DRUG DISCOVERY

Phytochemical and antibacterial activity of *Mormodica balsamina* leaves crude extract and fractions

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ABSTRACT

Momordica balsamina (Balsam apple) leaves is used in the management of several human diseases, it also serves as vegetable to many tribes in African countries, Nigeria inclusive. Bacterial infections are fundamental cause of mortality and morbidity globally. Therefore, the need to search for novel antibacterial agent from plants is vital. This research is aimed at determining the phytoconstituent and antibacterial activity of *Mormodica balsamina* leaves crude extract and its fractions. *Mormodica balsamina* leaves were extracted using methanol (crude extract) and later extracted using four solvents of varying polarities. The phytochemical screening was conducted using standard methods, while antibacterial activity was carried out using agar well diffusion method. MIC and MBC were carried out using broth dilution method. The result for phytochemical screening of crude extract revealed presence of phenols, saponins, glycosides, tannins, terpenoids, cardiac glycosides and alkaloids while flavonoids and anthocyanins were not detected. The antibacterial activity of crude extract and its fractions showed varied degrees of sensitivity (zone of inhibition) against tested bacteria. The methanol crude extract and n-butanol fraction showed higher antibacterial activity in both concentrations (100 and 300mg/ml) compared to n-hexane, ethylacetate and aqueous fractions. The results indicated that *Mormodica balsamina* leaves might be used as natural antibacterial alternative against infections caused by these bacteria. These also provide preamble for the determination of antimicrobial agents from plants in subsequent research.

Keywords: Phytochemicals, Antibacterial Activity, *Mormodica balsamina*, MIC, MBC.

1. INTRODUCTION

Medicinal plant contains phytoconstituents that can be utilized for therapeutic approach they also serve as precursors for the synthesis of useful drugs (Alamgir, 2017). These agents from medicinal plants have been reported by Seanego, (2012) to be effective in curing various infectious diseases (Omwirhiren et al., 2017). Plants have been used in folklore medicines for several years, however, there are limited sufficient documented scientific data to validate their efficacy (Trotter and Logan, 2019). Human beings have used plants for the treatment of numerous diseases since ancient times (Mayekar et al., 2021).

DISCOVERY SCIENTIFIC SOCIETY

Infectious diseases have been considered as one of the major threats to human health globally. Most of these diseases are caused by microorganisms especially bacteria (Parola and Raoult, 2001). Bacterial infection is a propagation of a harmful strain of bacteria on the body or inside the biological system infecting any area within the body system. To mension but few, typhoid, meningitis, pneumonia and food poisoning are among the deadly deases caused by harmful bacteria (Nash et al., 2015). Bacterial infectious diseases are the fundamental cause of morbidity and mortality globally (Sani et al., 2014). Since anti-biotic resistance prevalence is thriving and becoming a major health threat almost everywhere in the world posing a big threat to human society (Bengtsson-Palme and Heß, 2019). Hence alternative antibiotic solutions from medicinal plants are vital.

Momordica balsamina L. (balsam apple) is a tendril-bearing annual vine originated from tropical African regions. It is an important medicinal and nutritional plant of family Cucurbitaceae (Thakur et al., 2009). *Momordica balsamina* L has long been used as a food and for medicine purposes. Balsam apple is known as balsam pear. In Africa, it has several names e.g., in Nigeria it is locally called "Garahuni" (Hausa), "Akbon-ndewe" (Igbo) and "Ejirin" (Yoruba). Mozambians called it Cacana, while South Africans called it nkaka (Sabiu et al., 2021). *Momordica balsamina* demonstrate an extraordinary antimicrobial property (Bacteria) and can be utilized outstanding source of antimicrobial agent targeting several diseases (Moniruzzaman et al., 2022). In traditional medicine, *Momordica balsamina* L. has been reported to treat various diseases such as diabetes, HIV, fever, diarrhea and in family planning (Moyo, 2016). Therefore, there is need to search for novel antibacterial agents from medicinal plants due to their availability, accessibility and affordability especially. Hence the need to search for antimicrobial drugs from natural resources is vital and urgently demanding.

2. MATERIALS AND METHODS

Plant Samples Collection and Identification

Momordica balsamina leaves were collected from Zuru Local Government Area of Kebbi State and the plant sample was authenticated by a Taxonomist from Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aleiro. A voucher specimen (KSUSTA/PSB/H/VOUCHER NO: 160B) is deposited in the same herbarium.

