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Full Length Research Paper

Antimicrobial activity of plant phenols from *Chlorophora excelsa* and *Virgilia oroboides*

Thiriloshani Padayachee[1]* and Bharti Odhav[2]

[1]Department of Biosciences, Vaal University of Technology, Vanderbijlpark South Africa.
[2]Department of Biotechnology and Food Technology, Durban University of Technology, South Africa.

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The anti-bacterial and anti-fungal activity of four aqueous plant extracts (1 x 10^4 µg/ml) of 2,3'4,5'-tetra hydroxy-4'-geranylstilbene (chlorophorin) and 3',4, 5' - trihydroxy - 4' - geranylstilbene (Iroko) from the tree *Chlorophora excelsa* and (6aR,11aR)-3-hydroxy-8,9-methylenedioxypterocarpan (Maackiain) and 7-hydroxy-4'-methoxyisoflavone (formononetin) from *Virgilia oroboides* were evaluated by the seeded agar overlay well diffusion method. The test organisms and bioautography used included: *Bacillus coagulans, Streptococcus pneumoniae*, *Klebsiella pneumoniae, Escherichia coli, Mycobacteria tuberculosis*, *Aspergillus flavus* and *Fusarium verticilloides.* Vancomycin, the drug of choice for these organisms was used as the control at 30 µg/ml. The extracts showed that chlorophorin at 1.95 µg/ml and Iroko at 3.125 and 6.25 µg/ml respectively were active in inhibiting the growth of *S. pneumoniae* and *B. coagulans* and not active against *K. pneumoniae* and *E. coli*. Maackiain; formononetin and formononetin acetate showed little activity against *S. pneumonia*, *B. coagulans, K. pneumoniae* and *E. coli.* None of the extracts showed activity against *M. tuberculosis.* Maackiain, formononetin, chlorophorin and Iroko inhibited *F. vertiicilloides*, maackiain being the most active compound. Formononetin, chlorophorin and Iroko inhibited *A. flavus*. *A. flavus* was most sensitive to chlorophorin and Iroko. The bioautography method confirmed these results and was attributed to the phenolic nature of the compounds.

Key words: Plant compounds, anti-bacterial, anti-fungal, chlorophorin, Iroko.

INTRODUCTION

According to the World Health Organisation (2005), herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries. Plants have the ability to produce a wide variety of bio-active molecules through secondary metabolic pathways (Kutchan and Dixon, 2005; Cox and Balick, 1994; Mitscher et al., 1987). Current problems associated with antibiotic resistance (Lu and Collins, 2009; Recio et al., 1989), lack of availability and poverty means that one has to seek alternatives. Extracts from plants have lead to the discovery of many drugs. Africa has a large untapped resource of indigenous plants whose extracts could lead to the discovery of potentially new drugs. These new antimicrobial agents are not restricted to products of microbial origin (Samoylenko et al., 2009; Zhu et al., 2009; McLaughlin, 2008; Hoffmann et al., 1993; Mitscher et al., 1987). Since their structures are different to those from microbial sources, they provide a new challenge to the microbe, particularly with regard to the development of resistance (Lee, 2004; Kubo and Kubo, 1995). In this study, plant compounds from two South African indigenous plants for antimicrobial activity against common bacterial pathogens and mycotoxin producing fungi were used. The compounds in this study were derived from two indigenous trees *Virgilia oroboides* and *Chlorophora excelsa*.

C. excelsa produces compounds 2,3'4,5'-tetra hydroxy-4'-geranylstilbene (chlorophorin), which was first isolated from an ether extract of the heartwood by King and Grundson (1949) and 3', 4, 5' - trihydroxy - 4' - geranylstilbene (Iroko), a stilbene classified as an aromatic phenolic compound. *V.*

*Corresponding author. E-mail: thiri@vut.ac.za. Tel: +27 016 950 9446. Fax: +27 086 691 0795.

