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Antibacterial and Phytochemical Properties of the Leaf and Fruit Extracts of *Mormodica charantia*

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Abstract

The methanol, ethylacetate, chloroform and aqueous extracts of the leaves and fruits of Mormodica charantia were screened for their antibacterial activity using the agar well diffusion method. The extracts were tested against three enteric bacteria, Staphylococcus aureus, Escherichia coli and Salmonella enterica. Chloroform and water extracts of both the leaves and fruits of the Mormodica charantia plant showed remarkable antimicrobial activities even at low concentrations (10µg/ml). The column fractions antibacterial activity testing shows the fractions from column 3 of the leaves part and column 1 and 2 of the fruits to contain the most active components with a significant activity on the organism tested. Phytochemical screening of the crude extract of the leaves and fruits revealed the presence of alkaloids, flavonoids and steroids as the most common components found in considerable amounts. Other constituents detected are glycosides, terpenes, carbohydrate and traces of antraquinones. The toxicological analysis as carried out by up and down procedure of the active components from the crude extracts of plant parts indicated that the plant has no toxic effect on rats. It is concluded that these plants could be a source of new antibiotic compounds and further work is needed to isolate the secondary metabolites from the extracts studied in order to test its specific antimicrobial activity.

Key word: antibacterial screening, phytochemicals, toxic effect

Introduction

Momordica charantia is commonly known as 'Garaafini' in Hausa and 'Efirin' among the Yoruba ethnic groups of Nigeria (Mann *et al.*, 2003). This plant belongs to the family *cucurbitacea*. Leaves

which are characteristically alternate, basally cordate and palm nerved with 7cm long petiole. The fruit is a fleshy dehiscent, yellow at maturity and consumed as food plant. It is also found as an ingredient in curries in some South Asian countries. The raw fruit is available in West Africa and Asian market where it is known as Karela. The leaves and fruits are traditionally used for the treatment of colitis, dysentery, intestinal worms, jaundice, enteric fever and occasionally diabetes (Grover and Yadav, 2004). Traditionally, plant preparation is used as sources of medication that is passed on through experience and superstition from generation to generation, virtually by the word of mouth (Ogundare et al., 2005). The extensive use of natural plants in primary health remedies due to their pharmacological properties is quite common (Sheila et al., 2008). The investigation of the efficacy of this plant-based drugs has been paid great attention because of their few side effects, cost effectiveness and easy availability (Kumara, 2001).

The growing interest in herbal medicine demands toxicity risk assessment of the various indigenous preparations used in the treatment of diseases. The increasing use of plant extracts in food, cosmetics and pharmaceutical industries suggest that, in order to find active compounds a systematic study of medicinal plants is very important. In this study extracts of different parts (leaves and fruits) of *M. charantia* plant were screened for their antimicrobial activity against gastrointestinal pathogens to assess their scientific basis, safety and effectiveness in humans or animals and as well to contribute to the development of safe and body tolerable plant- based antibacterial drugs.

Materials and Methods

Collection and identification of plant materials

For each of the leaves and fruits, 400g of *Momordica charantia* were collected from Dundaye Village of Usmanu Danfodiyo University, Sokoto State, Nigeria. The plant was botanically authenticated at the herbarium, Botany unit of UDUS where voucher specimens (pcg/udus/caes/0010) are kept. The leaves and fruits were washed, dried at room temperature over a period of two weeks and pulverized into powder (Hassan *et al.*, 2006).

Bacteria used

The bacteria tested were clinical isolates from stool samples taken directly from patients with gastrointestinal diseases attending the Usmanu Danfodiyo University Teaching Hospital Sokoto. The microorganisms used were *Escherichia coli*, *Salmonella enterica* and coagulase-positive *Staphylococcus aureus*.

The isolates were identified and characterized according to their morphological, cultural, physiological biochemical and characteristics (Monica, 2003). The tests were: Gram's reaction; production of catalase, cytochrome oxidase and hydrogen peroxide; growth at 37°C; acid production from carbohydrates, production of acid and gas from 1 % glucose, methyl red and Voges-Proskauer test in MRVP medium, H&L test in O/F medium; production of ammonia from arginine; nitrate reduction in nitrate broth; indole production in tryptone broth and growth on acetate and citrate agar (Monica, 2003).