Plant Preparation and Extraction

The fresh leaves of *Momordica balsamina* were rinsed in sterile distilled water and shed-dried for a week. The dried leaves were pulverized, using sterile laboratory mortar and pestle, into fine coarse form. One thousand grams (1000g) of the powdered plant samples was weighed and soaked in 3.5 liters of 90% methanol for 72hours. The extract was filtered with muslin cloth and then through a Whatman filter paper No. 1 and concentrated using a vacuum rotary evaporator set at 45°C (Dupont et al., 2006). The concentrated extract was transferred into an open container and allowed to stay until it dried. The percentage yield was determined using the expression as follows

$$\text{Percentage yield} = \frac{\text{weight of extract}}{\text{weight of ground plant material}} \times \frac{100}{1}$$

Test Bacteria

The isolates, *Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Department of Microbiology Laboratory, Faculty of Life Sciences, Kebbi State University of Science and Technology, Aliero. The organisms were collected on a sterile agar slant and were then incubated at 37°C for 24hours kept as stock culture in the refrigerator at 4°C.

Solvent Fractionation of *Momordica balsamina* Crude Extract

The crude extract of the *M. balsamina* was fractionated using solvents of increasing polarities (n-hexane, ethylacetate and n-butanol). 40.3g of the crude extract was dissolved in 500ml of distilled water in a 1L separating funnel and then partitioned sequentially with equal volume (500ml) of n-hexane, ethyl-acetate and n-butanol to obtained corresponding fractions. Each fraction was collected, dried, weighed and the percentage yield calculated. The resulting fractions were kept in refrigerator in air tight containers for biological assays. The percentage yield was determined using the expression below:

$$\%\ \text{yield} = \frac{\text{Weight of dried corresponding fractions}}{\text{Initial weight of powdered sample}} \times 100$$

Phytochemical Screening of *Momordica balsamina* L. Crude Extract

The Phytochemical screening for determination of alkaloids, cardiac glycosides, flavonoids, tannins, saponins, terpenoids, phenols, glycosides and anthocyanins in crude extract and fraction were carried out according to the methods described by Harbone, (1973), Safowara, (1983) and Trease and Evans, (1989).

Preparation of Inoculums

After the sub-culturing, to prepare the bacterial inoculums, the sub-cultures were then inoculated on fresh nutrient agar plates using sterile cotton swabs at 37^0C for 24 h. The pure cultures on the nutrient agar plates were used as the inoculums (Sani et al., 2016).

Determination of Anti-bacterial Activity

Antibacterial activity of the crude methanol extract and fractions were conducted using agar well diffusion method (ditch method) as reported by Russell and Hugo, (1984). Mueller Hinton agar (MHA) was used as nutrient agar. The isolate inoculums were introduced in nutrient agar plates using sterile cotton swabs and 6mm ditch holes were made with cork borer. Thereafter, the same 100µl of the varying concentration of prepared extracts was added into the holes. Septrin against *E. coli*, Tarivid against *P. aeruginosa*, Peploxacin against *S. epidermis*, Ciproflaxacin against *S. aureus* were used as the positive control while the solvent (Methanol) was used as negative control. Finally, all dishes were incubated at 37^oC for 24 hours and zone of inhibition was observed and measured using transparent ruler.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of plant extract and fractions were determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Tubes dilution method was used. Serial dilutions of the extracts were made in a liquid medium. The lowest concentration (highest dilution) of extract preventing microbial growth is considered minimal inhibitory concentration (MIC) (Fatope et al., 1993).

Minimum Bactericidal Concentration

Minimum bactericidal concentration was carried out by inoculating sample from the MIC tubes showing no bacterial growth on nutrient agar plates and was incubated at 37^0C for 24hrs. The plates were observed for the presence or absence of growth. The least concentration of extract showing no bacterial growth was considered the MBC (Fatope et al., 1993).

Data analysis

All values are expressed as mean ± standard error of mean (SEM).

3. RESULTS AND DISCUSSION

Results

Percentage Yield

Weight and percentage yield of *M. balsamina* leaves crude extract and its solvent fractions are presented in Table 1.

Results of Phytochemical Screening

The qualitative phytochemical screening of *Momordica balsamina* leaves of crude methanol extract and fractions are presented in Table 2.