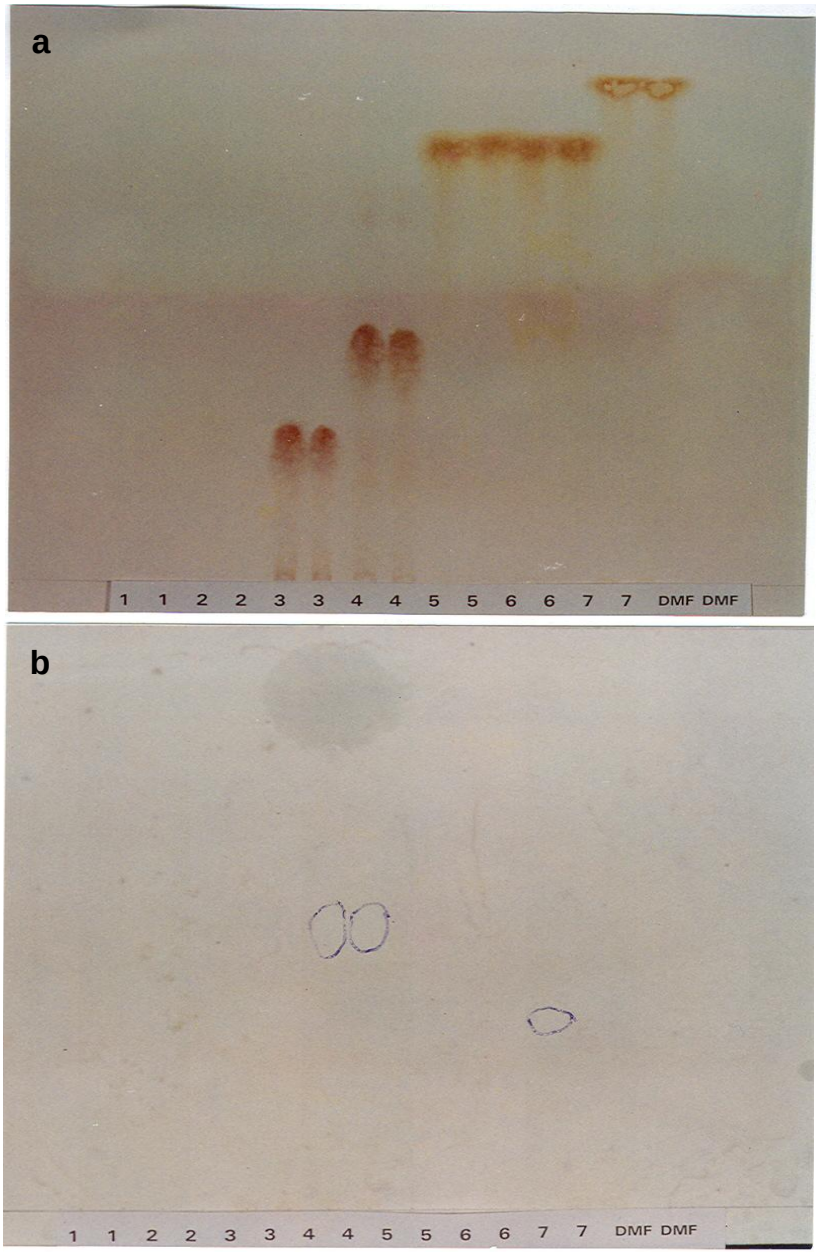

Figure 1. The effect of maackiain acetate,formononetin,chlorophorin and Iroko on *F. moniliforme*.TLC plates with separated plant compounds (a) template and (b) with *F. moniliforme* on the TLC plates showing areas of no growth (arrow) in the same region as where bands appear on the template. 1, Formononetin; 2, Formononetin acetate; 3, Iroko (stilbene); 4, Chlorophorin; 5, Maackiain; 6, Maackiain; 7, Maackiain acetate.

oroboides produces (6aR,11aR)-3-hydroxy-8,9-methylenedioxypterocarpan (Maackiain) which is classified as a pterocarpan (McMurphy et al.,1972; Pachler et al. 1967 cited by Swinny, 1989) and characterised by Swinny, (1989) and 7-hydroxy-4'-methoxyisoflavone classified as Formononetin (Bate-Smith et al.,1953; Braz et al., 1973; Letcher et al., 1976 cited by Swinny, 1989). The chemical structures of the compounds were elucidated by Swinny et al. (1989) illustrated in and were found to be phenolic compounds as shown in (Figure 1).

Phenols have long been known to have anti-septic activity and therefore have a large potential as therapeutic agents. Since these are produced by all vascular plants and well over 4000 distinct flavonoids have now been identified some of which display therapeutic activity (Yoon et al., 2007; Ja Kim et al., 2006; Walsh, 1988). In previous studies of

Table 1. Effect of plant compounds on gram positive and gram negative bacteria.

Parameter	*B. coagulans*	*S. pneumoniae*	*K. pneumoniae*	*E. coli*
Chlorophorin	+ + + [a] MIC	+ + + MIC	-	-
Iroko	+ + + MIC	+ +MIC	-	-
Maackiain methyl ether	-	-	-	-
Maackiain acetate	+	+	-	-
Formononetin	+	-	-	-
Formononetin acetate	-	-	+	-

[a]Zone of inhibition; - = 0 - 1 mm; + = 2 - 3 mm; ++ = 4 - 5 mm; +++ = 6 - 10 mm.

Acharya and Chatterjee (1974), both Chlorophorin and Iroko have been shown to be highly active against the gram positive organisms such as *Streptococcus pneumoniae* and *Bacillus coagulans*, and less active against the gram negative organisms such as *Klebsiella pneumoniae* and *E. coli.* An isoflavone, formononetin and a pterocarpan, maackiain showed minimal activity.

