

**ANTIMICROBIAL ACTIVITIES OF THE STEM BARK OF
TRICHILIA TESSMANNII (HARMS) AND *TRICHILIA MONADELPHA*
(THONN) J.J. DE WILDE BOTH OF THE FAMILY MELIACEAE**

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ABSTRACT

The present work has evaluated the antimicrobial activities of two medicinal plants; *Trichilia tessmannii* (Harms) and *Trichilia monadelpha* (Thonn) J.J. de Wilde both of the family Meliaceae. Dried pulverized plant materials of both plants were serially extracted by soxhlet extraction to obtain three extracts of each plant sample (i.e. petroleum ether, ethyl acetate and methanol extracts). The antimicrobial activity was determined by broth dilution method using standardized bacteria suspensions of *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and the fungus, *Candida albicans*. Broad-spectrum antimicrobial activity was observed for all extracts except the petroleum ether extract of *T. Tessmannii*. A minimum inhibitory concentration (MIC) of 0.625 mg/mL was determined as the highest activity for the ethyl acetate extract of *T. Tessmannii* against *Pseudomonas aeruginosa* and *Candida albicans*. Lowest MIC of >10 mg/mL was however recorded

by the petroleum ether extract of *T. Tessmannii* against *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

KEYWORDS: *Trichilia monadelpha*, *Trichilia tessmannii*, Infectious diseases, broth dilution, antimicrobial activity.

INTRODUCTION

According to the global burden of disease, infectious diseases are the world's biggest killers, affecting both children and adults. Infectious diseases account for 10% deaths in the industrialized countries while accounting for 60% in the developing countries.^[1] It is believed that about 33% of all reported deaths are caused by infectious and parasitic disease in Ghana.^[2] Against the constant background of established infections, epidemics of new and old infectious diseases periodically emerge, greatly magnifying the global burden of infectious diseases.^[3] The recent outbreak of Ebola Virus Disease (EVD) in some countries in West Africa attest to these facts. Interestingly, contemporary reports have directly or indirectly implicated the ethiology of some cancers to microbial and viral infections. In addition, coronary heart diseases have also been linked to some chronic infections with microorganisms.^[4] *Helicobacter pylori* is known to be associated with Peptic ulcer and gastric carcinoma.

The spread of multidrug-resistant pathogen is also a major setback to the treatment of infectious diseases the world over. Resistance to antibiotics is an inevitable challenge and the management of the process for all new antibiotics.^[5] There is therefore the need to search for new leads and novel compounds to ameliorate this public health issue. Plants have provided a source of inspiration for novel lead compounds and drugs and these have contributed to human health and well-being and as such considering them for development of new antimicrobial may be worthwhile.^[6]

Trichilia monadelpha (Thonn) JJ De Wilde (local 'Akan' name: Otanduro) is an evergreen, small to medium-sized tree of up to 12-20 m tall with smooth pale grey to greenish brown or dark brown bark surface and a spreading crown. The leaves are compound having 3–6 pairs of leaflets with stipules absent and petiole of about 4–13 cm long. Leaflets opposite, ovate to obovate, 4–26 cm × 1.5–9 cm, cuneate to obtuse at base, acuminate at apex, hairy below when young but glabrescent and pinnately veined. Flowers are unisexual, very similar in appearance, may be greenish yellow or greenish white in colour and usually scented. The fruits are often found in clusters, with obovoid to globose capsule of about 1.5–2.5 cm in diameter, 2–4-lobed, with up to 6-seeds.^[7]

