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Phytochemical screening and Antimicrobial activity of seeds and fruits extract of Momordica balsamina Linn

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Abstract:

Resistant strains are continuously appearing in the treatment of ailments and this necessitates the synthesis of new drugs especially from naturally occurring plants. The aim of the present study was to test the antimicrobial activity and screen the Phytochemical of herbal remedies *Momordica balsamina* linn. This medicinal plant is a very common indigenous plant of tropical and sub-tropical regions of the world. The plant fruits and seeds were extracted using soxhlet with three solvents with different polarity starting with petroleum ether, chloroform and ethanol. Preliminary Phytochemical screening of each part extracts revealed the presence of alkaloid, glycoside, flavanoide, phenols, diterpenes, protein, and amino acids. Bioassay of antimicrobial activity of all extracts was tested against standard pathogenic microbial strains four bacterial strains and two fungi *Candida albicans* and *Aspergillus niger.* Concentration 20 mg/ml showed significant activity in all extracts tested against at least three human pathogens bacteria strains and no significant activity against the fungal strain tested. The chloroform extract shows the higher clear zone of inhibition when compared to other extracts with a diameter of zones of inhibition of 25, 21, 19, and 18 mm for *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginesa*, respectively. Moderate activity against *Candida albicans* showed by some extracts but no activity against *Aspergilles niger.* The findings revealed the medicinal potential of Momordica balsamina.

Keywords:

Herbal remedies, *Momordica balsamina*, Cucurbitaceae, Phytochemistry, antimicrobial activity, drug development

Introduction:

Indigenous plants are the first source of therapy for most of the common ailments in developing countries like Sudan because of availability, economic status of the users and incidence of resistant or multi resistant strains. Out of the 3,50,000 plant species known so far, about 35,000 (some estimate up to 70,000) are used worldwide for medicinal purposes and less than about 0.5% of these have been investigated for their phytochemical and general pharmacological potential (Alkangar *etal*, 2015). This green inheritance thus represents an enormous reservoir of putative lead compounds to be discovered for various diseases. *Momordica balsamina linn* is a herbal remedies belonging to the family Cucurbitaceae and widespread in tropical environment and locally known as Balsam apple or Bitter melon, Dragon Flower while in Sudan known as Areer. It is indigenous to tropical and subtropical

regions of the world such as India, Asia, South America and Nigeria and widely used as food and medicine.

Momordica balsamina linn extracts and juice have been found suitable for different diseases and problems traditionally. Several researchers had reported the effectiveness of other species, *Momordica charantia* extracts in the treatment of ailments such as lowering of blood sugar or other actions of potential benefit against diabetes mellitus due to its hypoglycemic properties (Backok et al., 2014); controlling eye disorders and enhancing eyesight due to the presence of beta-carotene (Leatherdale et al., 2001); diarrhea, pyorrhea that is bleeding from the gums (Welhinda et al., 2002); piles and hemorrhoids (Srivastava et al., 1996); respiratory problems (Jayasooriya et al., 2000) and skin infections (Ahmad et al., 1999). Zhu et al., 1990 had also reported the anti-cancerous and anti-leukemic activity of bitter melon against numerous cell lines including liver cancer, human leukemia melanoma and solid sarcomas. Strong antimicrobial activity against wide range of gram positive and gram negative bacterial of *Momordica spp.* Was repored by Taylor (2000). Various extracts of water, ethanol and methanol of the leaves of other species, *Momordica charantia,* have demonstrated in vitro antibacterial activities against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruglnosa, Salmonella typhii, Klebsiella pneumoniae and Bacillus subtilis,* an extract of the entire plant was shown to have antiprotozal activity against *Entamoeba histolytica*. In other study, the fruit extract has demonstrated activity against the stomach ulcer-causing bacteria *Helicobacter pylori* (Yesilada *et al*., 1999).

Fungi and bacteria activities always cause ailments and need to synthesis new drugs especially from plants which will be effective against resistant or multi resistant strains. Systematic evaluation of plants used in traditional medicine to determine the effectiveness which may lead to new drug discovery or advance the use of indigenous herbal medicines for orthodox treatment.

No data on phytochemical screening and antimicrobial activities had been conducted to establish the potency of *Momordica balsamina.* Therefore the aims of this study were phytochemical screening, and antimicrobial analysis in both fruits and seeds of *Momordica balsamina.*

Materials and Methods:

Sample Collection:

The fresh fruits of *Momordica balsamina* were collected from West of Sudan. The sample was identified and authenticated by Dr.Yahia Suleiman, Medicinal and Aromatic plants & Traditional Medicine Research Institute, National Center For research , Khartoum, Sudan

Sampling procedure:

Freshly collected fruits of *Momordica balsamina* were rinsed with water and air dried for 15 days in shade. After drying, pulverized with mortar and pestle after which was weighed.

Reagents:

Petroleum ether. ethanol, distilled water, chloroform, concentrated sulphuric acid, dilute ammonia, olive oil, ferric chloride, Mayer's reagent, Draggendorff's reagent, glacial acetic acid and nutrient agar.