Previous anti-fungal studies (Wu et al., 2008; Kordial et al., 2005; Harrison et al*.,* 2003; Davidson, 1993) have shown that phenolic compounds do have anti-fungal properties and it was also stated by Shan et al. (2007) and McCutcheon et al. (1994) that most plants that exhibit anti-bacterial properties are also usually anti-fungal. Studies of Chipley and Uriah (1980) have also shown that the phenolic compounds, caffeic acid and chlorogenic acid inhibited the *Fusarium* species (Ja Kim et al., 2006; Harrison et al., 2003; Valle,1957) and that ferulic acid inhibits aflatoxin B_1 and G_1 production of *A. flavus* by approximately 50% and *A. parasiticus* by 75%. This study investigated the antimicrobial activity of four plant extracts: chlorophorin and Iroko from *Chlorophora excelsa* and, maackian and formononetin from *Virgrilia oroboides*, with a view to develop future biocontrol agents.

MATERIALS AND METHODS

Preparation and storage of microorganisms

B. coagulans, S. pneumoniae, *K. pneumoniae, E. coli,* and three strains of *M. tuberculosis, viz.,* A169 (Karachi strain- known resistance used as a control), H37 (ATCC virulent strain) and R9001 were obtained from the Department of Microbiology, University of Natal, Medical School. *A. flavus* and *F. verticilloides* were obtained as pure cultures from the Department of Physiology, University of KwaZulu Natal Medical School, Durban, RSA. The gram positive and gram negative bacteria were stored as stock cultures in Microbank vials (Prolab Diagnostics) at -70°C. *M. tuberculosis* strains were maintained on A. J. Lone Stein Jensen agar medium at 4°C. Fungal stock cultures were maintained and stored on Saboroud Dextrose agar (SDA) slants (Oxoid) at 4°C until they were required.

All experiments were conducted according to the Manual of Clinical Microbiology (Balows et al., 1991). The gram positive organisms (*B. coagulans* and *S. pneumoniae*) and gram negative organisms (*K. pneumoniae* and *E. coli*) were plated on Brain Heart Infusion agar plates and acid fast bacteria was plated on Middlebrook agar (Biolife) and supplemented with 5% glycerol and 100 ml oleic acid albumin and dextrose (OADC) (Biolab, ART No: C 70) and incubated at 37°C for six weeks, and stored at 4°C. The inoculum was standardised using McFarlands No. 2 standard in saline. This is equivalent to a bacterial concentration of 6 x 10^8 cells/ml (Balows et al., 1991; Washington et al., 1972).

Broth cultures of the strains of *M. tuberculosis* were prepared in Dubos broth (Biolife) and were incubated at 37°C for 24 h, after which the inoculum was standardised using McFarlands No. 1 standard. This was further diluted in Dubos broth to give a final *M. tuberculosis* concentration of 3 x 10^8 cells/ml (Balows et al., 1991; Washington et al., 1972). This was used as the inoculum.

Fugal cultures (*A. flavus* and *F. verticilloides*) were prepared by plating them on SDA plates, which were incubated for at 25°C for six days. The spores were harvested in 2 ml of sterile saline and counted with a Neubauer counting chamber to give a spore concentration of 4 x 10^7 spores /ml.

Preparation and storage of plant compounds

Iroko and chlorophorin from *C. excelsa* and Maackiain and Formononetin acetate from *V. oroboides* were obtained in powder form (Swinny, 1989). They were prepared in dimethylformamide (DMF) at 1.000 µg/ml (w/v), using a non-pyrogenic sterile filter 0.22 µm, (Millipore) and stored as stock solutions at ambient temperature until needed. Their identity and purity were verified by thin layer chromatography. These plant extracts were previously tested for their toxicity to human cells by *in-vitro* toxicity test against Hep -2 cells and their mutagenic activity was ascertained using the Ames test, as outlined by Franson (1992).

Evaluation of antimicrobial activities

The antimicrobial activities of these compounds were tested using the modified well diffusion assay (Leven et al., 1979). Each test was carried out in triplicate, and the test results expressed as a mean of each triplicate. The compounds were tested against the following gram positive organisms; viz., *B. coagulans* and *S. pneumoniae*, and gram negative bacteria viz., *K. pneumoniae* and *E. coli.* Dimethylformamide was used as a negative control, vancomycin (30 µg/ml) was used as positive control for *B. coagulans* and *S. pneumoniae* and ampicillin (25 µg/ml) was used for *K. pneumoniae* and *E. coli* and Rifampicin (1.0 µg/ml) was used against *M. tuberculosis* (Table 1).

Verification against *M. tuberculosis* was also tested using a sensitivity method described by Lsenberg, (1980) using Middlebrook 7H10 (BioLife). In this method, Middlebrook agar containing 6 x 10^8 colony forming units (CFU) of the test organism was over laid onto molten sensitivity agar (Biolife). Six equidistant wells, 6 mm in diameter, were made in the agar plates. Four wells were filled with 25 µl of the respective positive and negative controls, each in duplicate. The MIC's were established for the most active compounds using the method described (Lsenberg, 1980). Plant compounds were diluted in

Table 2. Minimum inhibitory concentration (MIC) in µg/ml of the plant compounds and the effect of antibiotics on the test bacteria.

