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Phytochemicals, anti-microbial and free radical scavenging activities of *Momordica charantia* Linn (Palisota Reichb) seeds

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Extractives from the seeds of *Momordica charantia* Linn were screened for phytochemical, antioxidant and anti-microbial activities against *Escherichia coli, Candida albicans, Staphylococcus aureus, Staphylococcus epidedermis, Klebsiella pneumonia* and *Proteus mirabilis.* The phytochemical screening of *Momordica charantia* seeds showed the presence of flavonoids, glycosides, sterols, fat and oil in hexane, ethyl acetate and ethanol extracts. At concentration of 1 mg/ml the radical scavenging activities of the plant extract was comparable to that of vitamin C used as control but at lower concentrations, the scavenging activity decreases. Therefore, it is a concentration dependent. The crude extract was found active against *E. coli at* 325 µg/ml, *C. albicans* at 162.5 µg/ml, *S. aureus* at 325 µg/ml, *S. epidedermis* at 500 µg/ml *and K. pneumonia* at 1500 µg/ml. These results suggest that the active principles in the extract possess broad spectrum antimicrobial activity, thus the plant could be used as a crude herbal drug.

Key words: *Momordica charantia*, diphenyl-1-picrylhyrazyl radical (DPPH), inhibition, *Cucurbitaceae*, phytochemicals.

INTRODUCTION

Medicinal plants are a source of great economic value all over the world. The world is rich in all the three levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. All over the world, thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been since ancient times (Rastogi and Mehrotra, 2002). Herbal medicine is still the mainstay of about 75 to 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents. The drugs which are already in use to treat infectious diseases is of concern because, drug safety remains an enormous global issue. It was estimated that 2.22 million hospitalized patients had serious Adverse Drug Reactions (ADR) and 106,000 died in a single year in the USA (Nair et al., 2005). Herbal and natural products have been used in folk medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, this coupled with their reduced cost make it encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair et al., 2005). The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years. Many of the current pharmaceuticals are derived from plants but very few are used as antimicrobials.

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Traditional healers are known to have long used plants to prevent or cure infectious condition.

Momordica charantia Linn. (Palisota Reichb) commonly known as bitter melon, bitter gourd or karela, was originated from India and carried to China in the 14th century (Indrani, 2005). It is a tropical and subtropical vine of the family *Cucurbitaceae*, widely grown in Asia, Africa, and the Caribbean. The fruit juice and/or a leaf tea is employed for diabetes, malaria, colic, sores and wounds, infections, measles, hepatitis, and fevers. Leaves are used for treating catarrh, constipation, dermatitis, diabetes, diarrhoea, eczema, fever, leprosy, malaria, rheumatism, breast cancer, snake bite , anaemia, dysentery, gonorrhoea, measles, rheumatoid arthritis (Taylor, 2002). Bitter melon has been shown to increase the number of beta cells in the pancreas thereby improving the body's ability to produce insulin. The fruit has also shown the ability to enhance cells' uptake of glucose, to promote insulin release, and potentiate the effect of insulin (Lotlikar et al., 1966).

The plant contains several biologically active compounds chiefly momordicin, cucurbitacin and glycosides such as momordin, charantin, momordicosides and other terpenoids compounds such as momordenol, momordol, momordicin-28 and momordicilin (Fatope et al., 1990; Ortigao et al., 1792). It is used as a folk medicine in Togo to treat gastrointestinal diseases and extracts have shown activity in vivo against the nematode worm, *Caenorhabditis elegans* (Okabe et al., 1982).

The aqueous extract powder of fresh unripe whole fruit at a dose 20 mg/kg body weight was found to reduce fasting blood glucose by 48%, an effect comparable to that of glibenclamide, a known synthetic drug. This extract was tested for nephrotoxicity, hepatoxicity, biochemical parameters and lipid profile. The extract did not show any signs of nephrotoxicity and hepatoxicity as judged by histological and biochemical parameters. Thus aqueous extract powder of the plant, an edible vegetable appears to be a safe alternative to reducing blood glucose (Virdi et al., 2003). As part of our investigation on some medicinal plants of Nigeria, we report for the first time in this paper biological activity from the seeds of *M. charantia.*

MATERIALS AND METHODS

Plant materials

The matured gourds of *M. charantia* were harvested at Chika, along Airport Road, Abuja and identified at The Herbarium Unit of Pharmaceutical Research and Development, Idu -Abuja, where voucher specimens were deposited.

