

**Antimicrobial activity of Balsam Apple (*Momordica balsamina* L.)**

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Abstract

Momordica balsamina L. commonly known as African pumpkin or Balsam apple (*Cucurbitaceae*) is used as vegetable in many African countries and traditionally in the treatment of skin diseases. In this study, phytochemical prospective and antimicrobial activities of its leaf extracts were evaluated with the view to validating its medicinal potentials. Acetone, ethanol and water were used as the extracting solvents. Presence of various secondary metabolites as tannins, flavonoids, proanthocyanidins, alkaloids and saponins were observed. Acetone extract indicated a significant inhibition in all the bacterial isolate (*Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*) tested at 100 mg/ml ($P < 0.05$) followed by ethanol extract which inhibited the growth of *E. coli* and *S. aureus*. The water extract showed a significant ($P < 0.05$) inhibition on the growth of *E. coli* and *Sa. typhi*. The result of antifungal assay showed that acetone extract suppressed the growth of *Aspergillus niger* and *Rhizopus sp* at 100 mg/ml. The results obtained provided a scientific support for the claimed ethnomedicinal uses of the leaves of *M. balsamina* and suggested its potential as a source of a lead compound in the treatment of microbial infections. Key words: Acetone, Bacteria, ethnobotany, Fungi, Methanol, water

INTRODUCTION

In the health system of many countries, herbal remedies have played a key role (UNESCO, 1996). The World Health Organization (WHO, 2001) has asserted that, more than 80% of the world population depends on traditional medicine for their primary health care requirements. Moreover, there is a global consensus on the benefits of phytopharmacy and at present, medicinal plants occupy a key position in the plant research and medicine. Many researchers have reported multiple roles of wild local vegetables as both food and medicinal sources (Sunday *et al.*, 2008; Jegessar *et al.*, 2008; Aliero and Wara, 2009, Bello *et al.* 2011a, Bello *et al.* 2011b).

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al.*, 2005). The most important of these bioactive compounds of plant are alkaloid, flavonoids, tannins and phenolic compounds. The phytochemical research based on medicinal information is generally considered an effective approach in the discovery of new drugs from plants (Duraipandiyan *et al.*, 2006).

Bacteria and fungi resistance to antimicrobial drug has continued to grow in the last decade (Cohen, 1992; Nascimento *et al.*, 2000). The increased prevalence of their resistance is due to extensive use and misuse of antimicrobial. The plant produces spindle shaped fruits (dark green when unripe and bright to deep orange when ripe) (John & Antony 2010). The seeds are embedded into a sweet edible red fleshy pulp

agents inadequate to control microbial infections (Cowan, 1999) thereby resulting in major public health problem (Alade and Irobi, 1993, Bax and Mullan, 2000). This development has led to increased search to unfold new broad spectrum and potent antimicrobial agents. Furthermore, antimicrobial resistance to antibiotic agents has led to treatment failure and the shift of medical care from orthodox to herbal medicine (Sunday *et al.*, 2008). Majority of the herbal therapies in use today, awaits validation of their claimed effects and possibly the development of novel antimicrobial drugs from them (Vadhana *et al.*, 2015).

Momordica balsamina L. commonly known as African pumpkin or Balsam apple and locally called Garahuni (Hausa language) (Fig 1), belongs to the family Cucurbitaceae (Burkill 1994). The plant is perennial herb with soft stems and tendrils that climbs up shrubs, boundary fields and fences. The green leaves are deeply palmately 5-7 lobed, with toothed margin and about 12 cm long stalked (John & Antony 2010). In the Republic, the leaves are cooked as part of green vegetables soup for lactating mother to regenerate her lost blood during labour and to purify her breast milk (Hassan and Umar, 2006). The leaves and young fruits of *M. balsamina* are eaten as vegetable in Cameroun, Sudan and southern Africa (Sunday *et al.*, 2008).

testing like water melon (Welman, 2004). In Northern part of Nigeria and some part of Niger The fruits, leaves and seeds extracts are used as anti-helminthic (Gills, 1992). Leaf extract is

used for the management of high fever, excessive uterine bleeding and for the treatment of syphilis (Gills, 1992). It is also used in the treatment of rheumatism, hepatitis, skin disease, diabetes and Gastro-enteritis (Gills, 1992).