Compound	*B. coagulans*	*S. pneumoniae*	*K. pneumoniae*	*E. coli*	*M. tuberculosis*
Chlorophorin	1.953± 0.06[a]	1.953± 0.06	50± 0.06	No effect	No effect
Iroko	3.125± 0.15	6.25± 0.015	No effect	No effect	No effect
Maackiain acetate	25± 0.26	12.5± 0.015	No effect	No effect	No effect
Formononetin	50± 0.025	No effect	25± 0.15	No effect	No effect
Ampicillin	No effect	No effect	No effect	25 ± 0.15	No effect
Rifampicin	-	-	-	-	1.0
Vancomycin (control)	30	30	30	No effect	No effect

[a] ± = Standard deviation of the mean.

dimethylformamide (DMF) to give concentrations of each compound ranging from 3.125 100 µg/ml (w/v). 5 ml of molten sensitivity agar and 1 ml of the respective plant compounds were poured into sterile quadrant Petri dishes. 30 µl of the standardised inoculum was spread over the surface of each quadrant. The plates were then incubated for three weeks at 37°C in a CO_2 incubator. The last two wells were inoculated with 0.025 ml of each test compound, at concentrations ranging from of 3.125 to100 µg/ml (w/v). The plates were incubated for 24 h at 37°C. The results were analysed by measuring the zones of inhibition (clearing around each well in mm), of the test compounds relative to the clearing around DMF, using the following formula. The results were assessed according to the ability of test compounds to inhibit the growth of the microorganisms.

Activity of tested compounds (zone of inhibition of test compounds - zone of inhibition of DMF)

Preliminary screening tests were carried out to establish the activity of the plant compounds on the test fungi, using the seeded agar overlay method. 10 ml SDA plates were overlaid with 5 ml of molten SDA that was seeded with 1 ml of previously prepared spore suspension (4,07 x 10^7 spores/ml) of *A. flavus* and *F. verticilloides* respectively. Six wells (6 mm diameter) were punched into each plate (0.25 µl; 100 µg/ml) and the plant test compounds was placed in each well and incubated at 25°C for four and six days for *A. flavus* and *F. verticilloides* respectively. DMF was used as the negative control and amphotericin B (5 mg/ml) was used as the positive control. The results were analysed by measuring the zones of inhibition of the test compounds. The antifungal effect was confirmed using bioautography (Betina, 1973). Two silica gel 60 TLC aluminium sheets (Merck) were spotted with 5 µl of 10 µg/ml (w/v) of each compound, 5 µl of saline (0.9 %w/v), and amphotericin B (Oxoid) (5 µg/ml). The compounds were separated using benzene: acetone (8:4 v/v) for 1 h. The plates were then air dried. One plate, which was used as the template, was developed with anisaldehyde solution and dried in a hot air oven at 80°C for 2 min. The second plate which was used for evaluating the effect of the plant compounds on fungal growth was sprayed with a fungal spore suspension which was prepared using 2 ml of the inoculum (4 x 10^7 spores /ml) suspended in 70 ml of fungal culture media that is glucose-mineral salts medium. This plate was placed in a sterile humidified container and incubated at 25°C for 4 days. The results were obtained by comparing the zone on the template plate to the zones of inhibition on the test plate. These were compared to the zones of inhibition obtained for DMF.

Determination of minimum inhibitory concentrations (MIC)

The MIC of the active compounds was determined using the procedure described by Hailu et al. (2005) and Atlas et al. (1984). Two fold serial dilutions of each compound were made in DMF at concentrations ranging from 3.125 µg/ml to100 µg/ml (w/v). The lowest concentration of the test compound in which no growth occurred was defined as the MIC.

The method using the seeded agar well diffusion by Hailu et al. (2005) and Atlas et al. (1984) were used to measure MIC of the active compounds against *A. flavus* and *F. verticilloide*. A single well 6 mm in diameter was cut out from SDA plate, to which 0.25 µl of test compound or control was added. Two fold serial dilutions of each active compound were made in DMF at concentrations ranging from1.5625 to100 µg/ml (w/v). DMF was used as the negative control, at a volume of 0.25 µl. Amphotericin B (80% purity) was used as a positive control at a concentration of 5 mg/ml. The agar was seeded with 4 x 10^7spores/ml. The lowest concentration of the test compound in which no inhibition occurred was defined as the MIC.

RESULTS

Evaluation of antimicrobial activities

Chlorophorin and Iroko (1 x10^4 µg/ml) demonstrated high anti-bacterial activity against *S. pneumoniae* and *B. coagulans.* These compounds did not have the same effect against *K. pneumoniae* and *E. coli.* Maackiain; Formononetin and Formononetin acetate showed no activity against *S. pneumonia*, *B. coagulan, K. pneumoniae* and *E. coli*. The MIC's for each of the plant compounds showed that chlorophorin was the statistically more active and was more effective than Vancomycin (the drug of choice for these organisms); (1.953 µg/ml) was required to inhibit the growth of *B. coagulans* and *S. pneumoniae* as compared to that required for Vancomycin to have the same effect at 30 µg/ml. Iroko was active against *B. coagulans* and *S. pneumoniae* and their MIC were 3.125 and 6.25 µg/ml respectively.