Extraction of plant materials and preparation of leaves and fruits extract concentrations

All the dried samples were milled into coarse powder by pounding manually with a clean pestle and mortar. The powder was collected into sterile cellophane bags and labeled. The Soxhlet apparatus and the Soxhelt method of extraction was employed in extracting the leaves and fruits material using methanol, chloroform, ethyl acetate and water. Forty grams of each of the powdered plant part was placed in the upper chamber in a thrimble, and 400ml of the solvent in the bottom flask. The flask and the vapour condensed to dryness using steam evaporator. The dried extracts were weighed and kept in well labeled sterile samples bottles (Oyeleke and Manga, 2008).

Antibacterial testing using the leaf and fruit crude extract

The test clinical bacterial isolates were screened for antibacterial activity against the plant extracts using the well diffusion method as described by Booth (1997) and NCCL Standard (1987). Pure culture of the organisms were inoculated into sterile nutrient broth to a density of 9×10^8 cfu/ml equivalents to McFarland Standard test-tube number 3 and incubated for 24hrs at 37°C. The suspension was used to streak for confluent growth on the surface of Muller-Hinton Agar plates with sterile swab.

The antibacterial agent used as control was the commercially prepared Amocillin (500mg/kg body weight) dry powder. Different concentrations of the extracts and control antibiotics was made by serial dilution into four (4) sterile test-tube to make dilutions of 100, 80, 60, and 30 µg/ml. The suspension of the bacterial inoculums was used to streak for confluent growth on the surface of nutrient agar plates with sterile swab. Wells (6.0mm) were punched out of the solid media using a sterile corkborer. Each of the dilution was place into each well. Petri dishes were incubated at 37°c, for 18-24hrs. Each test was carried out in triplicate. Antibacterial activity was recorded, if the zone of inhibition was greater than 6mm. The significance or otherwise of the difference of antibacterial activity was tested by one way analysis of variance (ANOVA) (Hassan et al., 2006).

Determination of minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC)

The MIC was determined using different dilutions of the extracts as follows: 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.8, 0.4 and 0.2 μ g/ml. The lowest concentration which did not show any growth of the test organism was interpreted as the MIC. The MBC was determined by sub-culturing 0.1ml of the highest concentration of the agent, which shows visible growth and all the tubes showing no visible sign of growth in the MIC tube dilution test. The MBC was the lowest concentration that results in killing 99.9% bacteria (Ochei and Kolahatkar, 2003).

Separation of plant components and antibacterial testing of the bioactive components from the column chromatographic fractions

The chromatographic media (silica gel) was used for the chromatographic separation. From the resultant column chromatographic fractions, the activity of reconstituted extract was tested against the test organisms. A quantity (0.01 g) of the dried fractions was dissolved into 5ml of water to give a concentration of 100μ g/ml. Wells of the inoculated Muller-Hinton culture plate were filled with the extract and incubated for 24hrs at 37° c. The zones of inhibition were measured (Oyeleke and Manga, 2008).



Phytochemical screening

The leaves and fruits water extracts were screened for the presence of phytochemical substances using the procedures of Oyeleke and Manga (2008). Alkaloids were detected by mixing 0.5gram of the leaf and fruit extracts with 1% aqueous hydrochloric acid on a steam bath. A quantity (1ml) of the filtered mixture was then treated with Hager's, Meyer's and Wagner's reagents to produce a deep brown creamy precipitate indicating positive test for alkaloids. Flavonoids were detected by the appearance of vellow solution when 1ml of the either the leaf or fruit extract was dissolved in Sodium Hydroxide solution and its subsequent disappearance on addition of Hydrochloric Acid. When 10ml amount of chloroform was added to 0.5gram of powdered extract in a test tube and filtered after shaking for 5 minutes to present a bright-pink color in the upper aqueous layer, this indicated the presence of anthraquinones. Steroids were detected by adding 15ml of concentrated H₂SO₄ to 1ml each of the extracts to produce reddish brown color. Glycosides from the extracts were detected when 0.5 gram of the extracts was mixed with 3ml FeCl₃ in glacial acetic acid and leaf was allowed to stand for 1minutes which then produce blue layer inter-phase upon running down 1.5ml of H_2SO_4 by the side of the tube. A 1:4 dilution each for the leaf and fruit in water when mixed with few drops 10% FeCl₃ produced a blue or green color indicating the presence of tannins.

