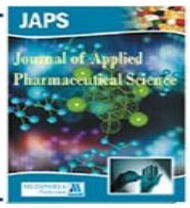
Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 20-03-2012
Revised on: 27-03-2012
Accepted on: 25-04-2012
DOI: 10.7324/JAPS.2012.2511

In-Vitro Antibacterial Properties and Pre-Liminary Phytochemical Analysis of *Amomum subulatum* Roxburg (Large Cardamom)

Mustapha A. Tijjani, Goni A. Dimari, Sule W. Buba and Irfan Z. Khan

Mustapha A. Tijjani, Goni A. Dimari, Irfan Z. Khan
Department of Chemistry, Faculty of Science, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria.

Sule W. Buba
Department of Biological Sciences, Faculty of Science, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria.

For Correspondence
Mustapha A. Tijjani
Department of Chemistry, Faculty of Science, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria.

ABSTRACT

The efficacy of methanolic seed extracts of *Amomum subulatum* Roxb. activity against bacterias such *Staphylococcus aureus, Streptococcus pneumoniae, Bacillus subtilis, Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aerugenosa and Candida albican* senegalensis was studied. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts was determined using standard methods. Results obtained showed considerable inhibition against the bacteria tested except *Salmonella pyrogenes* and *Escheria Coli* which showed considerable resistance at all concentrations of the extract. It can also observe that the extract exhibited greater inhibition on *Klebsiella pneumonia* and *Pseudomonas aerugenosa* (18±0.2 and 17±0.3). However the standard antibacterial drug tetracycline exhibit superior activity than the extract. Both the MICs and MBCs of the extract ranges from 50 to 200 mg/ml. Its further reveals that *Bacillus subtilis, Klebsiella pneumonia* and *Staphylococcus aureus* exhibit broadest activity at MIC and MBC concentrations of 50mg/ml. While the rest *Salmonella typhii* and *Pseudomonas aerogunosa* and *candida albicans* of the bacteria shows negative turbidity and resistance at MIC and MBC of 100mg/ml. This activity was indicative of the possible means of finding pure active principles from natural source with possible high potency that could serve as a lead to the pharmaceuticals. The low concentration (MIC and MBC) activity of the methanolic extracts give credence and scientific base for the claim therapeutic capabilities of *Amomum subulatum* as an anti-bacterial agent. The extract concentrate yield of the methanolic extracts was estimated to be 9.8%w/w which is brown in colour and oily in texture. Preliminary screening analysis of the powdered methanolic seed extracts showed the presence of Carbohydrate, tannins, cardioactive glycosides, tepenes, flavonoids, alkaloids and saponins. Anthraquinone was not found in the extract. The study provides the basis of use of this plant *Amomum subulam* in treatment of infections caused by pathogens (bacteria) and the phytochemical found are implicated in having anti-bacterial properties.

Keywords: Anti-bacterial, phytochemical, *Amomum subulatum*, extract.

INTRODUCTION

The use of plants in treatment of various ailments as old as time. Historically plants has provided man with not only food but also medicine in treatment of diseases and disorders when arises. Medicinal plants have important chemical compounds with pharmacological and toxicological value (Tijjani *et al.*, 2010). Plants have provided a good source of anti-infective agents; emetine, quinine, berberine, tannins, terpenoids, alkaloids and flavonoids remain highly effective instruments in the fight against microbial infections (Marjorie, 1999).