Maackiain was active against *S. pneumoniae* and had a MIC of 12.5 µg/ml. Formononetin was the only compound that was active against *K. pneumoniae* and it had a MIC of 25 µg/ml. The MIC's are shown in Table 2. None of the compounds showed activity against *M. tuberculosis* or *E. coli.* The plant extracts demonstrated significantly higher activity as compared to the control, Vancomycin. Chlorophorin against *B. coagulans* and *S. pneumoniae demonstrated* 93.5% more activity, while Iroko against *B. coagulans* demonstrated 89.58% and against *S.*

Table 3. Activity and the MIC for maackiain acetate, formononetin acetate, chlorophorin and Iroko against *F. verticilloides and A. flavus.*

Organism	Plant compound	Zones of inhibition ($1 X 10^4$)	MIC (µG/ML)	Total inhibition expressed as a % of control
F. verticilloides	Maackiain acetate	13 mm	1.56	99.97
	Formononetin acetate	2.5 mm	12.5	99.75
	Chlorophorin	9 mm	6.25	99.87
	Iroko	4.5 mm	12.5	99.75
	Amphotericin B	Control	5000	100
A. flavus	Maackiain acetate	negative	No effect	0
	Formononetin acetate	1 mm	25	99.5
	Chlorophorin	3 mm	6.25	99.87
	Iroko	2.7 mm	12.5	99.75
	Amphotericin B	CONTROL	5000	100

pneumoniae 79.17% more activity.

The seeded agar overlay method showed that maackiain acetate, formononetin acetate, chlorophorin and Iroko at 1 x 10^4 µg/ml inhibited the growth of *F. verticilloide,* with maackiain being the significantly more active compound. In contrast, formononetin acetate, chlorophorin and Iroko inhibited *A. flavus* while maackiain had no effect*. A. flavus* was most sensitive to chlorophorin and Iroko. Amphotericin B at a standardized concentration of 5 mg/ml was used as the control. Chlorophorin was effective at a concentration of 6.25 µg/ml against both *F. verticilloides* and *A. flavus* and that of Iroko demonstrated a twofold enhanced activity on the same strains as compared to chlorophorin. These results and the MIC's are summarised in Table 3. The bioautography method confirmed the results of the well diffusion seeded agar overlay test and showed that *F. verticilloides* was sensitive to maackiain acetate, formononetin, chlorophorin and Iroko (Figure 1) and *A. flavus* was sensitive to chlorophorin, Iroko and formononetin acetate and resistant to maackiain (Figure 2).

DISCUSSION

Medicinal plants possess many potentially valuable therapeutic agents that provide an impetus for further research (Samoylenko et al., 2009; Zhu et al., 2009; Kutchan and Dixon, 2005; Rios et al., 2005; Recio et al., 1989). Testing micro-organisms for their susceptibility to antimicrobials serves as an important aid to chemotherapeutic intervention. The methods commonly used are agar diffusion and broth dilution method (Jawez, 1989). Pellecuer et al. (1976) and Kordali et al. (2005) showed that different results can be obtained for different samples and to demonstrate this he used phenols and essential oils. In his study, it was shown that the agar diffusion method was more effective for phenolic compounds because of its polarity and less effectiveness for non-polar extracts like essential oils. The compounds tested in this study were previously defined as phenols (Oliver-Bever, 1986; Swinny, 1989). Maackiain was defined as a pterocarpan, formononetin an isoflavone and Iroko and chlorophorin aromatic phenolic compounds.

Hence, in this study, the agar diffusion method was used. The advantage of using this method was that small sample sizes could be used in the screening and the possibility of testing five or six compounds against a single micro-organism at any given time (Rios et al., 1988).

Maackiain and formononetin exhibited minimal activity against all the test bacteria, which could be attributed to their complex structures. Prindle (1983) reported several generalisations that can be made about the structural activity relation of simple and complexed phenols. The position of the alkyl chain may or may not influence activity; separation of the alkyl group from the phenol nucleus by an oxygen (methoxy) decreases activity (Veldhuizen et al., 2006; Ja Kim et al., 2006; Prindle, 1983; Suter, 1941; Klarmann and Shternov, 1936).

Maackiain and formononetin both have alkyl groups that are separated from the phenol nucleus by a methoxy group, which could possibly be the reason why these compounds showed minimal activity against the test bacteria.