Table 1 Physical Characteristics and Percentage yield of *M. balsamina* Leaves Crude Extract and Solvent Fractions

Extracts	Color, texture and solubility	Yield of extract (g)	Percentage yield (%)
Crude Methanol Extract	Dark green, gummy and soluble in water	40.3	4.03
n-hexane Fraction	Dark green, flaky and insoluble in water	3.4	0.34
Ethylacetate Fraction	Dark brown, gummy and soluble in water	5.5	0.55
n-butanol Fraction	Reddish brown, fine texture and soluble in water	12.3	1.23
Aqueous Fraction	Brown, fine texture and soluble in water	14.5	1.45

Table 2 Phytochemical Constituents of *M. balsamina* leaves

Phytochemical	Crude Extract	n-hexane	n-butanol	Ethylacetate	Aqueous
Saponins	+	-	+	+	+
Phenols	+	-	-	-	-
Glycosides	+	+	-	+	-
Flavonoids	-	-	-	-	-
Tannins	+	+	+	+	-
Terpenoids	+	+	+	+	+
Anthocyanins	-	-	-	-	-
Cardiac Glycosides	+	+	+	+	+
Alkaloids	+	-	+	+	+

KEY: + = Present, - = Not detected

Drug Susceptibility Test against Some Selected Bacteria

The drugs found to exhibit highest activity against certain test bacteria used are as follows; Ciprofloxacin (CPX) against *S. aureus*, peploxacin (PEF) against *S. epidermis*, Tarivid (OFX) against *P. aeruginosa* and Septrin (SXT) against *E. coli* as presented in Table 3.

Anti-bacterial Activities M. balsamina Leaves Crude Methanol Extract and Solvent Fractions

Anti-bacterial activities of *Momordica balsamina* leaves of crude methanol extract and fractions are presented in Table 4. The result revealed that methanol and n-butanol extracts have the highest zones of inhibition against tested bacteria. Standard drugs (STX, OFX, PEF and CPX) also exhibited higher mean zone of inhibitions against *E. coli, P. aeruginosa, S. epidermis* and *S. aureus* while methanol as negative control showed no activity.

Minimum Inhibitory Concentration of Momordica balsamina Leaves Crude Methanol Extract and Solvent Fractions against Tested Bacteria

Methanol and aqueous extracts at 0.78mg/ml (lowest concentration) inhibits *S. aureus* and *E. coli* while aqueous extract at 100mg/ml (higher) concentration inhibits *P. aeruginosa* as presented in Table 5.

Minimum Bacterial Concentration of Momordica balsamina Leaves Crude Methanol Extract and Solvent Fractions against Tested Bacteria

n-butanol fractions showed an MBC of 25mh/mL against *S.aureus.* Also ethylacetate and crude methanol extract showed an MBC of 25mg/mL against *S.aureus* and *S.epidermis*. Crude methanol extract, n-hexane, ethylacetate, n-butanol fractions showed an MBC of 100mg/mL against *P. aeruginosa*. Crude methanol extract, n-butanol and Aqueous fraction showed an MBC of 100mg/mL against *S. epidermis*. And finally, n-hexane and Aqueous fraction showed an MBC of 100mg/mL against *S. aureus* as presented in Table 6.

Table 3 Drug Susceptibility Test against Selected Bacteria

Drugs	Zone of Inhibition (mm)			
	S. aureus	*S. epidermis*	*P. aeruginosa*	*E. coli*
PEF (10ug)	25.67 ± 5.24	30.00 ± 0.58	20.00 ± 2.31	0.00 ± 0.00
GN (10ug)	18.00 ± 3.06	18.00 ± 2.31	20.00 ± 0.58	14.33 ± 2.03
APX (30ug)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
AM (30ug)	21.88 ± 0.58	0.00 ± 0.00	21.00 ± 0.58	19.00 ± 2.89
Z (20ug)	13.00 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
R (25ug)	28.00 ± 2.31	25.00 ± 2.89	0.00 ± 0.00	0.00 ± 0.00
CPX (10ug)	28.00 ± 7.21	20.33 ± 6.89	24.33 ± 5.90	17.33 ± 2.19
S (30ug)	21.00 ± 0.58	22.33 ± 1.20	16.67 ± 1.45	15.00 ± 0.58
SXT (30ug)	0.00 ± 0.00	0.00 ± 0.00	18.33 ± 0.88	22.33 ± 1.45
E (10ug)	17.67 ± 2.03	17.33 ± 1.45	0.00 ± 0.00	0.00 ± 0.00
ATM (30ug)	12.00 ± 1.53	10.00 ± 1.15	12.67 ± 1.20	1.33 ± 0.67
SP (10ug)	0.00 ± 0.00	0.00 ± 0.00	14.00 ± 4.04	0.00 ± 0.00

