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Research Paper

Antimicrobial and hypoglycemic effects of *Momordica balsamina.* Linn.

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ABSTRACT

We evaluated the antibacterial, antifungi and hypoglycemic effects of methanol and water (ratio 70 : 30) extract of *M. balsamina* leaves and stem. Antibacterial and antifungi activities were investigated using the cup bore agar diffusion method; while the hypoglycemic effect was evaluated in alloxan induced diabetic rats. The extract of *M. balsamina* significantly inhibited *Bacillus subtilis, Staphylococcus aureus* (Gram positive), *klebsiella pneumonia, Escherichia coli, Salmonella typhi, Proteus vulgaris* and *Pseudomonas aeruginosa* (Gram-negative) to varying degrees, but it had no inhibitory effect on fungal species (*Aspergillus niger, Aspergillus fumigatus*, *Mucor pusillus* (fungi), and *Candida albicans* (yeast). Gentamycin, the standard antibacterial drug used was effective in inhibiting these bacteria. The effect on *B. subtilis and E. coli* were comparable to that of gentamycin. Ketoconazole, the standard antifungi used was effective against all the fungi and yeast species. The extract of *M. balsamina* also significantly ($P<0.05$) reduced blood glucose level in alloxan induced diabetic rats twelve hours after administration. The effect of the extract at 1000mg/kg was comparable to that of the standard drug (chlorpropamide) used.
These results support some of the traditional uses of *M. balsamina* as an antiinfective herb and for the management of diabetic sores.

Keywords: Antibacterial, Antifungi, Hypoglycemic, *Momordica balsamina*.

INTRODUCTION

Bacteria and fungi resistance to antimicrobial drugs has continued to grow in the last decades (Cohen, 1992; Nascimento et al., 2000). The increased prevalence of their resistant is due to extensive use and misuse of antimicrobials. This has rendered the current available antimicrobial agents insufficient to control microbial infections (Cowan, 1999) and create major public health problem (Bax et al*.,* 2000; Alade, et al., 1993). This development has led to increased search to unfold new, broad spectrum, potent antimicrobial agents. Resistant to antibiotic due to extengens such as *Staphylococcus aureus* and Pseudomonas species is of great concern (Bax et al*.,* 2000; Chopra et al., 1992). Antimicrobial resistance to anti microbial agents has lead to treatment failure and the shift of medical care from orthodox to herbal medicine. Most of the herbal medicines in use await validation of their claimed effects and possibly the development of novel antimicrobial drugs from them.

Diabetes is another disease that is on the increase because of modern technology and change in our eating habits. Most diabetics are faced with the problem of getting solution to their diabetic sore, which in

most cases becomes infected. Their problem is further compounded with the growing number of resistant microorganisms to the existing antimicrobials. Drugs that have both anti-diabetic and potent antibacterial effect will be of advantage and also reduce polypharmacy and speed up healing of diabetics sores.

Momordica balsamina Linn. (Balsam apple; Family- Cucurbitacea) is commonly used in Africa. Their leaves and young fruits are eaten as vegetable in Cameroun, Sudan and Southern Africa. Their fruits, leaves and seeds extracts are used as anti-helmintic (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 and skin diseases, diabetics, and gastroenteritis (Gills, 1992). Despite the extensive study on the plants, there is still paucity of knowledge on the antimicrobial and anti diabetic actions of the plant's leaves and stems. The present study was therefore designed to find out the antimicrobial and hypoglycemic effects of *M balsamina.*

MATERIALS AND METHODS

Collection of plant materials: *M. balsamina* Linn leaves and stems were collected from a forest environment near University of Jos, Plateau State, Nigeria. Their identity was confirmed in Botany Department of the same University by Prof. Husaini and School of Forestry Jos by Mr. Kareem.

Extraction of plant materials: The leaves and stem were air dried under shade and then powdered using surface sterilized mortar and pestle. Powder plant material (1000 grams) was macerated in 70% methanol and left in air tight aspirator bottle for 72 hrs., (Sunday et al., 2007). The extract was filtered after 72 hrs, with the aid of sterile sieving cloth. The filtrate was then evaporated using a rotary evaporator at 45^0C. A yield of 21% (w/w) was obtained from the extraction process. The dried extract was labeled and stored in the refrigerator at 4^0C.

Bacteria and fungi species: *Bacillus Subtilis, Staphylococcus aureus* (Gram positive), *Klebsiella pneumonia, Escherichia coli, Salmonella typhi, Proteus vulgaris*, *Pseudomonas aeruginosa* (Gram-negative), *Aspergillus niger, Aspergillus fumigates*, *Mucor pusillus* (fingi), and *Candida albicas* (yeast) were used for this study. The test microorganisms were obtained from the stock cultures of microbiology unit of the Department of pharmaceutics and pharmaceutical technology, University of Jos, Nigeria.

