Erythrina latissima* E. Mey., *Scenecio serratuloides* DC. var. *serratuloides*, *Lippia javanica* (Burm. f.) Spreng.

Table 1 indicates plant name and variety collector, primary collector and collector number and botanical institutions where herbarium specimens are deposited. The plant material was divided into leaves, heartwood and bark and the active fractions were extracted by soxhlet extraction and an extraction method outlined by Kardono et al. (1990) with a few modifications. The extracted fractions were then tested for antibacterial and antifungal activity and then chemically characterized by UV and IR spectrophotometry, ^{1}H NMR spectroscopy and Gas chromatography chromatography - Mass spectroscopy (GC-MS).

Preparation of extract

The plant compounds were extracted by soxhlet extraction according to a method by Katsui and colleagues (1973). The leaves, bark and heartwood were air dried and blended. 100 g of powdered plant material was mixed with 95% (v/v) ethanol and was subjected to soxhlet extraction for 48 h. The total soxhlet extract was then mixed with 5% HCl/chloroform (v/v) to release the chloroform extractives, acids and neutrals (acid/base separation). 5% sodium hydroxide (v/v) was added to the chloroform extract releasing the neutral components. 90% methanol/hexane (v/v) was added to the neutral components causing the release of the methanol extractives, terpenes, sterols and polar lipids.

Modified extraction method

The second extraction method using a protocol outlined by Kardono et al. (1990) used solvents of differing boiling points. The plant material 50 g was initially mixed with 50 ml petroleum ether and boiled at 45°C to release the ether extractives. The marcerate from the petroleum ether extraction was re-extracted with methanol at 70°C to release methanol extractives. The macerate was then discarded. The methanol extracts were then dried and reconstituted in dichloromethane and hexane. These four extracts were filtered using Whatman No. 4 filter paper and concentrated to dryness using nitrogen.

Preparation of bacterial cultures

Four bacterial cultures, *Escherichia coli* (ATCC 8739), *Micrococcus luteus* (NCTC 8340), *Bacillus subtilis* (NCTC 3610), *Serratia marcescens* (ATCC 8100), were maintained on nutrient agar slants (Oxoid) and was recovered for testing by growth in nutrient broth for 24 h. *Staphylococcus aureus* (NCTC 6511) was maintained on blood agar (Oxoid).

Antibacterial test

The plates were filled with two layers of nutrient agar, each of approximately 15 ml. The lower base layer was poured and allowed to attain an even surface. The plate was allowed to cool and set. Once the base layer was set, 1 ml of the seven bacterial cultures was added aseptically to the molten agar (temperature ≥ 45°C). This was mixed by 'swirling' and poured evenly over the base layer.

The poured plate was then allowed to set. The settled plate was placed over a 6 X 6 latin square template and wells were cut at appropriate locations by means of a sterile well cutter. The agar plugs were carefully removed from the wells with a sterile needle, taking care not to damage the surface of the plate or the sides of the wells. 100 µl of the plant extracts was then added to these wells.

One blank plate containing nutrient agar and solvents without the extracts served as a negative control. Tetracycline 30 iu was used as a positive control for the gram positive bacteria and Penicillin G 5 iu for gram negative bacteria. Each test was carried out in triplicate.

Antifungal tests

The mycotoxigenic fungi *Fusarium moniliforme* (PPRI 1059) and *Aspergillus flavus* (ATCC 15546) were cultivated on Sabouraud Dextrose Agar slants (SDA), at 25°C for 14 and 21 days respectively. The spores were collected in 10 ml sterile distilled water and

the concentration was adjusted to approximately 10^6 spores/ml. SAB plates were poured and allowed to settle, to form a base layer. Fungal spores were added to molten SAB agar 50°C and poured to form a top layer.

Wells were cut out using a sterile well cutter, test extracts 100 µl, positive control, (amphotericin B, 5 µg/ml), and negative controls (solvents) only were added to the wells in triplicate. The results were evaluated by measuring the zones of clearing around the wells.

Chromatography of crude extracts

Extracted plant samples were reconstituted in 6 ml of methanol and heated at 60°C for 5 min. The sample was filtered with Whatman No. 4 filter paper and only the clear filtrate was used for thin layer chromatography (TLC) analysis.

Thin layer chromatography and detection

The plant material 5 µl was spotted on silica gel F_{254} precoated TLC plates (Merck). The solvent system used was ethyl acetate: methanol: water (100:13.5:10 v/v/v). Detection was achieved using UV light at 365 nm.

Secondary thin layer chromatography method

The plant material 5 µl was spotted on adsorbent silica gel 60 plates. The solvent system used for the first dimension was toluene: ethyl acetate (93:7, v/v), and benzene: acetone (2:1, v/v) was used for the second dimension. Three different spray reagents were used for detection: Spray reagent 1: Vanillin/sulphuric acid reagent (5% ethanolic sulphuric acid - solution 1, 1% ethanolic vanillin - solution 2. Plate was sprayed vigorously with 10 ml of solution 1 followed by 5 - 10 ml of solution 2. Plates were heated at 110°C for 5 - 10 min). Spray reagent 2:30% sulphuric acid spray reagent (Plates were heated at 100°C for 3 - 5 min and evaluated in visible or UV - 360 nm light).