Toxicity studies

Test animals

Twelve (12) male Rats of between 8 and 12 weeks old and weight falling in an interval within $\pm 20\%$ of the mean weight (178g) were used after being certified healthy by a Veterinary Doctor. The animals were kept at animal house in cages, were they were maintained under veterinary supervision and fed well with pellet diet and tap water ad libitum for one week to acclimatize them before starting the experiment. Investigations using experimental animals were conducted in accordance with internationally accepted principles for laboratory animal use and care (Hassan et al., 2006; Organization for Economic and Cultural Development (OECD) (2001).

Acute toxicity studies

The toxicological effect was assessed on the basis of toxicity signs: tremor, salivation, weakness, off-feed, depression and mortality, which were expressed as LD_{50} and calculated by using the limit test dose up and down procedure of Organization for Economic and Cultural Development (OECD) (Hassan et al., 2005). The Limit Test is a sequential test that uses a maximum of 5 animals of both gender with the female being nulliparous and non-pregnant; at the commencement of dosing. The temperature in the experimental animal room was at $22^{\circ}C (\pm 3^{\circ}C)$ with the relative humidity of 40-60% and adequate lighting sequence of 12 hours light and 12 hours dark. Animal were fasted prior to dosing with the food but not water withheld overnight. The fasted body weight of each animal was determined and the dose was calculated according to the body weight. Water extract of crude extract of the leaves and fruits was administered orally in concentrations of 3000mg/kg, body weights dose to five groups of animals (each consisting of one animal), using a stomach tube one after the other at the grace observation period of 24hrs, 48hrs and up to 14 days in a single oral dose while the control group received distilled water only. After the substance has been administered, food was withheld for a further 3-4 hours and observation of toxic symptoms was made and recorded systematically at one, two, four and six hours after administration. Finally the number of death and survivors was noted after 24hrs, 48hrs and up to 14 days for each animal.

Results

The results of antibacterial activity of crude aqueous and organic extracts of the leaves and fruits of Mordica charantia is presented in table 1-2 indicated that chloroform and water extracts of both the leaves and fruits of the Mordica charantia plant have a remarkable antimicrobial activities even at low concentrations (10µg/ml) with all the bacterial species tested for, except Salmonella enterica. The methanol and ethylacetate residues showed less significant activity with all of the organisms tested having activities at 80-100µg/ml in some cases. The inhibitory zones were recorded in millimeters. The MIC of the leaf aqueous extract was found to be 12.5µg/ml against both S. aureus and E. coli while MIC for S. enterica was found to be 25µg/ml. This was the same for the chloroform leaf extract except that a less MIC value of 6.25µg/ml was recorded against E. coli. The methanol extract indicated an MIC of 100µg/ml for both S. aureus and S. enterica.

The MBC values (Table 4) indicated that the chloroform leaf extract produced the highest bactericidal effect against *S. aureus* at a concentration of 6.25μ g/ml, while fruits gave MBC



value at 12.50μ g/ml for the same organism. The methanol extract of the fruit part of the plant showed no bactericidal effect at concentration of 100μ g/ml for both *S. aureus* and *S. enterica* but indicated a bactericidal effect against *E. coli* at a concentration of 50μ g/ml.

The antibacterial activity testing from the column chromatographic fractions of the leaves and fruits showed column 1-3 to contain the active components with a significant (p<0.05) activity against all the organisms tested. The results indicated that *Staphylococcus aureus* was most susceptible to the leaf fraction with highest zone of inhibition of 25.30mm, *Escherichia coli* had 22.83mm and *S. enterica* was found to be less susceptible with zone of 13.63mm. Fractions of columns 5 to 8 were found not to contain bioactive compound in significant amounts as the result indicated less or complete resistance to the fraction by all of the organisms used.

The phytochemical screening of the crude extract of the leaves and fruits revealed the presence of alkaloids, flavonoids and steroids as the most common components found in considerable amounts in both plant parts tested. Other constituents detected were glycosides, terpenes, carbohydrate and traces of antraquinones. The screening showed that saponins were found in moderate amount in the leaves than in the fruits. Other plants phytochemicals tested and found not presents in all the extracts are balsam, resins, and tannins.