Preparation of plant extracts:

Two methods of extraction were used. The first one, a weight (150g) of the dried coarsely powdered samples were subjected to successive extraction with organic solvents (1000 ml) such as petroleum ether, chloroform and methanol by soxhlet method. The second method, two weight (100g) of the coarsely powdered plant material was macerated with 500ml distilled water, and 500 ml methanol respectively, in a conical flask at room temperature for 48 hours, gentle shaking every period of time was done. The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was

removed in vacuo and stored at 4˚C. They were used for preliminary 3hytochemical screening. The concentrations (200mg/ml) of different extracts were prepared for the bioassay.

Phytochemical Screening:

Phytochemical analysis of the different plant extracts was performed using the methods described (Trease and Evans, 1983; Harbourne, 1998).

Test for Alkaloids: 0.5g of extract was diluted to 10ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into three portions. Mayer's reagent was added to one portion, Draggendortf's reagent to the second and Hager's to the third portion. The formation of a cream (with Meyer's reagent) or reddish brown precipitate with Draggendortf's reagent and the formation of yellow precipitate with Hager's reagent was regarded as positive for the presence of alkaloids.

Test for flavonoids: 0.5g of extract diluted with 3ml of distilled water and filtered. Dilute ammonia (5ml) was added to the filtrate of the extract. 1ml of concentrated surphuric acid was added. A yellow colouration that disappear on standing indicates the presence of flavonoids.

Test for Saponins: 0.5g of extract was added 5 ml of distilled water in a test tube. Solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigourously after which it was observed for the formation of an emulsion.

Test for Tannins: About 0.5g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Anthraquinones: 0.5g of the extract was boiled with 10 ml of sulphuric acid (H2SO4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test For Terpenoids (Salkowski Test): 0.5g each of the extract was added 2ml of chloroform. Concentrated H2SO4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for Cardiac glycosides (Keller-Killiani Test): 0.5g of extract diluted to 5ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer

Test for carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Test for phytosterols: two reagents were used first one Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes. The second was Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Test for proteins and aminoacids: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Test for diterpenes : the extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Antimicrobial Activity*:*

Test Organisms:

All the microbial strains of human pathogens used in the antimicrobial bioassay were obtained from the Department of Microbiology, Medicinal and Aromatic Plants &Traditional Medicine Research Institute, National Center for research, Khartoum, Sudan. These organisms include the Gram- negative such as *Escherichia coli (*ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853); the Gram- positive as *Bacillus subtilis* (NCTC8236), *Staphylococcus aureus* (ATCC25923) and fungus *Candida albicans,* .

Bioassay for antimicrobial activity:

Agar well-diffusion method by Perez et al. (1990) was followed to determine the antimicrobial activity. Nutrient agar (NA) was swabbed (sterile cotton swabs) with 8hours old – broth culture of respective bacteria and fungi. Two wells (10mm diameter) were made in each of these plates using sterile cork borer. About 0.3 ml of prepared concentrations of plant solvent extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2h. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for fungal pathogens. Diameter of the inhibition zones was recorded. Triplicates were maintained and the experiment was repeated thrice and the average values were recorded.

Results and Discussion:

Table **(**1**)**Yield percent of Momordica balsamina fruits and seeds extracts**:**

Plant part	***Extraction method***	***Plant extract***	***Yield %***
seed	Successive	Petroleum ether	6.92
		Chloroform	3.42
		Methanol	2.48
	Maceration	Distilled water	18.57
		Methanol	14.51
Fruit	Successive	Petroleum ether	1.12
		Chloroform	0.56
		Methanol	19.73
	Maceration	Distilled water	21.40
		Methanol	25.8

Table(2) Phytochemical screening result for Momordica balsamina fruit and seed extracts

Phytochemical group	Plant part									
	Seeds					Fruits				
	Method of extraction									
	Successive			Maceration		Successive			Maceration	
	P	C	M	W	M*	P	C	M	W	M*
Alkaloids	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve
Flavonoids	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve
Saponons	(+)ve	(+)ve	(-)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve
Tannins	(-)ve	(-)ve	(+)ve	(+)ve	(+)ve	(-)ve	(-)ve	(+)ve	(+)ve	(+)ve
Anthraquinones	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve
Cardiac glycosides	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Terpenoids	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve
Diterpenoids	(+)ve	(-)ve	(+)ve	(-)ve	(+)ve	(+)ve	(-)ve	(+)ve	(-)ve	(+)ve
Phytosterols	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve
Carbohydrates	(+)ve	(+)ve	(-)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(-)ve
Proteins	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve

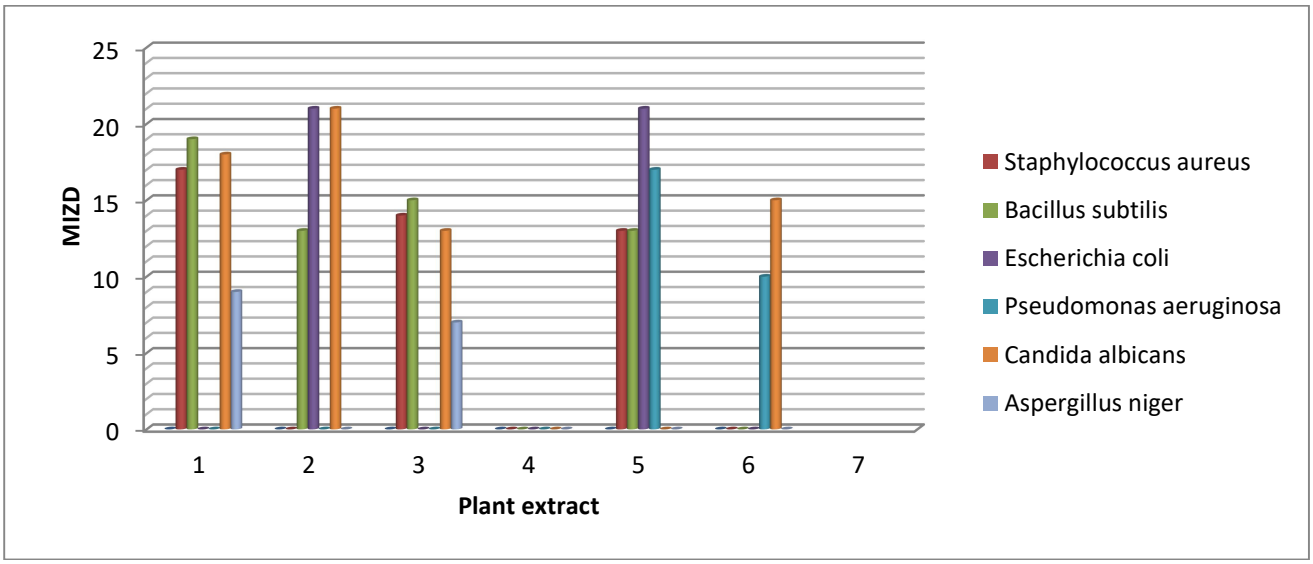
Key: (+)ve= Detected, (-)ve = not Detected, P= Petrolium ether, C = chloroform, M= Methanol, W = distilled water

The result of the phytochemical screening of the leaves extract of *Momordica balsamina* revealed the presence of alkaloids, flavonoids, saponins, tannins, anthraquinone and terpenoids in all extracts except cardiac glycoside as shown in the table 2. The presences of these secondary metabolites that are biologically active are responsible for the antimicrobial activities of *Momordica balsamina*. The presence of saponnis support the fact that *Momordica balsamina* seed can be used for cure of intestinal problems (Okwu and Okwu, 2004), this also gives the leaves the bitter taste. The presence of alkaloid in the fruits and seeds reflected the fact that this plant can be effectively used as anti-malaria drug, since alkaloid consists of quinine which is antimalaria. In Sudan this plant fruit was eaten as a vegetable and water and ethanol were two major solvent that are used in preparation of herbal remedies.

Antimicrobial activity

The crude extract and fractions were tested against the four types of bacteria using the disc diffusion method and two fungi (*Candida albicans and Aspergilles niger)*.the Mean Inhibition Zone Diameter (MIZD) were measured an
d reported.

Fig 1: the antimicrobial activity of *Momordica balsamina* fruits and seeds extracts at concentration 20mg/ml



In the antimicrobial analysis, concentration of 20mg/ml was done. The result revealed the highest clear zone of inhibition in chloroform extract of both seeds and fruits against *Escherichia coli*, also seeds chloroform extract showed high activity against *Candida albican* while chloroform extract of the fruits reflected a moderate activity against *Pseudomonas* aeruginosa. A moderate to high activity against Bacillus subtilis was showed by the seeds petroleum ether extract, while the fruit petroleum ether extracts revealed no activity against all microbial strains tested. Thus, the seed chloroform extract can be used as the active constituent of antimicrobial and antifungal natural products.

Although no data about the pharmacological effect of *Momordica balsamina* but Grover et al., 2004 reported other species *Momordica charantia* has ability to inhibit enzymes "guanylate cyclase" that is thought to be associated with psoriasis, Leukemia and tumol pathogenesis and suggested that drastic measures should be adopted to control the use of antibacterial agents to understand the genetic mechanisms of bacterial resistance and to continue studies to develop new drugs.

Conclusion:

The leaves extracts from *Momordica balsamina* contained the following phytochemical: alkaloids, flavonoids, saponins, tannins, anthraquinones and terpenoids which are responsible for the antimicrobial activity observed. *Momordica balsamina* plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals and cosmetic research activities. Further studies on the extracts of the whole plant using Mass Spectroscopy, and Nuclear Magnetic Resonance Techniques is recommended to elucidate the structure for drug synthesis.

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