Spray reagent 3: Dinitrophenylhydrazine reagent (DNPH): (0.1g of 2.4 dinitrophenylhydrazine was dissolved in 100 ml methanol, followed by addition of 1 ml of 36% HCL. After spraying with 10 ml, plate was evaluated under visible light. Dinitrophenylhydrazine spray reagent used for detection of ketones and aldehydes).

Circular thin layer chromatography

The solvent migrated circularly from the point of application to form arcs. Silica Gel F_{254}, precoated plates 20 x 20 cm (Merck and Germany) was used. Toluene/ethyl acetate 93:7 was used as the developing solvent. Two diagonal pencil lines were drawn from the corner of the TLC plate. The centre point of the plate was marked and the circle is drawn around it with a diameter of approximately 2 cm.

The circle was divided into four segments; the perimeter of each segment was used for the application of 5 µl of plant extract. Development: 100 ml of solvent was placed in 1000 ml beaker (ca. 20 cm diameter); a glass funnel was loosely packed with cotton wool which extended as a wick through the tube of the funnel. The funnel was placed on the solvent system, so that the solvent soaks into the cotton wool.

The loaded side of the plate faces downwards and the TLC plate was placed over the top of the beaker, so that the wick from the funnel makes contact exactly at the marked center. The solvent migrated as a circle on the TLC plate and the zones of the plant extract form arcs, which increased in length from the starting point to the periphery of the spreading solvents.

Detection of antifungal activity on TLC plates (bioautography)

Antifungal activity of the active components of the plant extracts was bio-assayed directly onto TLCs with *Aspergillus flavus* and *Fusarium moniliforme*. 15 - 20 µl of the plant extract was spotted on the TLC plate. The plate was air-dried and sprayed with the *A. flavus* and *F. moniliforme* spore suspension (10^6 spores/ml) in a mineral salt: 30% glucose media. The sprayed plate was placed in a closed humid tray and incubated at 25°C for 72 h. Antifungal activity of the plant components was indicated by the absence of mycelia around the migrated compound on the plate.

Preparative thin layer chromatography

Crude extract of active plant material was dissolved in chloroform and spotted on TLC plates (toluene: ethyl acetate, 93:7 v/v). Plates were run in duplicate and the template was used to identify the active components by developing one plate. Individual active bands were extracted and collected with chloroform. The extract was then filtered to remove the silica using Whatman No. 4 filter paper and concentrated by drying under nitrogen.

The isolated individual bands were then reconstituted and spotted on TLC plates and rechromatographed with a more polar solvent, toluene: ethyl acetate 50:50 v/v. These bands were collected and rechromatographed with toluene: ethyl acetate 7:93 v/v. The bands were scraped off the TLC plate and extracted with chloroform, filtered and concentrated by drying under nitrogen. These extracts were used for the structural determination of the individual components of the plant by Infrared, UV, ^{1}H NMR spectroscopy and GC-MS.

Structural identification of active compounds from medicinal plants

The λ_{max} for the compound was obtained by UV spectrophotometry using the Varian DMS 100 double-beam spectrophotometer with 1 cm path length quartz cuvettes using dichloromethane as the reference solvent (λ^{CHCl3}_{max} nm:229, 292 and 341). The experimental λ_{max} for the unknown compound was used to determine the absorbance in the UV range.

Infrared spectroscopy

The structure of the compound was confirmed with its IR spectrum (γ CH_2Cl2_{max} cm^{-1}: 3400, 2970, 2800, 2200, 1450 and 1000) in a Nicolet - OMNIC impact 410 system with a PIKE IR cell. Dichloromethane (MP -97°C; BP 40°C) was used as running solvent. Infrared beam was 9 mm in diameter and 9 cm from baseplate. Helium/Neon (He/Ne) laser was used with λ set at 350 nm.

^{1}H NMR spectroscopy

Electromagnets with a field of 14000 gauss were used between two pole pieces and the performance specifications for this magnet was stringent because of the high resolution work. The magnetic field sweep was used to maintain field homogeneity. Sweep range was set at 1175 milligauss for the 300 MHz instrument.

The radio frequency source (transmitter) had a power output less than 1 watt and the signal detector amplified the signal generated

by 10^5, which allowed for recording of signal. NMR sample cell: 5 mm (outer diameter) glass tube, which contained about 0.4 ml sample.

The sample probe (supplied with a driver turbine) rotated the sample tube along its longitudinal axis at 700 rpm^{-1}. This rotation was used to obtain sharper lines and better resolution and it also canceled out effect of impurities the magnetic field. Deuterized chlorine ($CDCl_3$) was used as the reference solvent.

Gas chromatography - mass spectroscopy

The extracted and purified plant sample was reconstituted by thoroughly shaking in dichloromethane 1 ml. Instrumentation: One microlitre of the above solution was injected into the Hewlett-Packard 6890 series Gas Chromatograph interfaced to a Hewlett-Packard 5793 mass selective detector (MSD) with Hewlett-Packard Chemstation software (Version b.02.05, 1989 - 1991). The chromatographic separation was achieved using the Hewlett-Packard Mass Spectroscopy Capillary column 30.0 m × 250 µm × 0.25 µm.