After oral administration of 1ml volume of 3000mg/kg body weight of the aqueous leaves and fruits extract of Mormodica charantia to the rats in a single oral dose at grace observation of 24hrs, 48hrs and up to 14 days no mortality and no any toxicity sign or behavioral changes such as excitement, restlessness, weakness, reduced reaction to noise, slow movement, resting at the corner of the cages, respiratory distress was observed. This indicates that the limit test dose and the LD₅₀ of the extract were greater than 3000mg/kg body weight. This result practically indicates that the plant Mormodica charantia was generally non- toxic at 3000mg/kg body weight. However, higher doses of up to 5000mg/kg body weight caused slow movement and resting at the corner of cages by the animals.

Extract Conc. (µg/ml)	Zone of Inhibitio	on (mm)/Bacteria (± :	= Standard deviation.)
	Staph. aureus	Esch. coli	Sal. enterica
100AE	20.28±0.07	24.00 ± 0.10	16.67±0.15
80AE	16.33±0.15	18.20±0.15	14.23±0.58
60AE	14.25±0.20	15.60±0.53	12.37±0.12
30AE	12.53±0.57	14.17 ± 0.30	10.10±0.11
10 <i>AE</i>	9.53±0.58	12.30±0.23	9.37±0.06
100 <i>ME</i>	11.50±0.26	12.23 ± 0.21	10.73±0.15
80 <i>ME</i>	10.60±0.21	10.77±0.25	9.5±0.10
60 <i>ME</i>	9.5±0.06	9.50±0.06	9.10±0.01
30 <i>ME</i>	8.87±0.32	8.70±0.32	8.67±0.28
10 <i>ME</i>	-	-	-
100 <i>EAE</i>	9.97±0.25	10.60 ± 0.36	9.63±0.32
80 <i>EAE</i>	8.63±0.32	9.50±0.50	8.50±0.58
60 EAE	-	-	-
30 <i>EAE</i>	-	-	-
10 EAE	-	-	-
100 <i>CHE</i>	26.77±0.25	25.57 ± 0.40	20.33±0.15
80 CHE	24.40±0.36	23.73±0.25	18.37±0.25
60 <i>CHE</i>	23.33±0.25	21.53±025	16.13±0.40
30 CHE	22.00±0.17	19.17±0.15	15.17±0.15
10 <i>CHE</i>	24.53±0.58	22.10±0.10	23.27±0.25
Amoxicilin 10µg/ml in water	4.53±0.58	22.10±0.10	23.27±0.25
Water	-	-	-

 Table 1: Antimicrobial activity of crude aqueous and organic extracts of the leaves of *M. charantia*.

AE=Aqueous Extract, ME=Methanol Extract, EAE=Ethyl Acetate Extract, CHE=Chloroform Extract, - =No activity. Values are represented as means of triplicates.

Table 2: Antimicrobial activity of crude aqueous and organic extracts of the fruits of M. charantia.

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Extract Conc. (µg/ml) Z	one of Inhibition (m	n)/Bacteria (± = Stand	lard deviation.)	
	Staph. aureus	Esch. coli	Sal. enterica	
100AE	22.27±0.25	23.46±0.15	17.37±0.32	
80AE	18.03±0.25	19.23±0.25	15.06±0.12	
60AE	13.30±0.26	14.33±0.58	13.37±0.35	
30AE	11.03±0.15	11.83±0.15	10.06±0.31	
10AE	9.23±0.21	9.17±0.21	-	
100 <i>ME</i>	10.80±0.32	11.23 ± 0.25	10.10±0.10	
80 <i>ME</i>	10.00±0.35	10.30±0.10	9.60±0.21	
60 <i>ME</i>	-	-	-	
30 <i>ME</i>	-	-	-	
10 <i>ME</i>	-	-	-	
100 <i>EAE</i>	10.01±0.10	10.87 ± 0.32	9.10±0.17	
80 <i>EAE</i>	9.23±0.21	9.30±0.10	-	
60 EAE	-	-	-	
30EAE	-	-	-	
10 EAE	-	-	-	
100 <i>CHE</i>	27.20±0.20	25.57 ± 0.51	20.87±0.15	
80 CHE	25.03±0.06	24.30±0.36	19.33±0.38	
60 <i>CHE</i>	23.33±0.28	22.13±0.15	16.83±0.15	
30 CHE	21.60±0.53	20.40±0.53	15.27±0.25	
10 <i>CHE</i>	20.73±0.25	18.77±0.25	13.57±0.51	
Amoxicilin 10µg/ml in wate	r 24.53±0.58	22.10±0.10	23.27±0.25	
Water	-	-	-	

AE=Aqueous Extract, ME=Methanol Extract, EAE=Ethyl Acetate Extract, CHE=Chloroform Extract, - =No activity. Values are represented as means of triplicates.