The results in this study show that the gram negative bacteria were more resistant than the gram positive bacteria. Previous studies have shown similar results where the gram positive bacteria were more susceptible to plant compounds. In a study carried out by Taniguchi et al. (1978), it was found that of 79 plant extracts tested from 72 species of plants belonging to 35 families, 44 extracts gave positive results against gram positive bacteria, while some of the extracts showed minimal activity against the gram negative bacteria and no activity against *E. coli*. A study by Meyer and Afolayan (1995), on the anti-bacterial activity of extracts from *Helichrysum aureoniteus*, showed that the extracts were active against five gram positive bacteria tested and none inhibited the growth of five gram negative bacteria tested. None of the compounds in this study or the above studies were active against *E. coli.*

The anti-bacterial activity of chlorophorin and Iroko confirmed the results reported by Oliver-Bever (1986) on

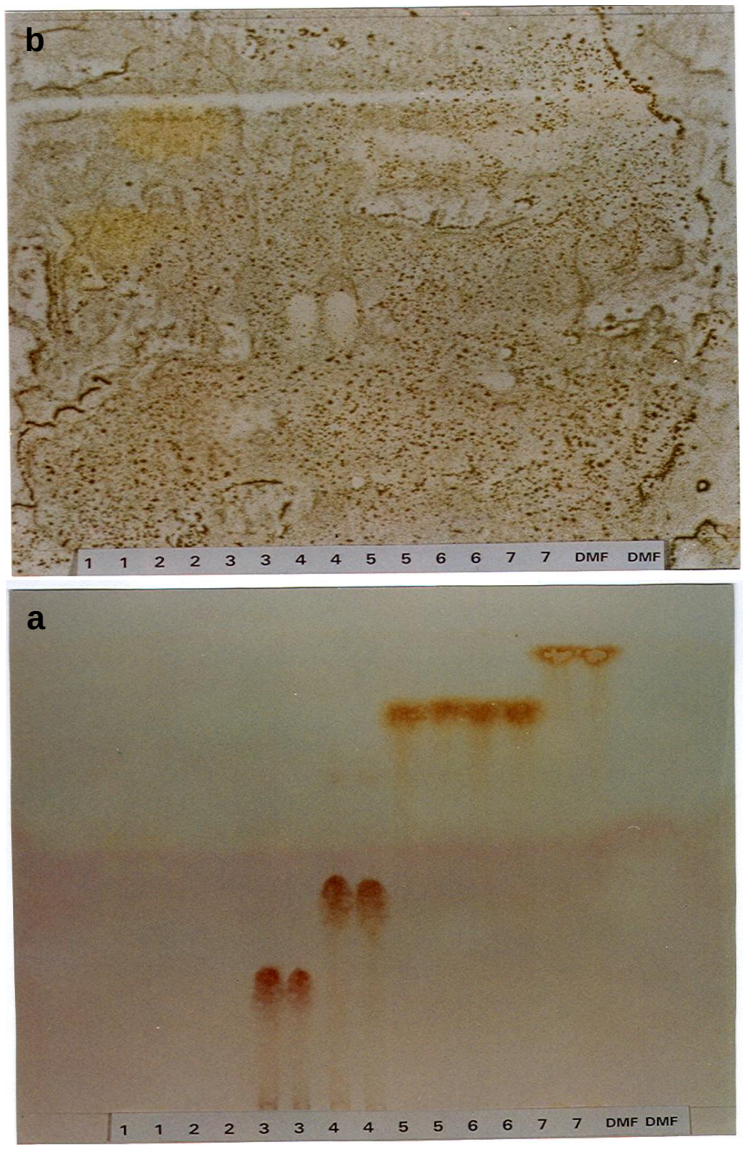

Figure 2. The effect of maackiain acetate, formononetin, chorophorin, Iroko on *A. flavus*. TLC plates with separated plant compounds (a) template and (b) with *A. flavus* grown on the TLC plates showing areas of no growth (arrow) in the same region as where the bands appear on the template.

other phenolic compounds. However, chlorophorin was more active than Iroko although both were phenolic compounds extracted from the same tree. To evaluate these compounds, the direct bioautographic method was used. According to Betina (1973), bioautography is the most important detection method for new or unidentified antimicrobial compounds. Fungi toxic products are measured qualitatively and quantitatively using this technique (Peterson and Edgington, 1969). In this study, two mycotoxin producing species *F. verticilloides* and *A. flavus* were tested against the four plant extracts maackiain, formononetin, chlorophorin and Iroko using the bioautographic method. In this study, all four compounds inhibited *A. flavus* with chlorophorin and Iroko being the most active against *F. verticilloides* was inhibited by maackiain.

The increased activity of chlorophorin could be attributed to the fact that chlorophorin has four hydroxyl groups, whereas Iroko has three hydroxyl groups. A study by Nick et al. (1995) showed that hydroxylated flavones have increased activity as opposed to those extracts that have fewer hydroxyl groups. Cushman et al. (1991), showed that hydroxylated flavones are known as inhibitors of protein kinases. This confirms the results reported by Yan et al. (2008) and Haslam (1996), that polyphenols in addition to giving the usual phenolic reaction they have the ability to precipitate proteins. In addition, Hagerman and Butler (1981) showed that phenols form irreversible complexes

with proline rich proteins, which could result in the inhibition of cell wall protein synthesis. These properties of phenols, may explain the mechanism of action of the plant extracts. These complexation reactions are of intrinsic scientific interest as studies in molecular recognition as the basis of possible functions.