The column stationery phase was a (5%-phenyl) – methylpolysiloxane. The gas chromatograph parameters were as follows: Oven temperature: 50°C held for 2.0 min and ramped at 20°C/min to 280°C held for 7.5 min. Injection was split-less at 250°C. Matches were obtained using the HPPest, PMW_TOXR and Wiley 275 libraries.

RESULTS

Antimicrobial activity

The crude extracts of the plants were investigated for antibacterial and antifungal properties. *B. micrantha* (heartwood and bark) showed antibacterial activity against *S. aureus* and *M. luteus*. It also showed activity against *M. luteus*. *W. salutaris* (bark) showed activity against *S. aureus* and *B. subtilus*. *W. salutaris* (leaves extract in dichloromethane) showed activity against *E. coli*, *S. aureus* and *B. subtilus*. *S. serratuloides* (leaf extract) showed activity against *S. aureus*, *B. subtilus*, *M. luteus* and *S. marcescens*. *L. javanica* (leaf extract) showed activity against *E. coli*, *S. aureus*, *B. subtilus*, *M. luteus* and *S. marcescens*. *E. lattisma* (leaf extract) showed excellent activity against *M. luteus*. *B. micrantha* and *W. salutaris* showed antifungal activity against *F. moniliforme.*

Table 1 shows the results of antimicrobial tests respectively. *W. salutaris* bark showed the most potent antifungal activity thus the most effort was placed on the isolation and identification of the active compounds found in *W. salutaris* bark. Further separation of these fractions using dinitrophenylhydrazine (DNPH) as a spray reagent and circular TLC confirmed the presence of four fractions (Figure 1A and B).

UV Spectroscopy

Ultra-Violet absorption for the four active fractions was determined to be at the highest at a wavelength of 300 nm. The maximum absorption value was 0.42 and the minimum value was 0.27. All four fractions contained compounds that absorbed UV light at wavelength 300 nm.

IR spectroscopy

The four active fractions absorbed light in the IR spectral range at identical wave numbers. They were found to have imides, condensed aromatic system, ortho-disubstituted aromatic group and aromatic esters by comparing to IR spectral correlation charts. The four fractions contained the ortho-disubstituted aromatic group at a wave number ~ 1200 -1300 cm^{-1}, this could be a probable site for the attachment of the methyl and hydroxyl group side chains.

The condensed aromatic system was also found in all four fractions at a wave number ~ 1670 - 1680 cm^{-1}, this observation is in agreement with that of Fukuyama et al. (1982) in which a group of three aromatic rings, two benzene rings and one furan ring make up the structural backbone of the compounds. These results are summarized in table 2.

Gas chromatography – mass spectroscopy

Fractions 1 and 2 contained 1, 2 benzenedicarboxylic acid, dibutyl ester andeicosane. 1, 2 benzenedicarboxylic acid, dibutyl ester is a phthalate derivative, which is used as plasticizers and is a common contaminant of mass spectra of compounds. The percentage quality matches for the four fractions verified these results, which is given in Table 3. Fractionation GC-MS scans are illustrated in Figures 2 and 3. The above identifications are based on a HPPest, PMW_TOXR and Wiley275 library search and are by no means absolute.

Fractions 1 and 2 did not contain the drimane-type sesquiterpenoid compound but contained a high level of the phthalate contaminant and fraction 3 contained a higher abundance of the contaminant (1,2 benzenedicarboxylic acid, dibutyl ester) than the compounds of interest (5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-Octahydro-6, 6, 9A-trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin). Further work with these three fractions could not be carried out because of the high level of contaminant in the sample.

Fractionations of the GC-MS scan

Fractionation GC-MS scans showed that two compounds isolated from *W. salutaris* fraction 4 were drimane-type sesquiterpenoids with known prolific antifungal compounds.

DISCUSSION

The antibacterial activity of the plant extracts tested was

Table 1. Results of antimicrobial activity.

Botanical Name	Tissue	Solvent	Zone of inhibition (mm)						
			Bacteria					Fungi	
			E. coli	*S. aureus*	*B. subtilus*	*M. luteus*	*S. marcescens*	*A. flavus*	*F. moniliforme*
B. micrantha	Heartwood	Methanol/ethyl acetate	-	5	2	4	2	-	8
	Bark	Methanol							
W. salutaris	Heartwood	Methanol/ethyl acetate	-	2	3	-	-	-	15
	Bark	Methanol/Hexane	-	5	6	-	-	-	12
	Leaf	Ethyl acetate	3	4	3	-	-	-	-
	Leaf	Dichloromethane	4	5	4	-	-	-	15
S. serratuloides	Leaf	Dichloromethane	-	3	2	-	-	-	10
C. aurea	Leaf	Dichloromethane	-	-	-	-	-	-	9
L. javanica	Leaf	Ethyl acetate	-	4	2	5	2	-	10
	Leaf	Dichloromethane	-	2	2	2	2	-	10
C. monilifera	Leaf	Dichloromethane	-	-	-	-	-	-	11
E. latissma	Leaf	Ethyl acetate	4	4	3	10	-	-	4
	Leaf	Dichloromethane	3	4	3	4	-	-	3
B. frutescens	Leaf	Ethyl acetate	-	5	-	-	-	-	-