In Northern Nigeria, there are many folklore practices most of which have no scientific background. For example, application of moist chopped leaf of *M. balsamina* on navel wound of newly born babies serves as its remedy. There is therefore an urgent need to correlate these folklore herbal practices with scientific evidences. The aim of this research therefore, is to determine the phytochemical constituents and evaluate the antimicrobial activity of *M. balsamina* leaves with the view to validating its medicinal potentials.

MATERIALS AND METHODS

Plant Collection and Extract Preparation

Fresh leaves of *M. balsamina* were collected from the wild of Umaru Musa Yar'adua University. The plant was identified and a voucher specimen (Bello 450) was prepared and deposited in the herbarium of the same institute. The plant material was allowed to air-dry at room temperature. The dried leaves were grounded into fine powder using mortar and pestle. Forty grams (40g) of the powdered plant was soaked into 400ml each of acetone, ethanol and water and agitated for 10-12 hrs and allowed to stand overnight. Each extract was filtered using filter paper and concentrated under reduced pressure to dryness below 40°C. The dried extracts obtained were used directly for phytochemical screening and evaluation of antimicrobial activity.

About 5g of the dried crude acetone, ethanol and water extracts of *M. balsamina* were separately weighed and dissolved in 10ml distilled water. This serves as the stock solution after which various concentrations of 0, 10, 50, and 100 mg/ml were made by dissolving each of 0, 0.1, 1 and 2 ml of the stock solution into 10ml distilled water.

Phytochemical Screening

Phytochemical test was conducted to verify the presence or absence of each of the following eight classes of secondary metabolites namely: phenolics glycosides, tannins, flavonoids, flavonols, proanthocyanidins, alkaloids, anthraquinones and saponins according to the method outlined by Trease and Evans (1999) and El-Olemy *et al.* (1994).

Source of Microorganism

The micro organisms used for this study were obtained from Microbiology laboratory of the General Hospital, Katsina. The organisms used

were: *Rhizopus sp* and *Aspergillus niger* (Fungi). *Escherichia coli* (gram -ve), *Staphylococcus aureus*, *Salmonella typhi* (gram +ve) (Bacteria).

Preparation of the Culture Medium

One (1) litre of Potato Dextrose Agar (PDA) was prepared according to manufacturer's instructions. The media was sterilized in an autoclave at 121°C for 15 minutes and allowed to cool at 50°C before pouring to sterile Petri dishes. Nutrient agar was prepared according to Murray *et al.*, (1995).

Antibacterial Activity Test

Stokes disc diffusion sensitivity technique of Murray *et al.* (1995) was adopted. Inoculums containing bacterial cells were applied onto nutrient agar plates. The discs were made by cutting disc (5-6mm) from a filter paper with a perforator, placing two of these in vial and impregnated with 0, 10, 50 and 100 mg/ml of anticipated antimicrobial plant extract solution. On each plate, Ampicillin was applied as a reference antibiotic. The reference antibiotic discs contained 50 mg of antibiotic. Each treatment was replicated trice. The discs were left to dry. This was then placed on a plate of sensitivity testing nutrient agar which was then incubated with the test organism (bacteria). The test organism was then streaked. Incubation was done at 37°C for 24 hours. The antimicrobial compound diffuses from the discs into the medium following overnight incubation. The culture was examined for areas of no growth around the discs (zone of inhibition).

The radius of the inhibition zone was measured from the edge of the discs to edge of the zone. The end point of inhibition is where growth starts. Larger the inhibition zone diameter, greater is the antimicrobial activity. It is anticipated through the antimicrobial activity of the plant extract that no area of growth will be induced around the disc. Microbial sensitivity to the antimicrobial is incubated at a distance from the disc where as resistant strains grow up to the edge of the disc.

Antifungal Activity Test

Pour plate method of Murray *et al.* (1995) was used to test the antifungal activity. About 10, 50 and 100 mg/ml each of the solvent type extract and control (0 mg/ml) were measured and placed in separate sterile Petri dishes containing the media. A 50 mg/ml of Ketoconazole tablet was used as a reference antibiotic. The organism were then streaked onto the plates and incubated at 37°C for 5 days. Results were taken by direct observation of the fungal growth on the Petri dishes.