OFX (10ug)	0.00 ± 0.00	0.00±0.00	29.00 ± 2.89	0.00 ± 0.00

Values are presented as mean ± Standard error of mean (SEM) (n = 3). Key: PEF=peploxacin, APX=Ampiclox GN=Gentamicin, Z=Zinnacef, AM=Amoxicillin, R= Rocephin, CPX=Ciprofloxacin, S=Streptomycin, SXT=Septrin, E=Erythromycin, ATM=Aztreonam, SP=Sponflaxacin, OFX=Tarivid.

Table 4 Anti-bacterial Activities of *Momordica balsamina* Leaves Crude Methanol Extract and Fractions

Zone of inhibition (mm)					
Bacterial strains	Fractions	300mg/ml	100mg/ml	Standard drugs	Methanol
E. coli	Methanol	18.00 ± 3.61	10.17 ± 1.69	SXT (30ug) 22.33 ± 1.45	0 ± 0.00
	N-hexane	10.33 ± 2.17	4.67 ± 2.17		
	Ethylacetate	11.67 ± 1.20	8.33 ± 0.88		
	n-butanol	19.17 ± 1.92	18.67 ± 4.42		
	Aqueous	14.00 ± 1.15	7.00 ± 1.89		
P. aeruginosa	Methanol	15.00 ± 3.46	12.00 ± 0.58	OFX (10ug) 29.00 ± 2.87	0 ± 0.00
	n-hexane	9.00 ± 3.51	14.42 ± 5.46		
	Ethylacetate	17.33 ± 0.88	12.83 ± 1.30		
	n-butanol	19.67 ± 4.04	16.67 ± 5.53		
	Aqueous	4.05 ± 2.31	1.33 ± 0.33		
S. epidermis	Methanol	17.33 ± 1.45	13.83 ± 0.88	PEF (10ug) 30.00 ± 0.58	0 ± 0.00
	n-hexane	10.33 ± 2.03	2.00 ± 1.00		
	Ethylacetate	8.33 ± 0.88	13.83 ± 4.26		
	n-butanol	19.50 ± 0.76	10.37 ± 0.12		
	Aqueous	10.00 ± 1.15	2.67 ± 1.45		
S. aureus	Methanol	14.67 ± 0.33	9.17 ± 2.40	CPX (10ug) 28.00 ± 4.16	0 ± 0.00
	n-hexane	8.00 ± 2.52	9.25 ± 7.00		
	Ethylacetate	19.33 ± 1.45	17.00 ± 5.92		
	n-butanol	16.83 ± 3.76	8.83 ± 4.46		
	Aqueous	9.67 ± 0.88	7.60 ± 2.02		

Table 5 Minimum Inhibitory Concentration of *Momordica balsamina* Leaves Crude Methanol Extract and Solvent Fractions against Tested Bacteria

Bacteria	MIC (mg/ml)				
	Methanol	N-hexane	Ethylacetate	N-butanol	Aqueous
E. Coli	12.5	12.5	50	6.25	0.78
Pseudomonas aeruginosa	6.25	1.56	6.25	50	100
Staphylococcus epidermis	0.78	50	1.56	3.13	50
Staphylococcus aureus	6.25	6.25	3.13	3.13	50

Table 6 Minimum Bacterial Concentration of *Momordica balsamina* Leaves Crude Methanol and Solvent Fractions

Extract/bacteria	MBC (mg/ml)				
	Methanol	N-hexane	Ethylacetate	N-butanol	Aqueous
E. coli	25	0	100	50	0
S. aureus	25	100	25	25	100
S. epidermis	100	0	0	100	100
P. aeruginosa	100	100	100	100	0