A decoction of the bark or the pulped bark is considered to have antiseptic and curative properties and so applied externally to wounds, cuts, sores and skin infections including yaws.^[8] A decoction of the bark is also used to treat gonorrhoea by mouth or by baths and

taken for syphilis and wash for chancroid and syphilitic sores, whereas small amounts of pulped bark are eaten or applied as an enema to treat gastro-intestinal complaints in Ghana.^[8,9] The stem bark of the plant has been used in Ghanaian traditional medicine to treat pain, psychoses, epilepsy and inflammation for many years and the efficacy is widely acclaimed in different communities in Ghana.^[8,10] Its analgesic property is applied in the treatment of arthritis, lumbago and oedema by massaging topically with the bark-pulp in Ghana.^[8] Pharmacological studies have revealed that the ethanolic stem bark extract of *T. monadelpha* has anti-trypanosomal and antiplasmodial activities.^[11] Various stem bark extracts of the plant have been shown to have anti-inflammatory^[12] as well as analgesic properties.^[13]

Trichilia tessmannii (Harms) (local 'Akan' name: Otanduro-nini) is on the other hand regarded traditionally as the male of *Trichilia monadelpha* and is expected to be more potent in medicinal properties. It is a medium-sized forest tree with a height of about 30 m. It has a straight cylindrical base of about 70 cm in diameter bearing a dense crown. The leaves are pari-pinnate having opposite leaflets with soft orange hairs beneath. The fruit is a three (3) chambers, sub-globose, stalked capsule of a size of about 3 cm across. The fruits are pinkish to purple or purplish red in colour. There are two (2) seeds in each chamber of the fruit. This tree is easily recognized by the grayish and slightly scaly bark which peels off in plates. The older trees slowly exude a little sweet-scented creamy or yellowish latex.^[14, 15] A decoction of the bark is used to treat stomach-aches and as a purgative (de Wilde). *Trichilia* bark extract has been reported to have therapeutic effect against microbes, dysentery, dyspepsia, sores/ulcers.^[16]

This study reports the antimicrobial spectrum of various extracts (petroleum ether, ethyl acetate and methanol extracts) of the stem barks of *Trichilia tessmannii* (Harms) and *Trichilia monadelpha* (Thonn) J.J. de wilde.

MATERIALS AND METHODS

2.1 Materials

All media (nutrient broth and sabouraud broth), chemicals (3-(4,5-dimethylthiazole-2yl)-2,5-diphenyltetrazolium bromide) (MTT), dimethyl sulfoxide (DMSO) and solvents (petroleum ether, ethyl acetate and methanol) used for this work were obtained from Oxoid Ltd, Basingstoke UK. *Escherichia coli* (NCTC-9002, *Pseudomonas aeruginosa* (ATCC 27853)), *Staphylococcus aureus* (ATCC 25923, and *Bacillus subtilis* (NCTC 10073)) were obtained

from the Department of Microbiology, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST. The fungus; *Candida albicans* (ATCC 10231) was a clinical strain obtained from Komfo Anokye Teaching Hospital (KATH), Kumasi Ghana.

2.2 Methods

2.2.1 Plant material collection and authentication

The stem barks of *T. tessmannii* and *T. monadelpha* were collected from Asakraka-Kwahu (Eastern Region) in January, 2013 and Bomaa (Brong Ahafo Region) in February, 2013 respectively. Both plant samples were authenticated at the Department of Herbal Medicine, in the Faculty of Pharmacy and Pharmaceutical sciences, Kwame Nkrumah University of Science and Technology (KNUST). Voucher specimen numbers KNUST/T.T/2013/S003 KNUST/T.M/2013/S006 were assigned respectively. The specimens were deposited at the Department's herbarium.

2.2.2 Preparation of the stem bark extract

The dead cells on the bark were scrapped and the remaining portions of the stem barks washed with water to rid of all unwanted materials. The barks were chopped into pieces and air-dried at room temperature for a week. The dried materials were coarsely powdered using a mechanical grinder. 200 g of pulverized plant material of each plant was extracted by successive extraction using the soxhlet apparatus with 500 mL each of petroleum ether, ethyl acetate and methanol successively for 48 hrs. The extracts obtained were filtered through No.1 Whatman's filter paper and evaporated to dryness using a rotary evaporator (R-114, Buchi, Switzerland) at reduced temperature of about 50 °C and a rotational speed of 5 revolutions per minute (rpm). The concentrates were further dried to obtain the dried petroleum ether, ethyl acetate and methanol extracts which were used in the preliminary antimicrobial screening.