Extraction/partitioning procedure

The gourds were cut open and seeds extracted by hand, washed with distilled water to remove slimy coatings and allowed to drip off water with a sieve and weighed. The weight was 917 g. The seeds were spread out, air-dried and weighed intermittently until a constant weight which was 694 g was obtained. The seeds were further sifted to remove immature fluffy seeds and weighed to obtain a final weight of 557 g.

Blended 500 g of the seeds was extracted by percolation as described by Singh et al. (2006) using Hexane, Ethyl Acetate and 95% Ethanol as extracting solvents which were in increasing order of polarity. The extracts were collected and concentrated with the aid of a Stuart rota-vapor and kept in a refrigerator.

Phytochemical screening of extract

For the purpose of this study, phytochemical screenings were carried out on the extracts to confirm the presence or absence of the following plant secondary metabolites: alkaloids, phenols, sterols, terpenes, tannins, flavonoids, anthraquinones, cardiac glycosides, saponins, fats and oil (Harborne, 1973; Trease and Evans, 1989).

Phenols

Equal volumes of each extract and ferric chloride solution (which is prepared by dissolving 135.2 g of $FeCl_3.6H_2O$ in distilled water containing 20 ml of concentrated HCl dilute to 1 L) are added together. A deep bluish green precipitate indicates the presence of phenol.

Alkaloids

Each extract was added to 1% aqueous HCl over water bath and filtered. The filtrate was treated with (2 g of Iodine in 6 g of potassium iodide in 100 ml of distilled water). Formation brown or reddish brown precipitate indicates presence of alkaloids.

Steroids

Each extract was added to 2 ml acetic anhydride and 2 ml H_2SO_4. Colour change from violet to blue or green indicates the presence of steroids.

Terpenes

Each extract was added to 0.5 ml acetic anhydride and few drops of concentrated H_2SO_4. A bluish green precipitate indicates the presence of terpenes.

Cardiac glycosides

Extract was treated with 2 ml glacial acetic acid with a drop of Ferric Chloride solution and underplayed with 1 ml H_2SO_4. A browning at the interface indicates the presence of cardiac glycosides.

Tannins

Each extract was boiled in 20 ml water and filtered. A few drops of 0.1% Ferric Chloride solution were added. Brownish green or blue-black color indicates the presence of Tannins.

Flavonoids

Five milliliter Ammonium solution was added to aqueous filtrate of each extract and then few drops of concentrated H_2SO_4. Yellow coloration indicates the presence of Flavonoids.

Anthroquinones

10ml benzene was added to each extract and filtered. 0.5 ml of 1% Ammonium solution was added and shaken. Pink, red, or violet color in the ammoniacal lower phase indicates the presence of Anthraquinones.

Saponins

1 g each extract was boiled with 5 ml distilled water and filtered. 3 ml distilled water was added to the filtrate and shaken vigorously for 5 min. Persistent frothing on warming indicates the presence of Saponins.

Fats and oils

Small quantity of each extract was pressed between two filter papers. Oily stains indicates the presence of Fats and Oils.

Antimicrobial screening of extract

The extract of the crude was used for antimicrobial tests to ascertain therapeutic effect of the crude. Sensitivity of different bacterial strains to various extracts was measured in terms of zone of inhibition using agar diffusion assay (ADA). The plates containing Mueller-Hinton Nutrient agar were spread with 0.2 ml of the inoculums. Wells (8 mm diameter) were cut out from agar plates using a sterilized stainless steel borer and filled with 0.1 ml of the extract. The plates inoculated with test organisms which were incubated at 37°C for 48 h and diameter of any resultant zone of inhibition was measured. For each combination of extract and the test organism strain, the experiment was performed in duplicate and repeated three times. The bacteria with a clear zone of inhibition of 11 mm or more were considered to be sensitive.

Determination of minimum inhibition concentrations

The minimum inhibition concentrations (MICs) can be defined as the lowest concentrations of *M. charantia* crude extract that will inhibit the visible growth of the selected micro-organisms after overnight incubation were also determined using agar dilution method. Minimum inhibitory concentration of the effective seed extract was worked out by agar dilution method. Nutrient agar plates containing varying concentrations (10 to 100 mg/ml aqueous extract; 1 to 50 mg/ml acetone) of different seed extracts were prepared and inoculated with 0.1 ml of the inoculum. The plates were incubated at 37°C for 24 h and the lowest concentration of the extract causing complete inhibition of the bacterial growth was taken as minimum inhibitory concentration (MIC). The results were compared with that of control using sterilized distilled water/acetone. The experiment was performed in duplicate and repeated three times (Andrews, 2001).

