ISSN: 2230-9926

Available online at http://www.journalijdr.com

IJDR

International Journal of Development Research
Vol. 5, Issue, 06, pp. 4655-4659, June, 2015

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIBACTERIAL ACTIVITY OF *ODONTONEMA STRICTUM (ACANTHACEAE)* AGAINST SELECTED BACTERIA

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ARTICLE INFO

Article History:
Received 02nd March, 2015
Received in revised form
29th April, 2015
Accepted 11th May, 2015
Published online 28th June, 2015

Key words:
Antibacterial activity,
Odontonema strictum,
Phytochemicals,
MRSA,
Klebsiella

ABSTRACT

The evaluation of the antibacterial activity using five bacterial strains (*Klebsiella, Shigela, Salmonella, Escherichia coli* and *Staphylococcus aureus*) showed that the most active leaf extract was that of *Odontonema strictum (Acanthaceae)* compared to that of *Solanum torvum*, *Symphytum officinale L.* and *Aphelandra squarrosa*. The inhibition diameters were measured and no resistance was observed at the concentration of 100 g/ml across all bacterial strains. The Activity Index (AI) values of *Odontonema strictum (OSM)* were determined using chloramphenicol as a positive standard antibiotic. The results showed that *OSM* is four fold more bactericidal than the standard on *Klebsiella*. Qualitative phytochemical screening of the extracts indicated the presence of flavonoids, carbohydrates, saponins, glycosides, tannins, steroids, and terpenoids.

INTRODUCTION

Plant natural products have always played an important role in discovery of new medicines. It has been estimated that between 25-50% of medicines have their origins in medicinal plants (David A. Akinpelu *et al*., 2009). Secondary metabolites which are ubiquitous in plants represent a special group of molecules in drug discovery research some of which possess antimicrobial properties. The recent emergence of multi-drug resistant microorganisms poses a serious challenge in Public Health. An increasing number of bacteria are now developing resistance to commercial antibiotics (Eloff *et al*., 2005). For instance, methicillin, a beta-lactam antibiotic, was used to treat infections caused by certain Gram-positive bacteria (*Staphylcoccus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes,* and *Streptococcus pneumonia)* but today, infections with Methicillin-Resistant *Staphylococcus Aureus* (MRSA) have become a major challenge , particularly in hospitals (Rand and Dale, 2007). Resistance to Vancomycin in the United States among patients infected with *Enterococcus faecium* amounts to approximately 20 to 30% (Thomas L. Lemke *et al*., 2008). According to Po-Ren Hsueh and Kwen-Tay Luh (2002), *Streptococcus pneumoniae* exhibits resistance to penicillins, cephalosporins, trimethoprim-sulfamethoxazole, macrolides and fluoroquinolones (ciprofloxacin). Furthermore, vancomycin-resistant *Staphylococcus aureus* (VRSA) and multidrug-resistant (MDR) strains of this organism have been reported (Simon Gibbons, 2004). Therefore, the emergence of drug resistant bacterial strains across most commercially marketed antibiotics, necessitates intensified research efforts in the discovery and development of novel antibiotics. Africa, with its rich fauna and flora, is a big resource for medicinal plants. However, there is a dearth of phytochemical knowledge on many herbal medicines mainly, due to lack of laboratory facilities, qualified scientists, and financial support. This research originated from an observation made on a dog which was found regularly digging and eating the roots of *OSM*, a plant mainly found in tropical regions of the world. This was very strange indeed since the dog selectively dug and ate the roots from *OSM* over other plants. It was further observed that, after consuming the roots, the dog did not suffer any immediate observable consequences. Since consumption of these roots neither resulted in death nor any observable biological response, it may be assumed that the plant material

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was safe at the dosage the dog was consuming. It is possible that the dog may have been using the roots for some kind of remedy or nutritional supplementation. Dogs usually do dig for small animals that hide in the holes. So the question that seeks an answer was "why would a dog eat these roots?" Many hypotheses were formulated to explain the dog's behavior towards the plant. The antimicrobial property of the plant was thought as the possible explanation. According to literature sources, *Odontonema callistachyum* has been shown to exhibit antimicrobial properties (Giovannini P. Heinreich *et al*., 2008). Moreover, *Odontonema tubiforme* has anti-inflammatory properties and is used to induce child birth in Panama (Caballero-George C and Mahabir P. Gupta, 2011). Furthermore, anti-hypertensive properties of *OSM* were established by S. Ouedraogo and co-workers (2005). The genus *Odontonema* is, therefore, a potential source of new bioactive secondary metabolites. The aim of this study was to evaluate the antimicrobial activity of leaf extracts of the species *OSM,* separate the fractions using chromatographic methods and hence determine the most active fraction. To achieve this, in addition to *OSM*, three other plants were selected for preliminary antibacterial testing. Among these three plants, two (*Solanum torvum and Symphytum officinale L.*) are used in folklore medicine in Zambia while the third is of the *Acanthaceae* family (*Aphelandra squarrosa*).