Table 2. Similarities of IR absorption wave numbers (cm^{-1}) for the four active fractions isolated from *Warburgia salutaris.*

	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Corresponding Functional groups
Peak 1	1718.05	1714.45	1716.57	1720.61	Imides
Peak 2	**1670.00**	**1680.53**	**1676.29**	**1676.11**	Condensed aromatic system
Peak 3	**1253.51**	**1282.84**	**1292.53**	**1239.91**	Ortho-disubstituted aromatic ring
Peak 4	1025.00	1023.26	1006.30	1026.27	Aromatic esters

found mainly against the gram-positive bacteria. The ethyl acetate and dichloromethane extracts of *W. salutaris* showed activity against *S. aureus, S. marcescens* and *Pseudomonas aeruginosa*. The negative results obtained against the gram negative bacteria were not surprising as, in general, these bacteria are more resistant than gram positive ones (Rios et al., 1991; Rabe and Van Staden, 1997)

The two different extraction techniques employed in this study resulted in the release of various volatile compounds. The heat-treated solvent extraction technique proved more effective in the release of isoprene compounds (terpenoids). *W. salutaris* has been reported to release a wide variety of isoprene compounds. The drimane type sesquiterpene dialdehyde, Warburganal isolated from *Polygonum hydropiper* exhibited potent antifeedant; antibiotic and molluscicidal activity (Fukuyama et al., 1972; Clarke and Appleton, 1997). Other drimane type sesquiterpenes

Table 3. Identification and corresponding percentage quality matches for the four active fractions from *arburgia salutaris.*

Compound Identity	Library Match Quality (%)
Fraction 1	
1,2 benzenedicarboxylic acid, dibutyl ester.	91
Fraction 2	
1,2 benzenedicarboxylic acid, dibutyl ester.	91
Fraction 3	97
Hexadecanoic acid, ethyl ester	
1,2 benzenedicarboxylrc acid, dibutyl ester	91
(5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-Octahydro-6, 6, 9A- trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin)	80
Fraction 4	
5, 10-Dihydro-6, 7-dimethyl-4H-benzo [5, 6] cyclohepta [1, 2-b]-furan.	91
(5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-Octahydro-6, 6, 9A- trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin).	98

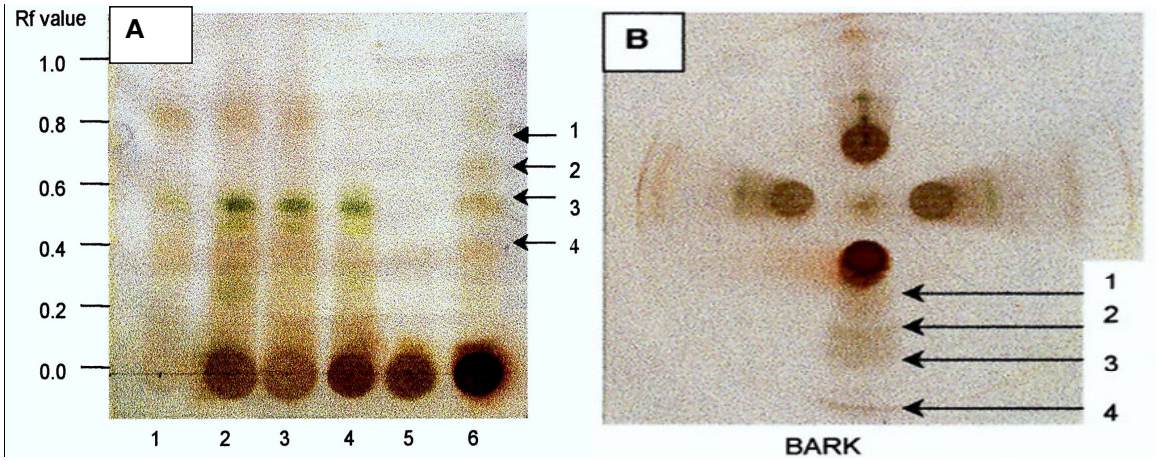

Figure 1. (A) Separation of individual components from *Warburgia salutaris* leaves and bark extracts. Lane 1 - *Warburgia salutaris* leaves (soxhlet extracted in dichloromethane, Lane 2 - *Warburgia salutaris* leaves (dichloromethane, 60°C) Lane 3 - *Warburgia salutaris* leaves (ethyl acetate, 60°C), Lane 4 - *Warburgia salutaris* leaves (hexane, 60°C), Lane 5 - *Warburgia salutaris* bark (Soxhlet extracted in dichloromethane) and Lane 6 - *Warburgia salutaris* bark (dichloromethane, 60°C). Arrows 1, 2, 3 and 4 are the components of interest found in *Warburgia salutes* bark. (B) Circular TLC verifies the presence of four compounds (arrowed 1-4). Compound migration is shown as arcs.

polygodial, isopolygodial, isodrimenol, drimenol, confertifolin and a nor-sesquiterpene monoaldehyde, polygonal have also been isolated from *Warburgia* spp. (Mashimbye et al., 1999).