The microorganisms have developed resistance to many antibiotics because of indiscriminate use of antimicrobial drugs that create a big problem in the treatment of infectious diseases (Davis, 1994). Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease; hence, further exploration of plant antimicrobials needs to occur (Parekh *et al*., 2007). Recently, scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. The efficacy of current antimicrobial agents has been reduced due to the continuing emergence of drug resistant organisms and the adaptations by microbial pathogens to commonly used antimicrobials. Therefore, the search for new drugs from plants continues to be a major source of commercial drugs. Plant based antimicrobials represent avast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease; hence, further exploration of plant antimicrobials needs to occur (Parekh *et al.*, 2007). The genus *Amomum* is the second largest genus and comes under the family *Zingiberaceae* (formerly known as *Scitamineae*) with 150 species (Thomas *et al.*, 2009). Large cardamom or Nepal cardamom (*Amomum subulatum)* Roxburgh (1820) was first to describe this plant in his 'Plants of the Coast of Coromandel' and in 'Flora Indica' (1820b). It contains 1.95 to 3.32% of essential oil (Gupta, 1986) having typical characteristic flavour and possesses medicinal properties like stimulant, stomachic, alexipharmic and astringent properties (Annon, 2006). For this reason, this species prescribed for the treatment of indigestion, vomiting, biliousness, abdominal pains and rectal diseases and also due to its pleasant aroma, it has been used as an essential ingredient in mixed spices (Nadkarni, 1976). Secondary metabolites like Essential oils are also known to possess antimicrobial activity and antioxidant properties. Thymol, carvacrol, *p*-cymene and *y*-terpinene (36.5%, 29.8%, 10.0%, 6.3%, respectively), isolated from ssential oil of *Thymus spathulifolium*, gave positive results (Sokmen *et al.*, 2004). Phenolic and flavanoid compounds separated from other constituents of crude extracts of plants have also been successfully tested for their antioxidant and antimicrobial activity (Mothana & Lindequist, 2005). The screening of plant extracts and their products for antimicrobial activity has shown that, higher plants represent potential sources of novel antibiotic prototypes (Agboke *et al.*, 2003). Even though hundreds of plants species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated. The objective of this study is to evaluate antimicrobial properties and phytochemical compositions of fruits of *Amomum subulatum* roxb.

METHODOLOGY

Plant collection and Identification

The seeds of *Amomum subulatum* Roxb. were randomly obtained at Monday market in Maiduguri, Borno State Nigeria. The plant specimen was identified and authenticated by a Plant Taxonomist, Prof. S.S. Sanusi of the Department of Biological Sciences, Faculty of Science, and University of Maiduguri. The herbarium specimen with a voucher number 563C was deposited at the Post Graduate Research Laboratory, Department of Chemistry. The seeds were air-dried in the laboratory and one kilogram (1kg) of it was pulverised into a coarse powder using mortar and pestle.

Extraction of stem bark and Phytochemical analysis

The weighed powdered sample (500g) was exhaustively soxhlet extracted with 95% methanol .The extract was concentrated in vacou to dryness using a rotatory evaporator and labelled. The methanolic extract was subjected to qualitative chemical screening for identification of the primary and secondary metabolites such as flavonoids, alkaloids, sterols, triterpenes, saponins, anthracenosides, tannins, polyuronides, emodol, etc as described by (Ioan, 1982; Sofowora,1993a&b and Trease and Evans 2002).

In-vitro antibacterial assay

The micro-organsims employed in this study were obtained from the Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary Science, University of Maiduguri. All the Micro-organisms were propagated and stored on nutrient agar on Sabourans Dextrose Agar (SDA) medium were obtained. The nutrient agar and SDA medium were obtained in dehydrated powdered form (Oxoid Ltd, England) and were prepared according to the Manufacturers specifications. All stock cultures were maintained in nutrient agar slant at 40c and subcultured in nutrient broth (Oxoid Ltd, England) at 37^0C for 24 hours prior to anti-microbial testing.

Test Organisms

The bacterial microbes used include *Staphylococcus aureus, Streptococcus pneumoniae, Bacillus subtilis, Salmonella typhi, Klebsiella pucumoniae, Pseudomonas aeroginosa and Candida albican.* These organisms were clinical isolates obtained from Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri. Standard and susceptibility antibiotic discs used was tetracycline.

Anti-bacterial Activity (Disc –diffusion technique)

The plate whole diffusion assay as described by (Kudi *et al.*, 1999 and Ogundife *et al.*, 2000) was used to determine the growth inhibition of bacteria by the plant extract. All the bacteria were maintained on 400^0C on nutrient agar plates before use. The test were carried out by using a stock solution concentration of 500mg/ml prepared by dissolving 1g of the methanolic extract into 2ml of distilled water. Nutrient agar was prepared and 25ml each was poured into sterile Petri dish. This was allowed to solidify and dry. The dilution ratio for grams positive and gram negative bacteria were 1.1000 and 1;5000 respectively using peptone water. (Usman *et al.*, 2007). Using a sterile cock-borer of 9mm diameter three equi-distant holes per plate were made in the set agar and were inoculated with 0.5ml overnight suspension of the bacteria. Thereafter, the holes were filled with the extract solution at varying

concentrations of 800mg/ml, 600mg/ml, 400mg/ml and 200mg/ml respectively. This was done in triplicate and the plates were incubated at 37^0C for 18 hours.The antibacterial activities were observed and measured using a transparent meter rule and recorded if the zone of inhibition was ≥ 10mm (Vlietink *et al.,* 1995 and Kudi *et al.,* 1999).

Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). The Vollekova *et al.,* (2001) method as modified by Usman *et al.,* (2007) was employed. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 500mg/ml (stock concentration) in sterile distilled water and serially diluted(two-fold) to a working concentration ranging from 3.12mg/ml to 200mg/ml using nutrient broth and later inoculated with 0.2ml suspension of the test organisms. After 18 hours of incubation at 37^oC, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value.

Minimum Bacterial Concentration (MBC)

The MBC is defined as the lowest concentration where no bacterial growth is observed (bacteriaocidal concentration). This was determined from the broth dilution resulting from the MIC tubes by sub-culturing to anti-microbial free agar as described by Vollekova *et al.,* (2001). In this technique, the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37^oC for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC

RESULTS AND DISCUSSION

The extract concentrate yield of the methanolic extracts was estimated to be 9.8%w/w which is brown in colour and oily in texture. Preliminary screening analysis results indicate the presence of Carbohydrate, tannins, cardioactive glycosides, tepenes, flavonoids, alkaloids and saponins. Anthraquinone was not found in the extract. Table **1** below shows the results of the phytochemical analysis.

Table. 1: Phytochemical analysis of methanolic seed extract of *Amomum subulatum* Roxb.

S/N	Constituents	Inference
1.	**Test for Flavonoid**	
Shinoda Test		-
Lead acetate test		++
Sodium hydroxide test		++
Ferric Chlorides test		++
2.	**Test for carbohydrate**	
General Test (Molish test)		++
Test of monosaccharide		-
Test for reducing sugar (Fehling test)		++
Combine reducing sugar test		++
Test for ketoses		+
Test for pentose		-
3.	**Test of Alkaloids**	
Dragendoff reagent		++
Mayers reagent		++
4.	**Test for free Anthraquinones (Bontrase)**	-
Test for combined anthraquinone		-
5.	**Test for tannins**	
Ferric chloride		++
Lead acetate		++
Hydrochloric acid test		-
6.	**Test for cardio active glycoside**	
Salkowski test		++
Liebermann Burchard test		++
7.	**Test for cardio active glycoside**	+
8.	**Test for soluble starch**	+
9.	**Test for phlobatannins**	+
10.	**Test for saponins**	
Frothing test		++
Fehling test		++

Key - Absent
+ Present in low concentration
++ Present in moderate concentration

The *In vitro* antimicrobial screening results is presented in table **2**. It shows the susceptibility of gram positive and gram negative organisms. The results of the minimum inhibitory concentration of the extract which can inhibit the growth of the bacteria under test and the results of the minimum bactericidal concentration assay is a shown in table 4. The *In-vitro* antibacterial screening of the extract showed considerable inhibition against the bacteria tested except *Salmonella pyrogenes* and *Escheria Coli* which showed considerable resistance at all concentrations of the extract. It can also observe that the extract exhibited greater inhibition on *Klebsiella pneumonia* and *Pseudomonas aerogenosa* (18±0.2 and 17± 0.3). However the standard antibacterial drug tetracycline exhibit superior activity than the extract. It was suggested that plants extracts exhibiting diameters of zones of inhibition ≥ 100mm were considered active (Zwardyk, 1972). The minimum Inhibitory concentration and minimum bacteriacidal concentrations assay (tables **3** and **4**) also reveals *that Bacillus subtilis, Klebsiella pneumonia* and *Staphylococcus aureus* exhibit broadest activity at MIC and MBC concentrations of 50mg/ml. While the rest *Salmonella typhii* and *Pseudomonas aerogunosa* and *candida albicans* of the bacteria shows negative turbidity and resistance at MIC and MBC of 100mg/ml. This activity was indicative of the possible means of finding pure active principles from natural source with possible high potency that could serve as a lead to the pharmaceuticals. The low concentration (MIC and MBC) activity of the methanolic extracts give credence and scientific base for the claim therapeutic capabilities of *Amomum subulatum* Roxb as an anti-bacterial agent.

This study also provides some validity for the use of the plant parts in African traditional medicine.The antibacterial activity of the extracts could be as a result of the secondary metabolites such as flavonoids, alkaloids, steroids , terpenes, tannins and saponins in the methanolic extracts. Terpenes, steroids, and flavonoids are compounds known to have antimicrobial and curative properties against several bacterial pathogens (Nwueze *et al.,* 2004; Hassan *et al.,* 2004; Nwaogu *et al.,* 2007). There was also a presence of cardiac glycosides and flavonoid in the extract which may be responsible for the antibacterial property as flavonoid play a major role in bacterial inhibition. (Wunwisa and Areeya, 2005).