Preparation of test organisms: An inoculums of size 10^8 colony forming units per milliliter (cfu/ml) of each of the isolates was prepared according to the method described by Bauer et al. (1966) This was effected by suspending loopful of inoculums from the stock into different labeled test tubes, each containing 10 ml of nutrient broth. A total of 3 test tubes were used for each test organism. The treated tubes were incubated at 37^0C for 24 hrs. The resultant cultures were then diluted with fresh nutrient broth in order to achieve optical densities corresponding to 10^8 cfu/ml.

Antimicrobial susceptibility test: Various concentrations (100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml) of the leaf and stem extract of *M. balsamina* were assayed for antimicrobial activity on the test organisms using modified cup plate method described by Collins et al. (1995). The Nutrient agar plates and Czapek-dox agar plates were prepared. Loopful (0.002 ml) of each bacterium inoculums corresponding to $1x10^8$ cfu were evenly streaked on the prepared agar plate containing Czapek-dox agar. The plates were then air dried for a period of five minutes. Prepared fungi spore suspension was evenly streaked on the prepared agar plate containing potato/glucose mixture. The plates were then air dried. Cups were bored in each solid media using sterile cork borer (number 3). A volume of 0.1 ml of the prepared dilutions of the extract (100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml, respectively) was introduced into each of the aseptically bored holes. The plates were replicated 5 times. The plates were then incubated at 37^0C for 24 hrs for bacteria and *C. albicans* and for a period of 5 days for the test fungi. The diameters of the zones of inhibition were measured and recorded.

***Anti-diabetic effect of M.balsamina*:** Wister albino rats (weight 150-220g) were injected with aloxan (100 mg/kg) through tail vein. Five days later, blood glucose levels of the animals were determined using a glucometer. The diabetic rats showing blood glucose levels in the range of 240–400 mg/dl were selected for the evaluation of *M. balsamina* for antidiabetic properties (Subramoniam et al., 1996). The diabetic rats were divided into four groups of six in each. The control group was given 0.1 ml of distilled water, ip. The test groups were given the extract of *M. balsamina* (500 mg/Kg and 1000 mg/Kg respectively).

The fourth group received chlopropramide (400 mg/kg, p.o.). Blood glucose levels were determined at 0, 1, 2, 3 and 12 hours. The rat`s blood was collected from it`s tail by massaging the whole length of the tail until sufficient blood accumulates at the tip. The glucometer was switched on and the glucometer code number was set to the code on the one touch glucose strip bottle. Then the strip was inserted into the glucometer as instructed by glucometer. With the aid of the surgical knife, the tip of the rat's tail was cut off and the blood dropped on the appropriate portion of the glucose strip inserted in the glucometer. After some seconds, the glucometer starts counting down from 45 seconds to 1 second and then displayed on its screen the glucose concentration in mg/dl. This procedure was repeated for all the rats used in the experiment and their blood glucose level noted.

RESULTS

Anti-microbial activities: The crude extract of *M. balsamina* inhibited ($P<0.05$) the tested bacterial strains (Table 1). The crude extract showed some activity against *B. subtilis, E. coli, P. aeruginosa, and P. mirabilis*, but showed marginal activity against *S. aureus K. pneumonia, and S. typhi.* The activity of the extract against *B. subtilis and E. coli* was comparable to that gentamycin (Table 1).

The crude extract of *M. balsamina* was unable to inhibit ($P>0.05$) the tested fungi and yeast strains (Table 2). The crude extract showed no activity against *A. fumigatus A. niger C. albicans and M. pusillus.* Ketoconazole was very effective in inhibiting all the fungi used for this study (Table 2).

M. balsamina crude extract has great potential as antimicrobial agent against bacteria but not against fungi and it can be used in the treatment of infectious diseases caused by resistant bacteria. *E. coli* showed maximum susceptibility.

Anti-diabetic effect: *M. balsamina* crude extract significantly ($P<0.05$) reduced blood glucose level in aloxan induced hyperglycemic rats. The effect of *M. balsamina* at 1000 mg/Kg was comparable to that of chlorpropamide (Table 3). The hypoglycemic effect of *M. balsamina* was not evident until the twelveth hour of administration; this implies that its hypoglyceamic effect is not immediate.

DISCUSSION

Over the last decades there has been a lot of work in the area of elucidating the active principle of herbal medicines and synthesizing the active constituent for medical use. Research has shown that a number of potent herbs don`t show activity or show reduced activity after separation and synthesis of the active principle. This largely has been attributed to the fact that some of the components in the plants acts synergistically or inhibit the actions of other components in the plant. The use of the plant material has been supported by this discovery. The crude extract of the stem and leaves of *M. balsamina* showed antibacterial and antidiabetic effects.

These results support the work of Jigam et al, (2004). They reported that the MeOH extracts of whole plant parts of *M. balsamina* was active against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* and not effective against *Saccharomyces cerevisiae* and *Aspergillus niger*. This work also established that the plant has activity against *B. subtilis, P. mirabilis and K. pneumonia,* in addition to the organism studied by Jigam et al, (2004). This suggests that *M. balsamina* has a broad spectrum anti bacterial activity against Gram negative and Gram positive bacteria. The study

also revealed that the extract of the mixture of the leaves and stems of *M. balsamina* as it is used in Nigeria is equally potent as the whole plant used by Jigam et al, 2004.