A typical pungent odour and bitter taste of *Warburgia* spp. was also exhibited by *W. salutaris*. The bitter characteristic is due to the bitter tasting monoterpenoid lactones known as iridoids. Iridoids are components of volatile oils, which have been used to stimulate actions within the body, such as mucosal or gastric secretion. Iridoids usually occur in angiosperms, especially valerian, gentian, blue flag and orris root and can have therapeutic uses or properties such as expectorants; anthelmintics; insecticides; antiseptics; antimicrobial; antileukemic properties and interferon-like activity (Smolarz and Skwarek, 1999) Fukuyama et al. (1982) reported the presence of various sesquiterpenoid dialdehydes from *Warburgia* spp. They also reported the distribution of seven different drimane-type sesquiterpenoids in *P. hydropiper* (Table 3).

The structural similarities between the sesquiterpenoids isolated from *W. stuhlmanii* and *W. ugandensis* and the constitutive compound isolated from *W. salutaris* is

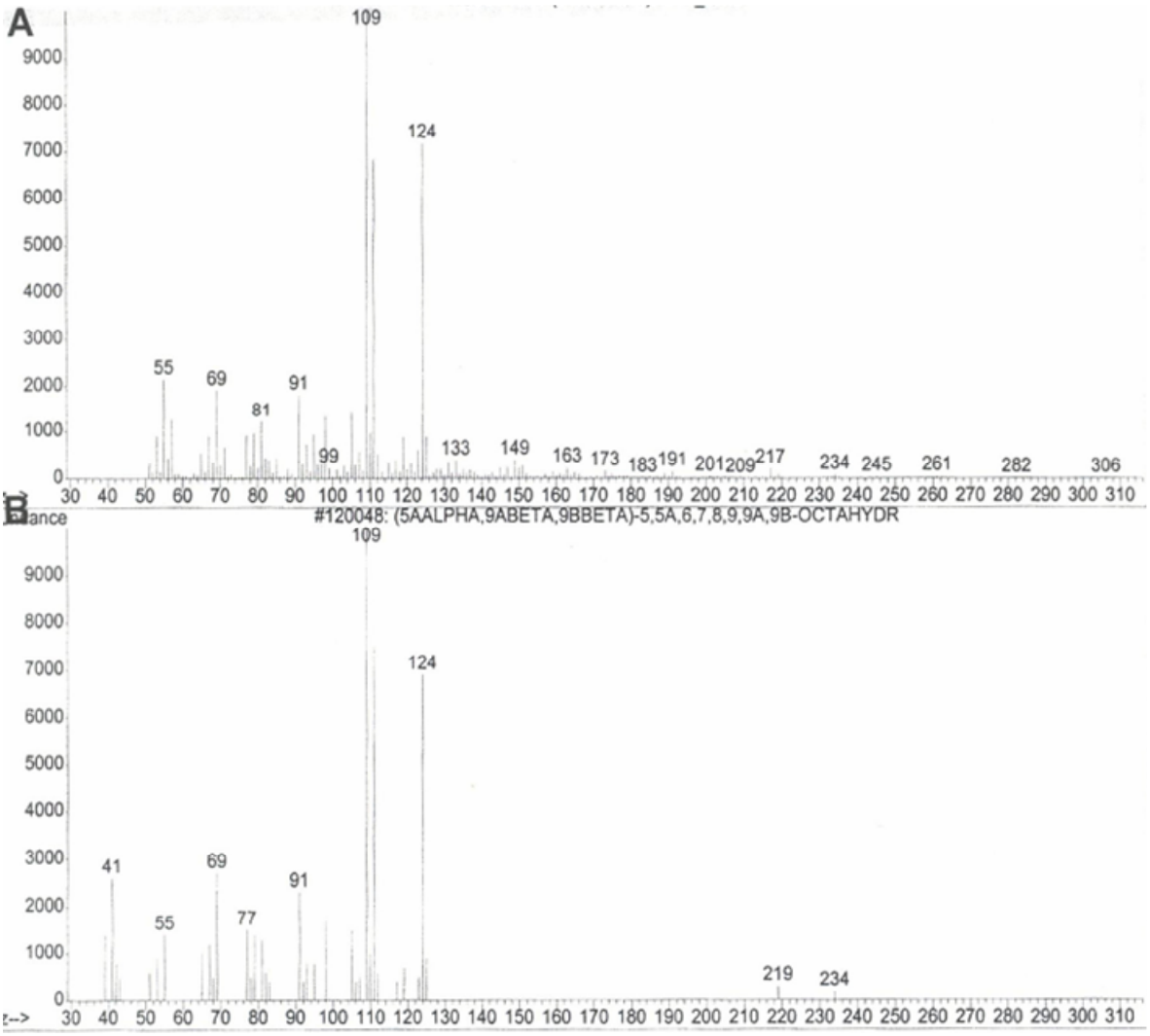

Figure 2. GC-MS fractionation scan comparing the purity of compound isolated from *Warburgia salutaris* (A) fraction 4 against a standard, (5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-Octahydro-6, 6, 9A- trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin).

shown in Figure 4. Warburganal (Figure 4.1), Polygodial (Figure 4.2), Ugandensidial (Figure 4.3), Muzigadial (Figure 4.4) and precursors of drimanolides (Figures 4.5 - 4.8) isolated from *W. stuhlmanii* and *W. ugandensis* were similar in structure in that they all contained condensed aromatic ring system, hydroxyl groups, an unsaturated aldehyde group and tertiary methyl groups.