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REFERENCES

Acharya TK, Chatterjee IB (1974). Science Culture, via Chermical Abstracts. 82(1975) 2861X, *Cassia tora.* 40:316.

Atlas RM, Brown AE, Dobra KW, Miller L (1984). Experimental Microbiology: Fundamentals and Applications. Exercise 48: Minimum Inhibitory Concentration (MIC) Method. pp. 267-269.

Balows A, Hausler WJ, Herrmann KL, Lsenberg HD, Shadomy HJ (1991). Manual of Clinical Microbiology. Fifth edition. American Society for microbiology. Washington, pp. 326-334.

Betina V (1973). Bioautography in paper and thin-layer chromatography and its scope in the antibiotic field. J. Chromatogr. 78:41-51.

Chipley JR, Uraih N (1980). Inhibition of *Aspergillus* growth and aflatoxin release by derivatives of benzoic acid. Appl. Environ. Microbiol. 40:352.

Cox PA, Balick MJ (1994). The ethnobotanical approach to drug discovery. Scientific J. America June. pp. 60-65.

Cushman M, Nagarathnam D, Geahlen RL (1991). Synthesis and evaluation of hydroxylated flavons and related compounds potential inhibitors of the protein-tyrosine kinase $P56^{lck}$. J. Nat. Prod. 54:1345-1352.

Davidson PM (1993). Antimicrobials in Foods. 2nd edition, In: P. M. Davidson and A. L. Branen (Eds.), Marcel Dekker, Inc. New York, pp. 263-305.

Franson RI (1992). Culture of animal cells: A manual of basic techniques. New York, Alan R. Liss Inc. pp. 124-136.

Hagerman AE, Butler LG (1981). The specificity of proanthocyanidin - protein interactions. J. Biol. Chem. 256:4494-4497.

Hailu T, Endris M, Kaleab A, Tsige GM (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. J. Ethnopharm. 100:168-175.

Harrison HF, Peterson JK, Snook ME, Bohac JR, Jackson DM (2003). Quantity and Potential Biological Activity of Caeffeic Acid in Sweet Potato [*Ipomoea batatas* (L.) Lam.] Storage Root Periderm. J. Agric. Food Chem. 51:2943-2948.

Haslam E (1996). Natural Poyphenols (Vegetable Tannins) as Drugs: Possible modes of action. J. Nat. Prod. 59:205-215.

Hoffmann JJ, Timmermann BN, McLaughlin SP, Punnapayak H (1993). Potential anti microbial activity of plants from the Southwestern United States. Int. J. Pharm. 31:101-115.

Ja Kim H, Jung Kim E, Seo SH, Shin CG, Jin C, Lee YS (2006). Vanillic Acid Glycoside and Quinic Acid Derivatives from Gardeniae Fructus. J. Nat. Prod. 69(4):600-603.

Jawez E (1989). Principles of antimicrobial drug action. In: B. G. Katzung(Ed.), Basic and Clinical Pharmacology, 4th Edition. Appleton and Lange, California. pp. 542-552.

Kim HJ, Chen F, Wang X, Rajapakse N, (2006). Effect of Methyl Jasmonate on Secondary Metabolites of Sweet Basil (*Ocimum basilicum* L). J. Agric. Food Chem. 54:2327-2332.

Klarmann EG, Shternov VA (1936). Bactericidal value of coal-tar disinfectants. Limitation of the *B. typhous* phenol coefficient as a measure. Ind. Eng. Chem. 8:369-372.

Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A (2005). Screening of Chemical Composition and Antifungal and Antioxidant Activities of the Essential oils from three Turkish *Artemisia* Species. 53:1408-1416.

Kubo A, Kubo I (1995). Anti-microbial agents from *Tanacetum balsamita*. J. Nat. Prod. 58:1565-1569.

Kutchan T, Dixon RA (2005). Physiology and metabolisim Secondary metabolism: nature's chemical reservoir under deconvolution. Curr. Opin. Plant Biol. 8:227-229.

Lee KH (2004). Current Developments in the Discovery and Design of New Drug Candidates from Plant Natural Product Leads. J. Nat. Prod. 67:273-283.

Leven M, Van den Berghe DA, Mertens F, Vlietinck A, Lammens E (1979). Screening of higher plants for biological activitites. In: Antimicrobial activity. Planta Medica. J. Med. Plant Res. 36:311-321.

Lsenberg HD (1980). Chemical Microbial Procedures Handbook. ASM, Publishers. Vol.15:13.1-13.13.

Lu TK, Collins JJ (2009). Team combats antibiotic resistance with engineered viruses. Proceedings of the national Academy of Sciences. March 2nd in Biology/Microbiology. Massachusetts Institute of Technology.