Table 3: Minimum Inhibitory Concentrations (MIC) of aqueous and organic solvent extracts of *M. charantia* plant.

	MIC va	alues in diff	erent solvents/org	ganisms (µg/m	l) ±standar	d deviation			
Plant Extract	Aqueo	us	Methanol		Chlorofo	rm			
	S. aureus	E. coli	Sal. enterica	S. aureus	E. coli	Sal. enterica	S. aureus	E. coli	Sal. enterica
Leaf	12.50	6.25	25.00	100	50	100	6.25	12.50	25.00
Fruit	25.00	6.25	25.00	100	50	100	6.25	6.25	25.00

Table 4: Minimum Bactericidal Concentrations (MBC) of aqueous and organic solvent extracts of *M. charantia* plant

	MIC v	alues in diffe	erent solvents/org	janisms (µg/m	l)±standard	deviation			
Plant Extract	Aque	ous	Methanol		Chlorofor	m			
	S. aureus	E. coli	Sal. enterica	S. aureus	E. coli	Sal. enterica	S. aureus	E. coli	Sal. enterica
Leaf	25.00	25.00	50.00	100	50	100	6.25	12.50	25.00
Fruit	50.00	25.00	50.00	100	50	100	12.50	12.50	25.00



 Table 5: Antimicrobial activity of the bioactive compounds from the column chromatographic fractions of chloroform leaf and fruit extract of *Momordica charantia*

Fractions	Zones of Inhibition (mm)						
		Leaves		Fruits			
	S. aurues	E. coli	Sal. enterica	S. aurues	E. coli	Sal. enterica	
Column 1	12.57±1.25	9.70±0.25	-	16.17±0.40	13.10±0.26	11.0±0.45	
Column 2	17.70±0.80	14.8±1.60	11.70±0.82	20.83± 0.96	17.00±0.92	13.07±0.21	
Column 3	25.30±1.30	22.83±0.85	13.63±0.93	9.30±0.30	9.20±0.35	-	
Column 4	10.23±0.68	9.50±0.62	-	-	-	-	
Column 5	9.07±0.12	8.80±0.35	-	-	-	-	
Column 6	-	-	-	-	-	-	
Column 7	-	-	-	-	-	-	
Column 8	-	-	-	-	-	-	

Values are represented as means of triplicates and ±Standard deviation.

Table 6: Photochem	ical properties of aque	ous and Organic	solvents extra	cts of M. cl	<i>harantia</i> leaves
Constituent	Aqueous Residue	Methanol	Ethyl	Acetate	Chloroform
		Residue	Residue		Residue
Alkaloids					
a. Drangedorff's	++	+	+		+++
b. Meyer's	+	+	+		+
c. Wagner's	+++	+	+		+++
Balsam	-	-	-		-
Carbonhydrate	++	+	+		+
Flavonoids	+++	++	+		+++
Anthraquinones	Traces	-	-		-
Cardiac glycoside	++	+	+		++
Tannins	-	-	-		-
Terpenes	++	+	+		+
Resins	-	-	-		-
Steroids	+++	++	+		+++
Saponins	++	+	+		+

-= Constituent not detected in the sample,+=Little amounts of the constituents in the sample,++= Moderate amounts of the constituents in the sample, +++ Numerous amounts of the constituents in the sample.