MATERIALS AND METHODS

Plant collection and identification

Plant specimens were collected in Lusaka (January-February 2014) and taxonomically identified by Dr. Chuba and his team of University of Zambia Department of Biological Sciences. Voucher specimens were prepared and deposited in the Herbarium of the Department of Biology.

Fig.1. The species *Odontonema strictum*

Extraction and fractionation

The plant samples were shade-dried at room temperature and finely powdered using a blender. The powder (310 g) was soaked in a mixture of methanol (MeOH) and dichloromethane (DCM) (1/1//v/v) for 24 h at room temperature, filtered and solid residue rinsed with the same mixture of solvents and evaporated at room temperature. The dried crude extracts were kept in air tight containers and stored at 4 °C. Each extract was used further for antimicrobial activity determination and chromatographic separations (Rabia Naz *et al*., 2011).

The microbiological strains

The organisms available for microbial studies (one gram negative and four gram positives) were: *Staphylococcus aureus (SA)* [Locally Isolated Organism (LIO)], *Escherichia coli (Ec) (*ATCC 25922), *Klebsiella ssp. (KlB)* (LIO), *Shigella ssp. (ShI)* (LIO) and *Salmonella ssp. (SaL)* (H9812). All the bacteria were obtained from the University Teaching Hospital (UTH), Lusaka Zambia. All the cultures were maintained on Mueller-Hilton agar at 4 ºC. The cells were inoculated and incubated at 37 ºC in Mueller-Hilton broth for 12 hours prior to the screening procedure.

Preliminary phytochemical screening of *OSM*

The dry powdered leaf material (50 g) of *OSM* was extracted with a 1:1 mixture of MeOH and DCM (800 mL) for 24 hours. The supernatant was filtered through Whatman N°1 filter paper, and the residue was rinsed with MeOH, and refiltered. The filtrates were combined and left to dry at room temperature. The evaporation of the solvent afforded a crude extract (4.5 g) which was later subjected to standard qualitative phytochemical analysis (Evans WC, 1996).

Table 1. Phytochemical screening of *OSM* extract

Chemical Constituent	Test	MeOH/DCM Extract
Alkaloids	Mayers	±
Carbohydrates	Fehling's	++
Glycosides	Keller-kiliani	+
Saponins	Foam	+++
Phytosterols	Libermann-Buchard	+
Tannins	Ferric chloride	+++
Flavonoids	Shinoda test	+

Legend: ± = low present; + = present; ++ = abundant; +++ = very abundant

Preparation of inocula (Agar well diffusion method)

Inoculants were prepared from subcultures of bacteria as follows (Ndip RN *et al*., 2007). Four to five colonies of the isolates were emulsified in sterile normal saline and turbidity adjusted to 1×10^8 CFU/ml (corresponding to 0.5 McFarland standards). A sterile cotton swab was dipped into the standardized bacterial suspension and used to evenly inoculate the Mueller Hinton agar plates (Oxoid, Basingstoke, England). The plates were allowed to dry for 3 to 5 min. Wells about 6 mm in diameter were aseptically punched with a sterile cork borer (5 holes per plate) and filled with 50 µl extracts of different concentrations (100, 10 and 1 mg/ml). The plates (in duplicate) were left for 30 min in order for the extracts to

diffuse into the agar before incubation. Thereafter, they were incubated at 37 °C for 24 h and the zone of inhibition measured to the nearest millimeter. The dry extracts were dissolved in 80% acetone (AcO) in distilled water giving a stock concentration of 100 mg/ml and the working concentrations were prepared by ten-fold serial dilution technique ranging from 1 to 100 mg/mL. The mean zone diameter of inhibition was calculated for each concentration of extract (1, 10 and 100 mg/ml) and 80% AcO as a negative control (Boyonova L. *et al*., 2005). Ampicillin, Tetracycline, Chloraphenicol and Amoxicillin (10 µg/discs) were used as positive reference standards to determine the sensitivity of each bacterial species tested. All tests were performed in ten replicates and repeated five times to minimize test error.