A compound isolated and identified from *W. salutaris* showed the presence of a condensed aromatic system in which a benzene ring was attached to a furan ring system, which was coupled with a 7-carbon aromatic ring. 5, 10-dihydro-6, 7-dimethyl-4H-benzo [5, 6] cyclohepta [1, 2-b]-furan (Figure 4.9) also contained two hydroxyl groups at positions C-5 and 10, and a pair of primary methyl groups at positions C-5 and 6.

Acetylation of isodrimenol yielded a labile monoacetate and a β, β-disubstituted furano compound. A similar compound was isolated from *W. salutaris*, (5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-octahydro-6, 6, 9A-trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin). (5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-Octahydro-6, 6, 9A- trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin) and 5, 10-dihydro-6, 7-dimethyl-4H-benzo [5, 6] cyclohepta [1, 2-b]-furan isolated from *W. salutaris* shows a good similarity in structure to the drimane-type unsaturated sesquiterpenoid dialdehydes (Figure 4.9) isolated from *W. stuhlmanii* and *W. ugandensis* which is responsible for the high antifungal activity, thus showing the potential of (5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-octahydro-6, 6, 9A- trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin) and 5, 10-dihydro-6, 7-dimethyl-4H-benzo [5, 6] cyclohepta [1, 2-b]-furan as antifungal agents.