McCutcheon AR, Ellis SM, Hanock REW, Towers GHN (1994). Anti-fungal screening of medicinal plants of British Columbian native peoples. J. Ethnopharm. 44:157-169.

McLaughlin JL (2008). Paw Paw and Cancer: Annonaceous Acetogenins from Discovery to Commercial Products. J. Nat. Prod. 71:1311-1321.

Meyer JJM, Afolayan AJ (1995). Antibacterial activity of *Helichrysum aureonitens* (*Asteraceae*). J. Ethnopharm. 47:109-111.

Mitscher LA, Drake S, Gollapudi SR, Okwute SK (1987). A modern look at Folkloric use of anti-infective agents. J. Nat. Prod. 50:1025-1040.

Nick A, Rali T, Sticher O (1995). Biological screening of traditional medicinal plants from Papua New Guinea. J. Ethnopharm. 49:147-156.

Oliver Bever BEP (1986). Medicinal plants in tropical West Africa. University Press. Cambridge. pp. 1-8.

Pellecuer S, Allergrini J, Simeon de Buochberg M (1976). Huiles essentielles bactericides and fungicides. Review from the Pasteur Institute de Lyon. 9:135-159.

Peterson CA, Edgington, LU (1969). Quantitative estimation of the fungicide benomyl using a bioautograph technique. J. Agric. Food Chem. 17:898-899.

Prindle RF (1983). Phenolic compounds. Disinfection, Sterilization and Preservation, 3rd ed., S. S. Block and Febiger (Editors). (Marcel Dekker, Inc) Philadelphia. pp. 197-205.

Recio MC, Rios JL, Villar A (1989). A review of some anti-microbial compounds isolated from medicinal plants reported in the literature 1978-1988. J. Phytother. Res. 3:117-125.

Rios JL, Recio MC (2005). Medicinal plants and antimicrobial activity. J. Ethnopharm. 100:80-84

Rios JL, Recio MC, Villar A (1988). Screening methods for natural products with antimicrobial activity. A review of the Literature. J. Ethnopharm. 23:127-149.

Samoylenko V, Ashfaq MK, Jacob MR, Tekwani BL, Khan SI, Manly SP, Joshi VC, Walker LA, Muhammad I (2009). Indolizidine, Antiinfective and Antiparasitic Compounds from *Prosopis glandulosa* var. *glandulosa*. J. Nat. Prod. 72:92-98.

Shan B, Cai YZ, Brooks JD, Corke H (2007). Antibacterial Properties and Major Bioactive Components of Cinnamon Stick (*Cinnamomum burmannii*): Activity against Foodborne Pathogenic Bacteria. J. Agric. Food Chem. 55:5484-5490.

Suter CM (1941). Relationships between the structures and bactericidal properties of phenols. Chem. Rev. 28:269-281.

Swinny EE (1989).The structure and synthesis of Metabolites from *Virgilia oroboides* and *Chlorophora excelsa* (Iroko).M Sc. Dissertation, University of Durban-Westville, Durban, South Africa.

Taniguchi M, Chapya A, Kubo I, Nakanishi K (1978). Screening of East African Plants for Antimicrobial activity. Chem. Pharm. Bull. 26:2910-2913.

Veldhuizen EJA, Tjeerdsma-van Bokhoven JLM, Zweijtzer C, Burt SA, Haagsman HP (2006). Structural requirements for the Antimicrobial Activity of Carvacrol. J. Agric. Food Chem. 54:1874-1879.

Valle E (1957). On anti-fungal factors in potato leaf. Acta Chem. Scandinavica. 11:395-399.

Walsh G (1988). Bio-pharmaceuticals : Biochemistry and Biotechnology. John Wiley and sons, Chichester. pp. 24 -56.

Washington JA, Warren E, Karlson AG (1972). Stability of Barium sulfate Turbidity standards. J. Appl. Microbiol. 24:1013-1017.

World Health Organisation (2005). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. WHO, Geneva, Switzerland.

Wu HS, Raza W, Fan JQ, Sun YG, Bao W, Shen QR (2008). Cinnamic Acid Inhibits Growth but Stimulates Production of Pathogenesis Factors by in Vitro Cultures of *Fusarium oxysporum* f.sp. niveum. J. Agric. Food Chem. 56:1316-1321.

Yan Y, Hu J, Yao (2009). Effects of Casien, Ovalbumin, and Dextran on the Astringency of Tea Polyphenols Determined by Quartz Crystal Microbalance with Dissipation. Langmuir American Chemical Society. 25:397-402.

Yoon KD, Jeong DG, Hwang YH, Ryu JM, Kim J (2007). Inhibitors of Osteoclast Differentiation from *Cephalotaxus koreana*. J. Nat. Prod. 70:2029-2032.

Zhu Y, Zhang P, Yu H, Li J, Wang MW, Zhao W (2009). Anti-*Helicobacter pylori* and Thrombin Inhibitory Components from Chinese Dragon's Blood, Dracaena cochinchinensis. J. Nat. Prod. 70:1570-1577.