Determination of anti-oxidant activity

The radical scavenging activities of the plant extracts against 2, 2-Diphenyl-1-picryl hydrazyl radical (Sigma-Aldrich) were determined by UV spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described by Ayoola et al. (2006). The following concentrations of the extract were used -0.0625, 0.125, 0.25, 0.5 and 1.0 mg/ml in methanol (Analar grade). The solutions formed colloidal to clear yellow solutions as the concentration increases. Vitamin C was used as the antioxidant standard at the same concentrations. 1 ml of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 0.1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = \{[Ab-Aa]/Ab\} \times 100$$

Where Ab is the absorption of the blank sample and Aa is the absorption of the extract. The results were tabulated.

RESULTS AND DISCUSSION

The phytochemical screening of seeds of *M. charantia* showed the presence of flavonoids, glycosides, sterols, fat and oil in hexane, ethyl acetate and ethanol extracts in Table 1. Only hexane and ethanol extracts contain alkaloids while phenols were not detected in all the extracts.

These chemical constituents present in the extracts have many therapeutic values. Flavonoids have both antifungal and antibacterial activity. They possess anti-inflammatory properties (Iwu et al., 1999). The therapeutic efficacy of the crude extract on microbes of importance was carried out by testing for its antimicrobial activity. In Table 2, the bacteria with a clear zone of inhibition of 11 mm or more were considered to be sensitive.

The crude extract was found active. Table 3 showed minimum inhibition concentration against *E. coli* at 325 µg/ml, *C. albicans* at 162.5 µg/ml, *S. aureus* at 325 µg/ml, *S. epidedermis* at 500 µg/ml *and K. pneumonia at* 1500 µg/ml. These results suggest that the active principle in the extract possesses broad spectrum antimicrobial activity.

This implies that the plant could be used as a crude herbal drug. This agrees with the ethno-medical use as crude antimicrobial for malaria and wounds (Harvey, 1999). The anti-oxidant activity was also determined as shown in Table 4. At concentration of 1 mg/ml the radical scavenging activities of the plant extracts was comparable to that vitamin C used as control but at lower concentrations, the scavenging activity decreases. Therefore, it is a concentration dependent. At these lower concentrations, it was observed that the solutions became colloidal. This was confirmed by Bushra (2009) who showed that solvent extraction as the most frequently used technique for isolation affects both the extract yields and resulting antioxidant activities of the plant. This suggests that the plant has anti-oxidative potential that can be of use to address cells *in-vivo* “oxidative stress”.

Table 1. Result for phytochemical screening of *Mormodica Charantia* seed extract.

Phytochemical	Result		
	Hexane	Ethyl acetate	95% Ethanol
Alkaloids	+	-	+
Flavonoids	+	+	+
Glycosides	+	+	+
Saponins	-	-	+
Taninns	-	+	-
Sterols	+	+	+
Terpenes	+	+	-
Phenols	-	-	-
Anthraquinones	+	+	-
Fats and Oils	+	+	+

Key: (+) = Present; (-) = Absent.

Table 2. Result of antimicrobial screening of *Mormodica Charantai* seed extract (mm).

Microbes	30 mg/well	15mg/well	7.5 mg/well	3.75 mg/well
E.coli	18	16	13	12
C. albicans	21	20	18	13
S. areus	16	14	13	12
S. epidedermis	13	11	-	-
K. pneumonia	16	14	-	-
P. mirabilis	-	-	-	-

Key: (-) = No activity.

Table 3. Result of determination of minimum inhibition concentrations of *M. **c**harantia* seed extract.

S/N	Micro-organism	Extract MIC (*µ*g/ml)
1	*E.coli*	325
2	*C. albicans*	162.5
3	*S. areus*	325
4	*S. epidedermis*	500
5	*K. pneumonia*	1500

Table 4. Result for Anti-oxidant activity of *E*xtract with Vitamin C as Control.

Concentration (mg/ml)	Vit C control	Seed extract	Vit C % inhibition (A)	Extract % inhibition (B)
0.0 (blank)	0.210	1.959		
1.0	0.074	0.614	64.76	68.66
0.5	0.088	1.076	58.10	45.07
0.25	0.089	1.146	57.62	41.55
0.125	0.090	1.225	57.14	37.47
0.0625	0.091	1.375	56.67	29.81

Conclusion

We concluded that the results of the work support the trado-medical and biological studies claims on this plant especially on the treatment of malaria, wounds and diabetes. The above observed properties demonstrate the great potentials of *M. charantia* for use in agriculture and medicine. Thus there is need to conduct more studies on the plant aimed at extensive investigation, isolation and purification of active constituents with broad spectrum activity for development of potential natural and synthetic pharmaceuticals for the therapy of infections and cells oxidative problems.

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