2.2.3. Antimicrobial assay

Preparation of standard suspension of microorganisms

The five (5) microorganisms were selected and used in this research based on their implication in most infections. The bacteria strains were cultured overnight at 37 °C in nutrient broth and the fungus was cultured overnight at 25 °C in sabourand dextrose agar. Standardized suspensions of microorganisms were prepared from the overnight broth cultures. Standardization was by serial dilutions of cultures in sterile normal saline to achieve

a suspension of equal turbidity using 0.5 Mc. Farland standards by visual comparison to achieve a dilution containing approximately 10^5 CFU/mL.

2.2.4 Preparation of media

Nutrient broth

25 g of nutrient broth powder was weighed into a beaker. It was dissolved in about 500 mL of distilled water and stirred to dissolve. Enough freshly prepared distilled water was added to produce 1000 mL. 10 mL quantities were poured into test tubes and plugged firmly with cotton wool. They were then sterilized by heating in an autoclave at 121 °C for 15 minutes.

Saborand dextrose broth.

65 g of the powder was weighed and dissolved in 1000 mL of freshly prepared distilled water. The mixture was heated while stirring until it dissolved completely. Aliquot of 20 mL of the mixture was poured into test tubes, plugged with cotton wool and sterilised in an autoclave at 121 °C for 15 minutes.

2.2.5 Micro-dilution assay (Broth dilution)

In the determination of the minimum inhibitory concentration (MIC), the method used was micro-well dilution as described by Ellof.^[17] Briefly, the inocula of microorganisms were prepared from a 12 -hour broth cultures and serial dilutions were made to achieve a suspension of 10^5 CFU/mL.

The various plant extracts were solubilised with 2 %v/v Dimethyl Sulfoxide (DMSO) to obtain concentrations of between 0.312 mg/mL to 10 mg/mL. The 96-well sterile plates were prepared by dispensing into each well 100 µL of double strength nutrient broth, 100 µL of the test samples and 20 µL of the inoculums of standardized suspensions of the various cultures of test organisms.

The micro-plates were then incubated at 37 °C (bacteria) and at 25 °C (fungus) for 24 hrs. Growth of the microorganisms was determined by adding 20 µL of a 0.125 %w/v solution of tetrazolium salt (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) (MTT)) and incubating for further 30 minutes. Dark wells indicate the presence of microorganisms as the dehydrogenase enzymes in the live bacteria react to form a dark complex with the tetrazolium salt.

Ciprofloxacin and ketoconazole were used as positive control and DMSO as negative control. All the experiments were triplicated.

3. RESULTS

3.1 Antimicrobial Assay

The antimicrobial activity of the stem barks of the two selected plants were tested against five microorganisms of which, two (2) were Gram positive, two (2) Gram negative and One (1) fungus, by the use of broth microdilution using the 96-well microtiter plates . The MIC was taken as the least concentration that inhibited any visible growth of organisms after a 24 hour incubation period.^[17, 18]

The antimicrobial activities were assessed using methanol, ethyl acetate and petroleum ether extracts of *T. Tessmannii* and *T. Monadelpha*. These were tested against the five (5) selected microorganisms. The results for the antimicrobial assays are summarized in Table 1.

Table 1: Minimum inhibitory concentrations (MIC) for the three extracts of *T. Tessmannii* (TT) and *T. Monadelpha* (TM)

Test Organisms	MIC (mg/mL)					
	<i>Methanol Extract</i>		<i>Ethyl acetate Extract</i>		<i>Pet ether Extract</i>	
	TT	TM	TT	TM	TT	TM
Staph. aureus	2.50	2.50	1.25	10.00	10.00	10.00
B. subtilis	1.25	2.50	1.25	5.00	>10.00	2.50
E. coli	2.50	1.25	1.25	5.00	>10.00	2.50
P. aeruginosa	1.25	2.50	0.625	5.00	>10.00	2.50
C. albicans	1.25	2.50	0.625	5.00	10.00	10.00

Table 2: Minimum inhibitory concentrations (MIC) for the reference drug.