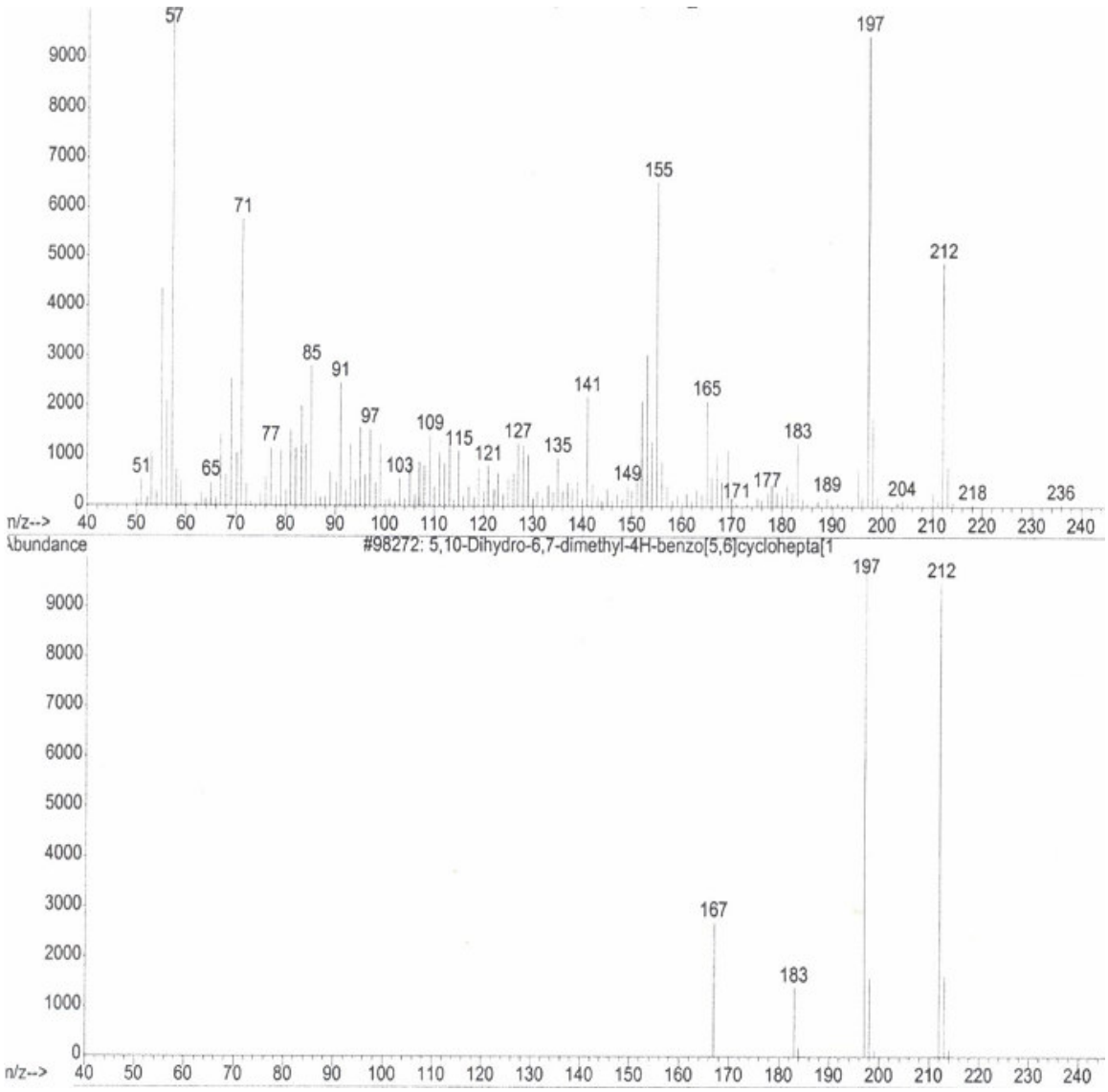

Figure 3. GC-MS fractionations scan comparing the purity of compound isolated from *Warburgia salutaris* (A) fraction 4 against a standard, 5, 10-Dihydro-6, 7-dimethyl-4H-benzo [5, 6] cyclohepta [1, 2-b]-furan.

The hot-tasting sesquiterpenoids isolated from *W. salutaris* were identical to potent African armyworm anti-feedants isolated from *W. ugandensis.* The (5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-octahydro-6, 6, 9A- trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin) and 5, 10-dihydro-6, 7-dimethyl-4H-benzo [5, 6] cyclohepta [1, 2-b]-furan isolated from *W. salutaris* form part of a large number of phytochemicals that have been isolated as antimicrobial agents. Their activity is usually not potent enough to be considered for practical application. Future studies to enhance their biological activities are needed. Applications could also include the treatment of systemic fungi. The difficulty involved with the treatment of systemic fungi is the similarity between fungal cells and mammalian cells, “What kills fungi also puts the host cell at risk” (Kamuhabwa et al., 2000). It is important therefore, to search for compounds that can target fungal cells specifically and be administered at low dosages. The fungicidal activity of (5Aα, 9Aα, 9Bβ)-5, 5A, 6, 7, 8, 9, 9A, 9B-octahydro-6, 6, 9A- trimethylnaphtho[1, 2c] furan-1-(3H)-one (drimenin) and 5, 10-dihydro-6, 7-dimethyl-4H-benzo [5, 6] cyclohepta [1, 2-b]-furan, with possible mechanism of synergism with other naturally occurring compounds, makes these two compounds promising antimicrobial agents.

ACKNOWLEDGEMENTS

The authors are grateful to National Research Foundation for funding this research. Prof. H. Baijnath of Univer-