Table. 2: Susceptibility test (zone of inhibition) results for the methanolic extract of *Amomum subulatum* Roxb on different microorganisms at different concentrations.

Dose Extract (mg/ml)	Inhibition zone (mean±sem)						
	S.A.	S.P.	B.S.	S.T.	E.C.	K.P.	P.A.
800mg/ml	15±0.2	R	17 ± 0.3	13± 0.4	R	18± 0.2	17± 0.3
600mg/ml	13 ±0.5	R	15±0.4	11± 0.5	R	15± 0.4	14±0.2
400mg/ml	11±0.3	R	13± 0.6	10±0.4	R	12± 0.3	18±0.2
200mg/ml	9 ±0.3	R	11± 0.4	8 ± 0.4	R	10± 0.4	8±0.3
Tetracycline	21 ± 0.4	R	18 ± 0.1	10± 0.2	R	25±0.2	15±0.4

S.p= *Salmonella pyrogenes,* E.c.= *Escheria Coli.* , S.a = *Staphylococcus aureus,* B.s = *Bacillus subtilis*
S.t = *Salmonella typhii,* K.p = *Klebsiella pneumonia* , P.a = *Pseudomonas aerogunosa*
C.a= *Candida albicans* , R =means resistant.

Table. 3: Minimum inhibitory concentrations of the methanolic extracts of *Amomum subulatum* Roxb.

Organism	200mg/ml	100mg/ml	50mg/ml	12.5mg/ml	6.25 mg/ml	3.125mg/ml
S.A	-	-	-	+	+	+
B.S	-	-	-	+	+	+
S.T	-	-	+	+	+	+
K.P	-	-	-	+	+	+
P.A	-	-	+	+	+	+
C.A	-	-	+	+	+	+

Positive (+) means turbid or cloudy.
Negative (-) means not turbid.
S.a = *Staphylococcus aureus,* B.s = Bacillus subtilis, S.t = *Salmonella typhi*
K.p = *Klebsiella pucumoniae,* P.a = *Pseudomonas aerogunosa*
C.a= *Candida albican*

Table. 4: Minimum bacterial Concentration (MBC) values for Bacterial isolates against the methanolic seed extract of *Amomum subulatim* Roxb.

Organism	200mg/ml	100mg/ml	50mg/ml	12.5mg/ml	6.25 mg/ml	3.125mg/ml
S.A	-	-	+	+	+	+
B.S	-	-	+	+	+	+
S.T	-	+	+	+	+	+
K.P	-	-	+	+	-	+
P.A	-	+	+	+	+	+
C.A	-	+	+	+	+	+

S.a = *Staphylococcus aureu,* B.s = *Bacillus subtilis* ,S.t = *Salmonella typhi* ,K.p = *Klebsiella pneumoniae* , P.a = *Pseudomonas aerogunosa,* C.a= *Candida albican,*
- = Resistance (growth of bacteria), + = growth

CONCLUSION

This research provides the basis of use of the plant *Amomum subulatum* Roxb. in treatment of infections caused by pathogens (bacteria). The methanolic seed extract showed varying inhibitory activities against all the test organisms. The study also shows the presences of secondary metabolites such as flavonoids, alkaloids, tannins, saponins cardiac glycosides, terpenoids and starch in the methanolic seed extract of *Amomum subulatum.* The folkloric use of this plant in treatment of infection caused by bacteria is validate

REFERENCES

Agboke A., Attama A. A. and Momoh M. A. Journal of Applied Pharmaceutical Science (09); 2011: 157-160

Anonymous . Wealth of India: a dictionary of Indian raw materials and industrial research. Revised Edition, 2006 .1: 226-229.

Davis J. Inactivation of antibiotics and the dissemination of resistance genes. Science, 1994; 264:375-382.

Gupta PN. Studies on capsule morphology of large cardamom cultivars (*Amomum subulatum Roxb*), J. Plantation Crops, 1986; 16: 371-375.

Hassan M M, Oyewale A O, Amuoitan, JO, Abdullah MS, and Okokwo. Preliminary Phytochemical and Anti-microbial Investigation of Crude Extracts of root bark of Deterium microcarpum. J. Chem.Sci. Nigeria. 2004; 29:36-39.