Constituent	Aqueous Residue	Methanol	Ethyl Residue	Acetate Chloroform Residue	
		Residue	Kesidue	Kesiuue	
Alkaloids					
a. Drangedorff's	+++	+	+	+++	
b. Meyer's	+++	+	+	+++	
c. Wagner's	+++	+	+	+++	
Balsam	-	-	-	-	
Carbonhydrate	+	+	+	+	
Flavonoids	++	+	+	+++	
Anthraquinones	Traces	-	-	-	
Cardiac glycoside	++	+	+	++	
Tannins	-	-	-	-	
Terpenes	++	+	+	+	
Resins	-	-	-	-	
Steroids	++	++	+	+	
Saponins	++	+	+	+	

Table 7: Phytochemical	properties of aqueous and O	Organic solvents extracts of <i>M. charantia</i> fruits
Table 7. Thy toenennear	properties of aqueous and o	and solvenus extracts of m. charanna in and

- = Constituent not detected in the sample,+=Little amounts of the constituents in the sample,++= Moderate amounts of the constituents in the sample, +++ Numerous amounts of the constituents in the sample.

Discussion

Indications of antibacterial activity observed in this study was based on an easy in-vitro system used for assessing the antibacterial activities of plants. At concentration from 10µg/ml, antibacterial activity was observed from both the leaves and fruits of the plant. Chloroform and water extracts exhibited significant (p<0.05) inhibitory activity on all the three bacteria isolates used. Hence different solvent extracts of some plants may have different pharmacological properties. This observation is consistent with previous report by Freiburghans and colleagues (1996). The antibacterial potential of the plant parts has been elucidated by the results obtained. The leaf and fruit extracts (water, ethanol, chloroform) experimentally methanol and demonstrated antimicrobial activity. This finding is in agreement with previous report by Omoregbe and colleagues (1996) which indicated in-vitro antimicrobial activity of leaf extract of wild bitter gourd (Momordica charantia L. var.), which is normally smaller than cultivated bitter gourd, use in this study. However from their finding the extracts showed no inhibitory activity against methicillinresistant Staphylococcus aureus or Pseudomonas aeruginosa but were found to be active against Escherichia coli, Salmonella paratyphi, Shigella dysenterae and Streptomyces griseus. Fruit extract has also been found to have activity against Helicobacter pylori organism with MICs ranging between 1.95 and 250 μ g/ml (Yesilada *et al.*, 1999). The MIC and MBC of the aqueous and organic solvents extract as judged by this study indicated that both the leaves and fruits of the plant bitter melon have a bacteriostatic activity against pathogens tested at concentration from 6.25 μ g/ml and bactericidal effect from concentration above that. The chloroform extract of the leaves and fruits was shown to have more potency with all the organisms tested. This observation does not seem to corroborate the practice of Aboriginal Northern Nigeria of using water to extract bioactive compounds (Hassan *et al.*, 2007).

We observed that *Salmonella enterica* shows a less MIC and MBC values with both the leaves and fruits compared to other organisms. This may be attributed to the fact that cell wall of Gram-positive bacteria were somewhat different from that of Gram-negative bacteria and the site of action of some of the most effective antibiotic medications is the cell wall (Jawetz*et al.*, 2004). These findings of low MIC values are comparable to those obtained by Grover and Yadav (2004) which indicated that extracts of leaves of *Mordica charantia have* antibacterial activity against pathogenic bacteria at low doses.

The phytochemicals found present in the leaf and fruit of the plant include: flavonoids, tannins, saponins, alkaloids, terpenoids, steroids and cardiac glycosides. Flavonoids and alkaloids present in this plant has been shown in a previous study (Maurice,



1999) to possess many pharmacological properties such as: antioxidant activities, anti-inflammatory activities, anti- cancer activities and anti-microbial effects hence, flavonoids and alkaloids may have a contributory effect to its anti-infective properties and other pharmacological effects the plant possesses.

The pharmacological evaluation of *M. charantia* include acute toxicity test to determine it efficacy and safety as a medicinal plant. The toxicity study on the aqueous *M. charantia* extract showed that administration of a single high dose of 3000mg/kg body weight to rats did not result in death, suggesting a high level of safety in the use of this plant at lower doses only. Although chronic toxicity study would give details of the cytotoxic activity of *M. charantia* plant.

This study therefore, supports the claims on the folkloric use of the plant part (leaf, fruit and stem) in infectious cases and could be of considerable interest to the development of new drugs. Further studies are in progress to elucidate the structure of the active compound and final answer on the safety of *M. charantia*.

Conflict of interest: No conflict of interest declared

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