Reference Drug	MIC (µg/ml)				
	Staph. aureus	B. subtilis	E. coli	P. aeruginosa	C. albicans
Ciprofloxacin	0.25	0.1	0.1	0.1	NT
Ketoconazole	NT	NT	NT	NT	5

KEY: NT = Not Tested.

4. DISCUSSIONS

4.1 Antimicrobial assay

Trichilia monadelpha known in Akan as “Otanduro” which means ‘hate medicine’ due to its bitter taste and *Trichilia tessmannii* also known in Akan as “Otanduro-nini” which means ‘the male hate medicine’ have long been used by the rural folks for their medicinal properties.

They have been used for the treatment of various skin infections and some sexually transmitted diseases such as gonorrhoea and syphilis.^[8] There is great similarity between the physical features of *T. tessmannii* and *Trichilia monadelpha* (Thonn) JJ De Wilde^[19] with the possibility of mistaking one for the other. In addition, the photochemical principles identified in both stem barks are similar. We have reported the following phytochemical principles in the stem bark of *T. Tessmannii*; tannins, reducing sugars, anthraquinones, alkaloids, saponins, glycosides, cardiac glycosides, terpenoids, coumarins and phytosterol.^[20] Ben *et al.*,^[21] have also reported from the stem bark of *Trichilia monadelpha* the same phytochemicals; tannins, reducing sugars, anthraquinones, alkaloids, saponins, glycosides, cardiac glycosides, terpenoids, coumarins and phytosterol.

The antimicrobial activity was assessed on the three extracts obtained from serial extraction of the stem bark of the two selected plants; *T. Tessmannii* and *T. Monadelpha*. The broth dilution method was used in determining the minimum inhibitory concentrations (MICs).^{[17,}
^{18]} Broth dilution was selected as a method for the antimicrobial assay because; dilution methods are more reproducible and yield better quantitative evaluation than agar disk diffusion method. In addition, broth micro-dilution using the 96-well microtiter plates is currently more preferred due to its fast, well standardized approach and reliability. Furthermore, it is now considered as the reference international testing method for antimicrobial activity.^[22]

The results from the antimicrobial assay performed showed that the three extracts of *T. Tessmannii* exhibited varying inhibitory effects against the five selected microorganisms (two Gram positive, two Gram negative and one fungus). The results observed with the use of the ethyl acetate extract (containing semi-polar constituents of the plant) were preeminent against all the selected microorganisms. The MICs recorded were between the ranges 0.625 mg/mL - 1.25 mg/mL. The best activity observed with the use of ethyl acetate extract was against *P. aeruginosa* and *C. albicans* with an MIC of 0.625 mg/mL.

The polar constituents of the plant were more susceptible to be extracted in methanol. The MICs recorded by the methanolic extract in the range 1.25 mg/mL to 2.5 mg/mL was however less in potency compared to the ethyl acetate extract. Notwithstanding this, varied inhibitory effect was observed against all the selected microorganisms as in Table 1. Activity was significantly high against *B. subtilis*, *P. aeruginosa* and *C. albicans* at MIC of 1.25 mg/mL in comparison to the other microbes. The pet ether extract on the other hand

demonstrated the lowest activity against all the selected microorganisms showing no inhibition against *B. subtilis*, *P. aeruginosa* and *E. coli* even at a high concentration of 10 mg/mL. There was however inhibition of *Staph. aureus* and *C. albicans* at MIC as high as 10 mg/mL. The implication of the results is the possible absence of non-polar constituents in the plants under investigation or that these non-polar constituents dissolvable in pet ether have no significant activity against the selected microorganisms. It is suggestive that the antimicrobial activity of *T. Tessmannii* may reside more in the ethyl acetate extract which would contain the semi-polar constituents of the plant as it gave the most potent activity at the lowest MICs compared to all the other extracts. The ethyl acetate extract would further be processed for isolation, purification, characterization and structural elucidation.