Ioan C. Methodology for Analysis of Vegetable Drugs. Chemical Industries Branch Division of Industrial Operations UNIDO, Romania, 1982; 128-141.

Kudi A C, Umoh J U, Eduvice I O and Gefu J. Screening of some Nigerian Medicinal plants for antibacterial activity .J. Ethnopharmacol, 1999. 67:225-228

Marjorie M C,. Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 1999, 12: 564-582

Mothana AA and Lindequist U. Antimicrobial activity of some medicinal plants of the island Soqotra. Journal of Ethnopharmacology, 2005, 1-2 (96): 177-181.

Murugan M and Mohan V.R : Evaluation of phytochemical analysis and antibacterial activity of *Bauhinia purpurea* L. and *Hiptage benghalensis* L. Kurz. Journal of Applied Pharmaceutical Science 2011, 01 (09);

Nadkarni AK. Indian materia medica. (1976); 1: 93.

Nwaogu,L A , Alisi, CO, and ibegbulem C.U. Phytochemical and Anti-bacterial activity of ethanolic extracts of *Ladoiphia oweriensis* leaf. Afr. J, Biotech. 2007; 6(7): 890-893

Nwez ET, Okafor JI and Njoku, O. Anti-microbial Activities of methanolic extracts of Trumeguineesis (Schumm and thorn) and Morinda Lucinda Benth used in Nigeria Herb. Medicinal Practise. J. Bio. Res. Biotechnol. 2004; 2(1): 890-893

Ogundipe O O, Moody J O, Fakeye T O, and ladip O B., Anti-microbial activity of Mallotus oppositifolium extracts. Afr J.Med. Sci. 2000, 29(3/4): 281-283

Parekh J, Darsharna J, and Chanda S.,.Efficacy of aqueous and methanol extracts of some medicinal plants for potential antimicrobial activity. *Turk. J. Bio*. 2007.29: 203-210.

Roxburgh W, Plants of the Coast of Coromandel. 1820a. Vol. 3. Mission Press, Serampore.

Roxburgh W, . Flora Indica. 1820b Vol. 1. W. Bulmer and Co., London.

Sarkar PR. Yogic treatment and natural remedies, II ed. AMPS Publication, Tiljala, Calcutta, (1986): 85.

Sokmen A M, Gulluce, H A Akpulat D, Daferera B, Tepe M, Polissiou, M. Sokmen and F. Sahin. The *In vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. *Food Control*, 2004. 15(8): 627-634

Sofowora A, Recent Trends in Research into African Medicinal plants. *J. Ethnopharmacol* 1993a**;** 38: 209-214. 26-100.

Sofowora A, Medicinal plant and Traditional Medicine in Africa. 2nd edition Spectrum Books Ibadan Nigeria..Pp. 1993b. 1-8, 81-93, 134-156.`

Thomas VP, Sabu M, Gupta U Taxonomic studies on cultivars of *Amomum subulatum* (Zingiberaceae). Rheedea, 2009; 19 (1&2): 25-36.

Trease and Evans. Pharmcognosy. Harcourt Publishers. Fifteen Edition. (2002) 80-87.

Tijjani, M. A Abdurahaman F.I. and Sandabe U.K. Chemical composition and anti-nociceptive effects of the leaf extract of *Vitex doniana* Sweet. *J. Chem Soc. of Nigeria*. 2011; 1 (2): 213-217.

Usman H, Abdurahman F I and Ladan A A. Phytochemical and antimicrobial evaluation of tribulus terrestris L. (*Zygophylaceae*) growing in Nigeria. Res. *J. Biosci*. (Medwell journals). 2007; 2(3): 244-247

Wunwisa K, Areeya K. Anti-bacterial Properties of Thai Traditional Flower Vegetable Extracts. AUJT. 2005; 9(2): 71-74

Vlietink, A. J., Van Hoof, L., Totte, J. Lasure, A, Vanden Breghe, D.Rwanagabo, P.C. and Mrukiyunmwami, J. Screening of hundred Rwandese medicinal plants for anti-microbial and antiviral properties, J. Ethnopharmacol. 1995, 46, 3-47.

Vollekova A D, Kastalova U and Sochorova R . Isoquinoline alkaloids from Mahonia aquifolium Stem barkb is active against Malassezia Spp. Folia Microbiol. 2001; 46: 107-111.

Zwadyk P. Enteriobacteriaceae in Zinsser Microbiology. 20th Ed. Goerge thiene Verlag, Stuttgart. (1972) 20-3.