RESULTS AND DISCUSSION

Table 2. Susceptibility test of different medicinal plant extracts (50µl/disc) against the test organisms (mm)

Bacterial Strains	Plant extracts														
	OSM (Leaves)			*OSM* (flowers)			*Aphelandra squarossa(leaves)*			*Solanum torvum(leaves)*			*Symphytum officinale* L(leaves)		
	Concentrations of extracts (mg/ml) in 80 % acetone														
	1	10	100	1	10	100	1	10	100	1	10	100	1	10	100
SA	-	11R	37	13R	26R	-	-	15R	17R	-	15R	18R	6	13	23R
Ec	-	18R	20	-	-	-	-	-	-	-	-	-	-	-	-
SaL	-	-	12	-	-	-	-	15R	18R	-	-	-	10R	17	20
ShI	9	15R	18	-	17R	37R	11R	14R	17R	-	19R	20R	-	18R	25R
KlB	11R	12R	25	-	-	-	-	-	-	-	-	-	-	-	-

Legend: R: resistance and -: No activity

Table 3. Zone of inhibition of positive controls (mm)

Bacterial strains	ANTIBIOTICS (0.025 mg/ml)			
	Chloramphenicol	Ampicilin	Amoxicillin	Tetracycline
SA	37	35	21	40
Ec	35	(-)	(-)	13
ShI	35	(-)	(-)	09
SaL	20	30R	15R	26
KlB	06	(-)	(-)	08

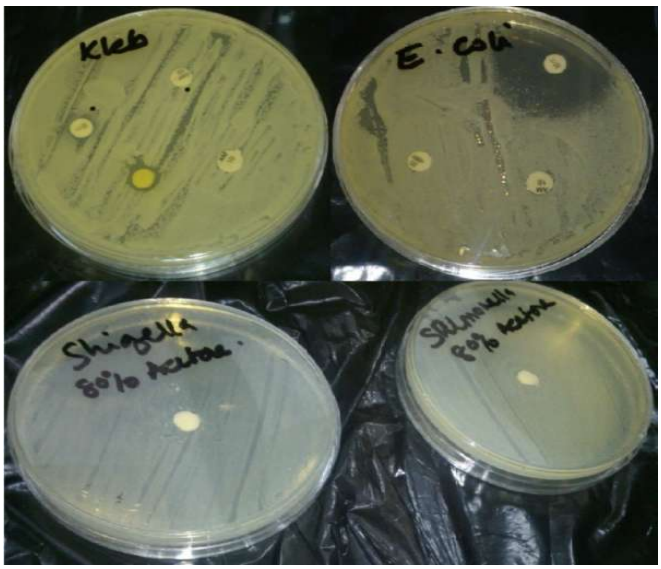

Fig.2. Zones of inhibition of positive and negative controls. The standards are not active against *KlB*. Only Chloramphenicol showed activity on *Ec*. 80% AcO did not show toxicity against the selected bacterial strains

Activity index (AI)

The activity index (AI) shows how potent a drug is compared to the standard. The AI values of *OSM* were determined using chloramphenicol as the standard antibiotic. Results are recorded in table 4.

$$AI = \frac{Inhibition\ zone\ of\ the\ sample}{Inhibition\ zone\ of\ the\ standard}$$

Table 4. AI values of *OSM* compared to Chloramphenicol

Bacterial strains	*OSM* (Leaves)
SA	1.00
Ec	0.57
SaL	0.04
ShI	0.90
KlB	4.17

Table 4 shows how potent *OSM* (100mg/ml) is compared to chloramphenicol standard. The relatively good activities observed on *SA*, *Ec*, *ShI*, and *KlB* warrant further investigations. Interestingly, *OSM* exhibited more potent activity against *KlB* than any other standard antibiotic or plant extracts.

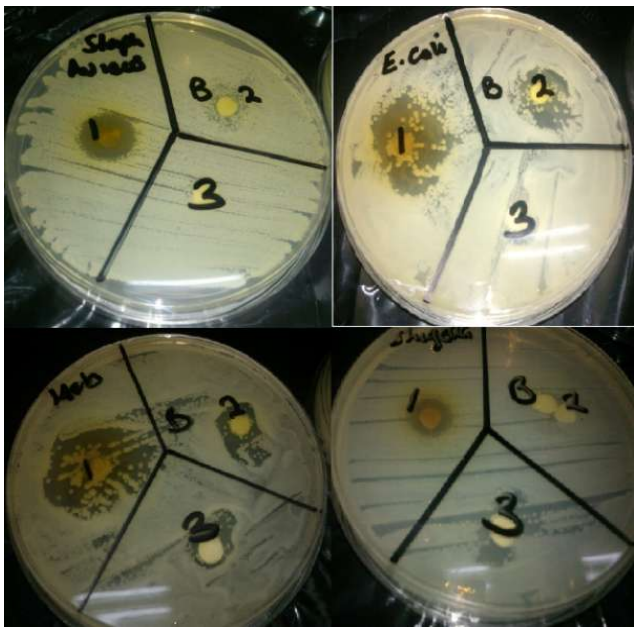

Fig.3. Zones of inhibition of *OSM* (1, 10 and 100 mg/ml) on *SA*, *Ec*, *KlB*, and *ShI*. *OSM* exhibited significant activity on all the selected bacteria