0 = not detected

Discussion

Phytochemicals are chemical compounds of plants origin, generally they produced by plants as defense mechanism against fungi, bacteria and plant virus infections, phytochemicals are also produced by plant in other to prevent insects and other animals from

consuming them (Kennedy and Wightman, 2011). The phytochemical screening of *M. balsamina* leaves extracts and fractions indicated the presence of various secondary metabolites that are well known to present different therapeutic applications (Othman et al., 2019). According to Liu, (2013) phytochemicals are bioactive plant chemicals in fruits, edible leaves and some plant parts that are beneficial not only nutritionally but also serves as mechanism of defense to plant. Phytochemical compounds such as alkaloids, saponins, tannins, flavonoids and steroids have been documented to be biologically active and thus partially responsible for the antimicrobial activities of plants, hence justifying the use of medicinal plant in traditional medicine (Vinoth et al., 2012).

The phytochemical composition of the crude methanol extract of *M. balsamina* indicated the presence of terpenoids, glycosides, cardiac glycosides, tannins, saponins, alkaloids and phenols. These metabolites are reported to have anti-bacterial activities in their isolated form and are responsible for the anti-bacterial activities of many plant extracts (Singh and Dubey, 2020). Terpenoids, alkaloids, tannins, saponins, glycosides, cardiac glycosides were only present in the most potent fraction (n-butanol fraction). n-hexane, Ethylacetate and aqeous fraction indicated the present and absence varying phytochemicals as shown in Table 2.

Plants rich in variety of phytochemicals (secondary metabolites), including terpenoids, alkaloids, tannins, flavonoids demonstrate biological and pharmacological activities and may have the potential to be used as antimicrobial and chemotherapeutic agents or serve as starting material in the synthesis of recent antibiotics (Maran et al., 2021). Tran et al., (2020) reported that plant-derived secondary metabolites are small molecules or macromolecules biosynthesized in plants including steroids, alkaloids, phenolic, lignans, carbohydrates and glycosides, etc. that provide diverse biological properties of benefit to humans, including their anti-allergenic, antitumor, antimicrobial, anti-inflammatory, anti-hyperglycemic and antioxidant activities.

In the present study, the antibacterial activities of *M. balsamina*, obtained from Zuru in Kebbi, were determined against wide range of bacteria (*Pseudomonas auruginosa, Escherchia coli, Staphylococcus aureus* and *Staphylococcus epidermis*) on the basis of well diffusion and micro dilution assays. The antibacterial activities of *M. balsamina* leaves crude methanol extracts and its fractions (n-hexane, Ethylacetate, n-butanol and aqueous) were examined in the present study and their potencies were quantitatively assessed (Table 4). In this study, *Momordica balsamina* leaves methanol and butanol extracts showed highest zone of inhibition, promising MIC and MBC against *E. coli, P. aeruginosa, S. epidermis, S. aureus* and further support the antimicrobial properties of these fractions. The methanol extract of *M.balsamina* in this study also, showed antibacterial activity more as indicated by the MIC and MBC which is similar to reports from other studies (Thakur et al., 2009).

According to Mickymara, (2019) *M. balsamina* exhibited antibacterial activities against pathogenic bacteria. *M. balsamina* extracts significantly prevent the growth of *Bacillus subtilis*, *S. aureus*, *Klebsiella pneumoniae*, *Escherchia coli*, *Salmonella typhi*, *Proteus vulgaris* and *Pseudomonas auruginosa* to varying degrees. Methanol extract of *M. balsamina* leaves and stem exhibited significant inhibitory property against *B. subtilis, E. coli, P. auruginosa* and *P. mirabilis* (Otimenyin et al., 2008) and whole plant was very effective against *S. aureus*, *E. coli*, *P. auruginosa* and *Salmonella typhi* (Jigam et al., 2004). The aqueous and ethanolic extract of *M. balsamina* was active against *Salmonella typhi* (Akinyemi et al., 2005). Thus, *M. balsamina* exhibited an extraordinary property against micro-organisms (Bacteria) which can well be used as an antibacterial agent against several diseases caused by study bacteria.

4. CONCLUSION

The results indicated that the *M. balsamina* crude methanol extract and its fractions especially n-butanol extracts have numerous phytochemicals and possesses antibacterial activity.

Informed consent

Not applicable.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for sample collection, identification & experimentation.

Conflicts of interests

The authors declare that there are no conflicts of interests.

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The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

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