The results obtained further supports the report by Jigam et al, 2004, that the methanolic extract of *M. balsamina* has no antifungi properties. The lack of antifungi activity against *Saccharomyces cerevisiae* and *Aspergillus niger* was extended to the other fungi and yeast strains tested in this study.

The antibacterial properties observed may augment the analgesic and anti-inflammatory properties (Otimenyin and Uguru, 2005) of the herb by reducing or eliminating the causative agents of inflammation and thus decreasing the nociceptive stimulants released from inflamed cell. This implies that the *M. balsamina* alone will effectively combat the infection and pain associated with infectious and diabetic diseases thus reducing the number of drugs used in pain, infection and diabetes management and preventing the complications of poly pharmacy.

There has been an increase in the incidence of diabetes over the years due to an increase in the consumption of processed food. This development has also led to an increase in research into herbal medicines for the discovery of effective and lead drugs for the management of this disease conditions (Jamal, 1997). Traditional medicines are used throughout the world for the management of diabetes, *M. balsamina* is one of the traditional medicines that has been in use for decades for the management of this disease condition (Gill, 1992). *Trgonella foenum-graecum* is an example of plants that has been validated to have antidiabetic properties and is still in use today for the management of diabetic conditions. (Shani et al., 1974). The results obtained from this work revealed that *M. balsamina* has the claimed antidiabetic properties.

CONCLUSION

M. balsamina crude extracts have great potential as antimicrobial agent against bacteria but not against fungi and it can be used in the treatment of infectious diseases caused by resistant bacteria strains.

The antibacterial and hypoglycemic properties of *M. balsamina* shown in this study support the traditional use of the plant in the management of diabetic sore.

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Table-1: Antibacterial effect of methanol/water extract of *M. balsamina* plant.

Organisms	Zones of Inhibition (mm)					
	Extract Concentration					
	100mg	75mg	50mg	25mg	12.5mg	Gent. (4mg)
E. coli	30.3±4.1*	26.0±2.2*	23.5±3.2*	19.9±0.2*	19.3±0.0	21.0±0.2
B. subtilis	22.1±3.3*	21.2±2.1*	16.1±2.3	10.1±0.2*	12.2±0.2	16.0±3.4
P. mirabilis	19.0 ±0.4	17.8 ± 3.0*	12.7 ± 1.0*	8.3 ± 1.1*	0.0±0.0*	23.0±2.4
P. aeruginosa	5.9±1.3*	5.7 ± 1.7*	5.1 ± 2.3*	4.7 ± 0.8*	0.9±0.2*	13.0±1.3
S. aureus	2.0 ±1.3*	2.0 ± 0.2*	1.7 ± 0.2*	1.3 ± 0.4*	0.0±0.0*	23.0±2.1
S. typhi	1.9±0.6*	0.5±0.1*	0.0±0.2*	0.0±0.2*	0.0±0.0*	22.0±0.2
K. pneumonia	1.7±0.3*	1.5±0.5*	1.2±0.2*	0.9±0.1*	1.3±0.2*	19.0±0.2

- Results were expressed as Mean ± SEM.
- *P<0.05. When compared with gentamycin.
- Gent.= Gentamycin

Table-2: Antifungal effects of methanol/water extract of *M. balsamina* plant.

Organisms	Zones of Inhibition (mm)					
	Extract Concentration					
	100mg	75mg	50mg	25mg	12.5mg	Keto. (1mg)
A fumigatus	0.4±0.2*	0.4±0.2*	0.2±0.1*	0.1±0.3*	0.1±0.2*	16±3.4
A. niger	0.2±0.2*	0.2±0.1*	0 ± 0*	0 ± 0*	0 ± 0*	13±1.3
C. albicans	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	23±2.4
M. pusillus	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*	23±2.1

- Results were expressed as Mean ± SEM.
- *P<0.05. When compared with ketoconazole.
- Keto. = Ketoconazole

Table-3: Effect of *M. balsamina* extract on BGC Level of hyperglycemic rats.

Treatment (mg/kg)	Mean BGC level ± S.E.M. (mg/dl)				
	Sampling time (hours)				
	0	**1**	**2**	**3**	**12**
Control	248.17±92.85	231.61±34.46	222.12±17.68	260.21±20.66	280.4±20.53
Chlorpropamide, 400mg/Kg	297.27±120.54	306.41±98.17	326.65±109.82	264.41±78.18	52.81±10.01*
M. balsamina 500mg/Kg	248.42±70.62	269.83±61.79	304.40±97.93	211.29±18.89	67.22±6.18*
M. balsamina 1000mg/Kg	353.75±72.73	413.45±135.0	351.31±62.38	344.25±106.82	34.25±4.11*

- *P < 0.05, there is significant difference.
- The values were expressed as mean ±S.E.M.
- BGC= Blood glucose concentration.