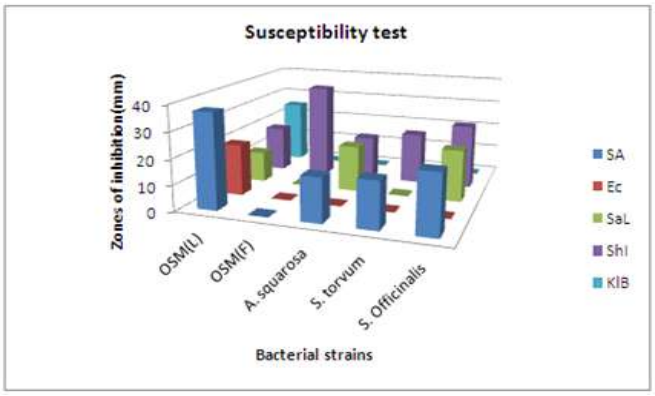

Fig.4. Zones of inhibition (mm) of different medicinal plant extracts against *SA*, *Ec*, *SaL*, *Shi* and *KlB* (mm) at a concentration of 100 mg/ml. At this concentration, *OSM* was the only plant species which exhibited significant antibacterial activity on the selected bacterial strains. In addition, *OSM* was the only plant active against *KlB*

Bio-guided fractionation

The powdered leaf materials (310 g) of *OSM (Acanthaceae)* were extracted with 1.6 L of MeOH and DCM, (1/1//v/v) for 24 hours. The supernatant was filtered through Whatman N°1 filter paper. The extract was then left to dry under room temperature to furnish a 25 g dry extract. Vacuum liquid chromatography (VLC) was chosen to separate compounds using silica gel as a stationary phase. Initially, 150 ml of n-hexane was gradually added into the VLC and flashed through to remove fats, waxes, and some chlorophyll. The polarity was then increased by adding EtOAc (0% -100 %) and later MeOH (0% - 50%). In total, 25 fractions were collected (50 ml each). Fractions were mixed according to the results obtained from thin layer chromatography (TLC) and weight. The fractions were left to dry at room temperature and later subjected to bioactivity measurements. Bioautography was performed and fractions 7-8 (870 mg) exhibited the highest anti-bacterial activity compared to other extracts. In other words, this result revealed that the active secondary metabolites are soluble in hexane-ethyl acetate mixtures. Qualitative phytochemical screening of the active fraction gave a positive test for steroids. Therefore, fraction 7-8 was selected for further purification and antimicrobial investigations.

Fig. 5. Bioautography of fractions obtained from column chromatography of fraction 7-8

OSM (leaf extract) was found to be the most potent plant extracts (Table 2). At a concentration of 100 mg/ml, no resistance was observed. The inhibition zones ranged from 0 to 37 mm. *OSM* seems to be active against both gram negative and gram positive bacteria: 37 mm for *SA*, *21* mm for *KlB*, and 20 mm for *Ec*. In addition, *OSM* leaf extract still exhibited notable activity at 10 mg/ml except for *Salmonella typhi*. Chloramphenicol appeared to be the best standard against selected bacteria. No resistance to this antibiotic was observed. Chloramphenicol exhibited the same sensitive on *SA* as *OSM* at the concentration of 100 mg/ml (37 mm). However, it is four times less potent against KlB (Table 4). The results in table 2 corroborated the use of one species of the genus *odontonema* for its antimicrobial properties. Most wounds are typically contaminated by bacteria and others microorganisms. The ability of healing open wounds can be an indication of the antibacterial properties of the plant extracts.

Conclusion

The aim of this research was to evaluate and compare the antibacterial properties of *OSM* and other selected plant species. This was prompted from the observation made on the behavior of a dog digging and eating the roots of *OSM*. One gram positive (*SA*) and four gram negative bacterial strains (*ShI*, *SaL*, *Ec* and *KlB*) were selected for the experiment. *OSM* was the most potent at a concentration of 100 mg/ml on all the microorganisms. Compared to a standard (Chloramphenicol), *OSM* exhibited impressive activity against *SA*, *Ec* and *KlB*. Bioguided fractionation using thin layer chromatography (TLC) and column chromatography (CC) led to the active fractions which were later subjected to qualitative phytochemical screening with the steroid test coming out positive. Further investigations will lead to the isolation and characterization of the active phytosterols.

Acknowledgments

The authors are thankful to the Society of Jesus (Provincia Africae Centralis) for the financial support of the research. Doctors Kaimoyo and Chuba, Mrs Florence Nyirenda, Mrs M.K Tembo, Mr R. Sikazwe (all of them from the department of Biological Sciences/University of Zambia, Ms Mirriam Chipeta and Rodney Chisha are as well acknowledged for their scientific contribution.

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