Statistical Analysis

The experimental results were expressed as mean \pm standard deviation (SD) of three replicates. The data were subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by Waller Duncan's multiple range tests using the statistical package for social sciences (SPSS) 1999 Version. $P < 0.05$ value was regarded as significant.

RESULTS

Extract yield and phytochemical constituents of *M. balsamina*

The percentage yield of acetone, ethanol and water extracts of *M. balsamina* leaf was found to be 2.50%, 4.77% and 15.05% respectively. The results of qualitative phytochemical constituents of *M. balsamina* leaf were presented in Table 1. The results indicated the presence of Tannin, flavonoids, saponins, flavonols and alkaloids and the absence of phenols, proanthocyanidins and anthraquinones.

Antibacterial Activity Test

The result of antibacterial activity test of *M. balsamina* by measuring the zone of inhibition of acetone, ethanol and water extracts was presented in Table 2. The result showed a significant ($P < 0.05$) inhibition in the highest concentration (100 mg/ml) of all the extracts used when compared with the reference antibiotics (Ampicillin). Acetone extract indicated a significant ($P < 0.05$) inhibition in all the bacterial isolate tested followed by ethanol extract that significantly ($P < 0.05$) inhibited the growth of *E. coli* and *S. aureus*. The water extract showed a significant ($P < 0.05$) inhibition on the growth of *E. coli* and *S. typhi*.

Antifungal Activity Test

The result of antifungal activity test by observing the growth of *Rhizopus sp* and *A. niger* was presented in Table 3. The result showed that acetone extract of *M. balsamina* has a very strong (+++) activity against *A. niger* at the highest concentration of 100 mg/ml. This is followed by 10 mg/ml which showed a strong (++) activity and 1mg/ml with less activity (+). The ethanol and water extract indicates a strong (++) activity against *A. niger* at 100 mg/ml and less activity (+) at 10 mg/ml. Strong activity (++) was observed in acetone extract against *Rhizopus sp* in 100 mg/ml treatment. In ethanol and water extracts, only little (+) activity was observed against the *Rhizopus sp* even in the highest (100 mg/ml) concentration of the extract. No activity (-) was observed in all the control plates where no extract was

added. The reference drug (Ketoconazole) plate showed only some strong (++) activity in *A. niger* and little (+) activity against *Rhizopus sp*.

DISCUSSION

The aim of this research is to determine the phytochemical constituents and evaluate the antimicrobial activity of *M. balsamina* leaves so as to validate its medicinal potentials. The phytochemical screening of *M. balsamina* fresh leaves extracts and fractions have indicated the presence of various secondary metabolites that are well known to present different therapeutic applications. For example Okwu and Okwu (2004) reported that tannins have a strong antimicrobial, antiviral, molluscicidal and antitumoral activity. Kandaswami *et al.* (1994) also reported that flavonoids possess anticarcinogenic, antiviral, antihemorrhagic and antioxidant activity. Presence of all these secondary metabolites in the fresh leaves of *M. balsamina* observed in this study can be an indication of its activities against the bacteria and fungi that were tested. This finding is in agreement with the work of Sunday *et al.* (2008) which observed an activity of methanolic extract of *M. balsamina* against *E. coli*, *Bacillus subtilis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *klebsiella pneumonia*. However, the current work revealed that other forms of extracts such as acetone may have a greater activity than methanolic extracts. Obviously, the disparity may not be unconnected with differences in polarity and thus, different extractability.

Over the last decades, several studies were carried out to discover the active principle of herbal therapies. The antibacterial and antifungal activity elucidated by this research is a contribution to this field. Although the results presented here showed high activity of *M. balsamina* against *Rhizopus sp*. the results contradicts that of Sunday *et al.* (2008) who reported only a little activity. The current study also indicated a strong activity of acetone extract against *A. niger* which is in agreement to the existing literature.

Thus, the application of the moist chopped leaves of *M. balsamina* on the navel wounds of newly born babies as a Folklore practice in northern Nigeria should be adopted. However, other extracting solvents like acetone may be used for the extraction instead of water since it showed a better activity as demonstrated in this study.