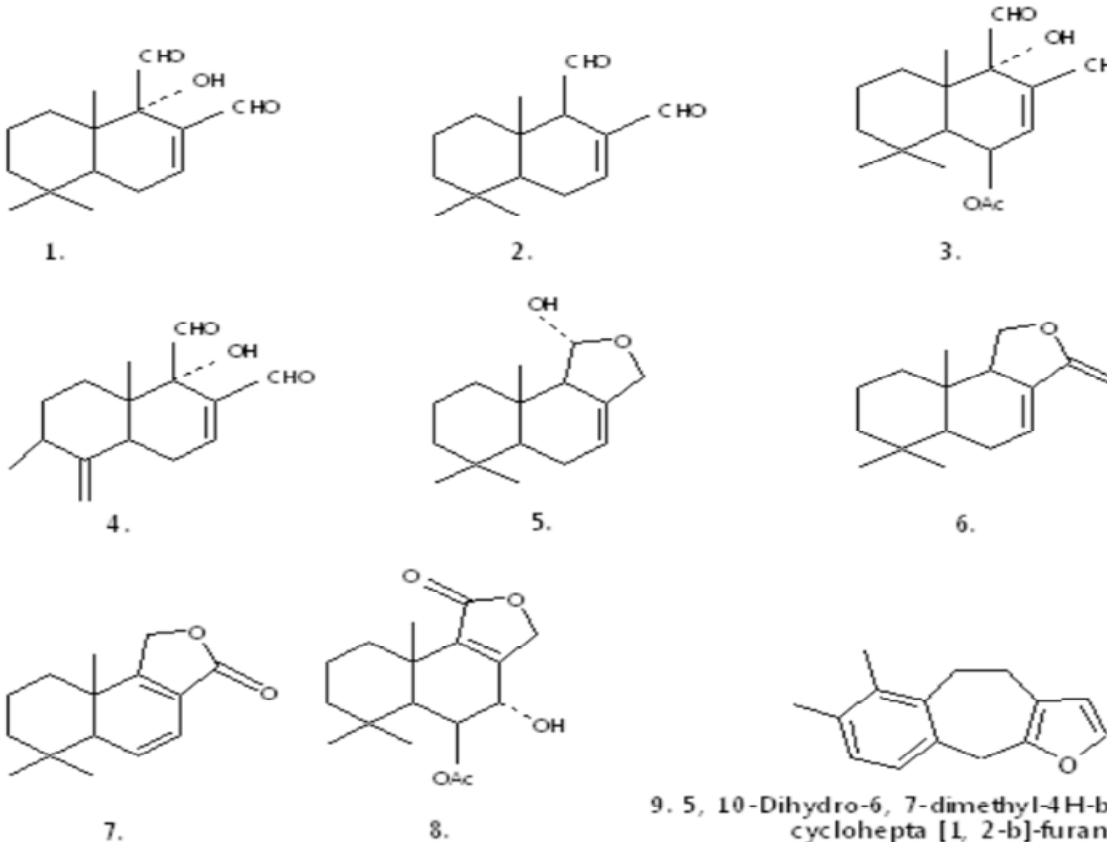

Figure 4. Structural configuration of drimane type sesquiterpenoids isolated from *W. stuhlmanii W. ugandensis, P. hydropiper* and *W. salutaris*. 1. warburganal, 2. Polygodial, 3. Ugansidial, 4. Muzigadial and 5. – 8. precursors of drimanolides. 9. 5, 10-Dihydro-6, 7-dimethyl-4H-benzo [5, 6] cyclohepta [1, 2-b]-furan isolated from *W. salutaris*.

sity of Kwazulu Natal is acknowledged for plant material identification and collection. Center for Research Management and Development, Durban University of Technology is appreciated for financial support.

REFERENCES

Al-Said, MS, El-Khawaja SM, El-Feraly FS, Hufford, CD (1990). 9-Deoxy drimane sesquiterpenes from *Canella winterana*. Phytochemistry 29:975

Braulio MF (2007). Natural sesquiterpenoids. Natural Product Reports. 24: 1350 – 1381

Bruno G, Sparapano, L (2007). Effects of three esca-associated fungi on *Vitis vinifera* L.: II. Characterization of biomolecules in xylem sap and leaves of healthy and diseased vines, Physiological and Molecular Plant Pathology. 69, 195-208.Cimino, G., De Rosa, S., De Stefano, S., Sodano, G., Villani, G., 1983. Medicinal plants of East Africa. Sci. 219 (4589): 1237-1238.

Clarke TE, Appleton CC (1997). The molluscicidal activity of *Apodytes dimidiate* E. Meyer ex Arn (Icacinaceae), *Gardenia thunbergia* L. F (Rubiaceae) and *Warburgia salutaris* (Berthol. F.) Chiov. (Canellaceae), three South African plants. J. Ethnopharmacol. 56: 15-30.

Fukuyama Y, Sato T, Asakawa Y, Takemoto T (1982). A potent cytotoxic warburganal and related drimane-type sesquiterpenoids from *Polygonum hydropiper*. Phytochemistry 12: 2895-2898.

Grayer RJ, Harborne JB (1994). A survey of antifungal compounds from higher plants. Phytochemistry 37: 19-42.

Ingham JL (1973). Disease resistance in higher plants. Phytopathol. 78: 314-335.

Kamuhabwa A, Nshimo C, De Witte P (2000). Cytotoxicity of some medicinal plant extracts used in Tanzanian traditional medicine. J. Ethnopharmacol. 70: 143-149.

Kardono LBS, Tsauri S, Padmawinata K, Pezzuto JM, Kinghorn AB (1990). Methods for drug evaluation. J. Nat. Prod. 53: 1447-1455.

Katsui N, Matsunaga A, Imaizumi K, Masamune T (1973). The structure and synthesis of rishitinol, a new sesquiterpene alcohol from diseased potato tubers. Tetrahedron Lett. 2: 83-86.

Kott V, Barbini L, Cruanes M, Munoz JD, Vivot E, Cruanes J, Martino V, Ferraro G, Cavallaor L, Campos R (1999). Antiviral activity in Argentine medicinal plants. J. Ethnopharmacol. 64: 79-84.

Kubo I, Miura I, Petteri MJ, Lee YW, Pilkiewicz F, Nakanishi K (1977). Host plant resistance in pests. Tetrahedron Lett. 18: 4553-4556.

Ma WC (1977). Natural plant chemistry- A mechanistic and biosynthetic approach to secondary metabolism. Physiol. Entomol. 2: 199-207.

Mashimbye MJ, Maumela MC, Drewes SE (1999). A drimane sesquiterpenoid lactone from *Warburgia salutaris*. Phytochemistry. 51(3): 435-438.

Munakata K (1975). Insect antifeeding substances in plant leaves. Pure Applied Chemistry. 42: 57-66.

Nakanishi K (1975). Natural Products Chemistry. Kodansha/Academic Press, Tokyo. 2:586.

Powell G, Hardie J, Pickett JA (1995). The effects of antifeedant compounds and mineral oil on stylet penetration and transmission of

potato virus Y by *Myzus persicae* (Sulz.) (Hom., Aphididae). Physiol. Entomol. 20: 141-146.
Rabe T, Van Staden J (1997). Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 56:81-87.
Rios JL, Recio MC, Villar A (1991). Isolation and identification of the antibacterial compounds from *Helichrysum stoechas*. J. Ethnopharmacol. 33: 51-55.
Smolarz HD, Skwarek T (1999). The investigation into the interferon-like activity of *Polygonum* L. genus. Acta Poloniae Pharmaceutica. 56: 459-462.
Taniguchi M, Kubo I (1993). Ethnobotanical drug discovery based on medicine men's trials in the African savanna: screening of East African plants for antimicrobial activity II. J. Nat. Prod. 56: 1539-1546.