A rather interesting scenario emerges with the results of the antimicrobial activities obtained for the three extracts of *T. Monadelpha*. Whereas greater activity was observed with the ethyl acetate extract of *T. Tessmannii*, the unsurpassed activity was obtained for the methanolic extract of *T. Monadelpha*, exhibiting varying inhibitory effects against the five selected microorganisms. The minimum inhibitory concentrations (MICs) were in the range 1.25 mg/mL - 2.5 mg/mL (Table 1) with the highest activity observed against *E. coli* at a MIC of 1.25 mg/mL. The pet ether extract followed in intensity of activity by showing varied inhibitory effect against all the selected microbes as a consequence of *T. Monadelpha*'s non-polar constituents. The MIC range of 2.5 mg/mL - 10 mg/mL was obtained with greater activity against *B. subtilis*, *P. aeruginosa* and *E. coli* at MIC of 2.5 mg/mL. A highlighted distinction between the antimicrobial activities of the two plants under study was seen with the low activity exhibited by the ethyl acetate extract of *T. Monadelpha* against all the selected microorganisms. There was however inhibition of *B. subtilis*, *P. aeruginosa*, *E. coli* and *C. albicans* at MIC of 5 mg/mL and *Staph. aureus* at MIC of 10 mg/mL. These results suggest the poor antimicrobial activities of the semi-polar constituents of *T. Monadelpha* compared to the polar and non-polar constituents. Furthermore, it is implicit that the polar constituents as contained in the methanolic extract of *T. Monadelpha* exhibit potent antimicrobial activities and might further be exploited for isolation, purification, characterization and structural elucidation.

Several researchers have demonstrated that some phytochemicals principles such as terpenoids,^[23] flavonoids,^[24] coumarins,^[25] saponins,^[26, 27] tannins and alkaloids,^[28] possess certain antimicrobial properties. The presence of these phytochemicals such as tannins,

reducing sugars, anthraquinones, alkaloids, saponins, glycosides, cardiac glycosides, terpenoids, coumarins and phytosterol^[20, 21] in these plants may be responsible for the observed antimicrobial activities.

A comparative evaluation of the activities of the extracts of both plants to the reference drugs revealed that all the extracts with the exception of the petroleum ether extract of *T. Tessmannii* showed both broad spectrum antibacterial and antifungal activity while ciprofloxacin and ketoconazole (reference drugs) only showed their respective characteristic antibacterial and antifungal activities (Table 2). In terms of antimicrobial spectrum, the extracts from both plants may be said to have better antimicrobial spectrum than each of the reference drugs since the extracts showed both antibacterial and antifungal properties.

In relation to the MICs, those of reference drugs were better than the extracts. However the reference drugs are single compounds while the extracts are crude; thus the extracts are a mixture of unidentified active compounds. Therefore the effects observed by these extracts may be as a result of one or more of these. The other compounds may be masking or antagonising the effect produced by these extracts. Again, the higher MICs of the extracts as compared to the lower MICs for the reference drugs could be as a result of the responsible compound(s) in the extracts being in lower concentration. In order to expound the full effect of the extracts, isolation purification and characterisation of the active compound(s) responsible for the observed activity will be the quest for further research which is underway.

6. CONCLUSION

From the results of the antimicrobial assay, it may be concluded that all the extracts except the petroleum ether extract of *T. Tessmannii* had broad-spectrum antimicrobial activity. The highest activity was obtained by the ethyl acetate extract of *T. Tessmannii* against *Pseudomonas aeruginosa* and fungus, *Candida albicans* at a minimum inhibitory concentration (MIC) of 0.625 mg/mL. The lowest activity was obtained by the petroleum ether extract of *T. Tessmannii* given a MIC of >10 mg/mL against *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The implication of these results is that both plants; *Trichilia tessmannii* (Harms) and *Trichilia monadelpha* (Thonn) J.J. de wilde might be exploited as natural drug products for managing infections caused by the tested organisms.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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