CONCLUSION

The results obtained in this study provided a scientific support for the claimed ethnomedicinal uses of *M. balsamina* extracts and suggested its potential as antifungal and antibacterial agent that could be useful in the ongoing search for antimicrobial agent from plants. Our results showed that leaf extracts of *M. balsamina* has various secondary metabolites that are well known to present different

therapeutic applications. We also showed that leaf extracts of *M. balsamina* has strong antimicrobial activity and could be used to treat some bacterial and fungal diseases. We recommend the standardization of proper doses as this might help in improving the potential health benefit of *M. balsamina*. We also recommend the isolation and structure elucidation of bioactive agents with antimicrobial properties.

REFERENCES

- Alade, P.I. and Irobi, O.N. (1993). Antimicrobial Activities of Crude leaf extract of *Acalypha wicensiana*. *J. ethnopharmacol* **39**:71-174.
- Aliero, A.A. and Wara, S.H. (2009). Validating the Medicinal Potential of *Leptadenia hastata*. *African Journal of Pharmacy and Pharmacology*. **3**(6): 335-338
- Bello A., Aliero A.A., Saidu Y., Muhammad S. (2011a). Evaluation of Polyphenolic Content and Alpha-Glucosidase Inhibitory Effect of *Leptadenia hastata* (Pers.) Decne. *Nigerian Journal of Botany*, **24** (2): 331-339.
- Bello A., Aliero A.A., Saidu Y., Muhammad S. (2011b). Hypoglycaemic and Hypolipidaemic Effects of *Leptadenia hastata* (Pers.) Decne in Alloxan Induced Diabetic Rats. *Nigerian Journal of Basic and Applied Science*, **19** (2): 187-192.
- Burkill, H. M. (1994). *The useful plants of west tropical Africa. Volume 2: Families El* (No. Edn 2). Royal Botanic Gardens.
- Bax, R, and Mullan, V. (2000). The Millennium bugs 'the need for and development of new Antibacterials' *Interm. J Antimicrob Agent.*, **16**:51-59
- Cohen, M.L. (1992). Epidemiology of drug resistance 'Implications for a Post Antimicrobial Era. *Science* **257**:1050-1055.
- Duraipandiyan, V., Muniappan, A., and Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complimentary and Alternative Medicine*. **6**: 35
- Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*, **4**(7), 685-688.
- El-Olemy, M.M, Farid, J.A. and Abdel-fattah, A.A. (1994). *Experimental Phytochemistry. A laboratory manual*. College of Pharmacy, King said University; Riyadh p 3-61
- Gill LS (1992). Ethnomedicinal uses of plants in Nigeria. University of Benin press, Benin, Nigeria, p. 223.
- Hassan, L.G. and Umar, K.J (2006). Nutritional Value of Balsam Apple (*Momordica balsamina* L.) Leaves. *Pakistan Journal of Nutrition*. **5** (6): 522-529.
- Jagessar, R.C., Mohammed, A. and Gomes, G. (2008). An Evaluation of the Antibacterial and Antifungal activity of leaf extracts of *Momodica charantia* against *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli*. *Nature and science*, **6** (1): 1-4.
- John, K. J., & Antony, V. T. (2010). A taxonomic revision of the genus *Momordica* L.(Cucurbitaceae) in India. *Indian Journal of Plant Genetic Resources*, **23**(2), 172-184.
- Jose Galberto, M. Eidl, M.M., Adriana, R.C. and Fabiola, F.G., (2011). Antibacterial activity of *Momordica charantia* (Cucurbitaceae) Extracts and fractions. *Journal of basic and Clinical pharmacy* **002** (1): 45-51.
- Kandaswami C.E., Perskins D.S., Solonink G, Arzewiecki, Middle E. (1994). Anti-preventive effects of citrus flavonoids on human squamous cell carcinoma *in vitro*. *Cancer lett* **56**: 147-157.
- Murray, P.R, Baron, E.J, Ptalber, M.A., Tenover, F.C. and Tenover, R.H. (1995). *Manual of clinical Microbiology* 6th ed. Mosty year Book London.
- Nascimento, G.F, Juliana, L., Paulo, C.F. and Givliana, L.S. (2000). Antibacterial activity of plant extract and phytochemicals on antibiotic resistant bacteria. *Braz. J. Microbial.*, **31**: 247-256
- Okwu, D. E. and Okwu, M. E. (2004). Chemical composition of *Spondias mombin* Linn. Plants parts. *Journal of Sustainable Agriculture and Environment*, **6**(2): 140-147.

Sunday, O.O., Uguru, M.O. and Ogbonna A. (2008). Antimicrobial and hypoglycemic Effects of *Momordica balsamina*. *Linn. Journal of natural products*. 1:3-9

Trease, G.E. and Evans, W.C. (1985). *Pharmacognosy*, 12th edition, English language Books Society, Bailliere Tindall. P.394

UNESCO, (1996). Culture and health, Orientation Texts, World Decade for Cultural Development 1988-1997. Document CLT/ DEC/PRO - 1996, Paris, France. P. 129.

Vadhana, P., Singh, B. R., Bharadwaj, M., & Singh, S. V. (2015). Emergence of herbal antimicrobial drug resistance in clinical bacterial isolates. *Pharm Anal Acta*, 6(10), 434.

Welman, M. (2004). *Momordica balsamina* L. Nutritional Herbarium, Pretoria. www.plantzafrica.com/platk/m/momordabalsam.htm. Retrieve on 17/05/2005

WHO (2001). Legal status of traditional Medicine and Complementary Alternative medicine A World wide review. WHO Publishing 1



Fig 1. Growth habit of *Mormodica balsamina*

Table 1: Phytochemical constituents of *M. balsamina*

Phytochemical	Result	Inference
Phenolic glycosides	Negative	Phenolic glycosides are absent
Tannins	Positive	Tannins are present
Flavonoids	Positive	Flavonoids are present
Flavonols	Positive	Flavonols are present
Proanthocyanidins	Negative	Proanthocyanidins are absent
Alkaloids	Positive	Alkaloids are present
Anthraquinones	Negative	Anthraquinones are absent
Saponins	Positive	Saponins are present

Table 2: Antibacterial activity of *M. balsamina* extracts

		Zones of Inhibition (mm)				
		Concentration of extract (mg/ml)				
Extract	Organism	100	50	10	Amp (50)	Control
Acetone	<i>E. coli</i>	5.3±1.0*	5.0±0.9*	NZ	4.5±1.1	NZ
	<i>S. typhi</i>	6.0±0.1*	4.0±0.2	2.6±0.2	5.6±0.3	NZ
	<i>S. aureus</i>	4.2 ±0.2*	3.2±0.1*	1.8±0.1	3.8±0.2	NZ
Ethanol	<i>E. coli</i>	7.4±1.3*	6.3±0.9	2.2±1.1	6.2±0.9	NZ
	<i>S. typhi</i>	6.5± 1.0	6.0 ±0.9	2.2 ±0.8	7.2 ±1.0	NZ
	<i>S. aureus</i>	5.2±1.0*	4.1±1.2	2.0 ±0.9	4.3 ±0.9	NZ
Water	<i>E. coli</i>	5.0 ±0.9*	3.0 ±0.8	NZ	4.5 ±0.2	NZ
	<i>S. Typhi</i>	5.4 ±1.1*	3.1 ±0.9	1.9 ±0.9	5.1 ±1.2	NZ
	<i>S. aureus</i>	1.9 ±0.2	NZ	NZ	4.2 ±0.2	NZ

Values are mean ± S.D (n = 3)

Mean followed by * across the row are significantly different when compared with the standard (Ampicillin). P<0.05. Amp: Ampicillin. NZ: No zone of inhibition

Table 3: Antifungal activity of *M. balsamina* extracts

		Activity based on fungal growth				
		Concentration of extract (mg/ml)				
Extract	Organism	100	50	10	Ketoc (50)	Control
Acetone	<i>Rhizopus sp</i>	++	+	-	+	-
	<i>A. niger</i>	+++	++	+	++	-
Ethanol	<i>Rhizopus sp</i>	+	-	-	+	-
	<i>A. niger</i>	++	+	-	+	-
Water	<i>Rhizopus</i>	+	+	-	+	-
	<i>A. niger</i>	++	+	-	+	-

Key: (+++) = Very strong activity (No growth), (++) = Strong activity (Little growth), (+) = little activity (Mild growth) and (-) = No activity (presence of growth), Ketoc = Ketoconazole.