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# INVESTIGATION ON THE BIOEFFECTS OF ETHANOL EXTRACTS OF LEAF AND STEM OF *MOMORDICA CHARANTIA* ON CLINICAL STRAINS OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

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***ABSTRACT***
***The bio-effects of the ethanol extracts from the leaf and stem of Momordica charantia were studied with the view to ascertain the medical usefulness ascribed to the plant by the locals. The plant parts, stem and leaf, revealed remarkable activity against Escherichia coli and Staphlococcus aureus. The leaves extracts showed activity at a concentration as low as 10mg/ml against E. coli and 15mg/ml against S. aureus. The Minimum Inhibitory Concentration (MIC) ranges from 10-15mg/ml while Minimum Bactericidal Concentration (MBC) from 30-45mg/ml. The activity of these extracts compared favourably with those of standard antibiotics, Tetracyclines (0.33mg) and Ampicillin (10µg), used in this study. The phytochemical analysis of the extracts showed the presence of tannins, alkaloids, cardiac glycosides and steroids. The presence of these chemicals in the extracts may have been responsible for the activity possessed by the plant extracts.***

***Keywords: Bioeffects, Ethanol extracts, Clinical strains, Momordica charantia***

## INTRODUCTION

The emergence of resistant strains of microbial pathogens and high cost of orthodox medicine as well as the menace of fake and adulterated drugs in our society today have led to so many untold and unbearable hardships to man in terms of health care delivery. These have led to the death of so many individuals while others have been maimed for life. The reality of these problems has led to search for solutions and so far it is gradually being accepted that man should go back to basics i.e back to nature.

Man has largely depended on his environment for solutions to his problems. Through the ages man has procure health care deliveries to himself and livestock as well as plants around him through the use of plant chemicals.

Plants are believed to be natural reservoir of chemicals with medicinal values and a number of modern drugs are said to have been isolated from natural sources except the synthetic ones. Isolation of these phytochemicals is based on information provided by the locals on the medicinal uses of such plants (Moshahid *et al.,* 2009).

The consumption of plant materials as alternative medicine has been encouraged because they are cheap and they could significantly contribute to the improvement of human health in terms of cure and prevention of diseases Okoko and Oruambo (2008). Plants have been useful as food and medicine [Lee, *et al.,* 2003; Ogle, *et al.,* 2003; Adebooye and Opabode, 2004; Ayodele, 2005]. They contain vitamins needed by human body for healthy living (Szeto, *et al.,* 2002; Jimoh, *et al*., 2004). The native country of *Momordica charantia* is uncertain, but the plant is cultivated throughout the tropics, particularly in India, China, East Africa and Central and South America (Sofowora, 1993). It is occasionally grown as an ornamental creeper, but more commonly cultivated for the use of the unripe fruit as a vegetable. The fruit has a number of different local names – bitter gourd, bitter-melon, balsam-pear, cundeamor (South America), karela (India), carilla or goo-fah (Jamaica); the reported spelling of the local names is often variable. The wild variety (*M. charantia* var. abbreviata) grows as a weed in the West Indies, where the plant is known as cerasee (Jamaica) or sorossie (Dominican Republic). This variety has smaller fruit than the Indian one. With the emergence of resistant strains of *Escherichia coli* such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC) and coagulase negative *Staphylococcus aureus* which have become a great medical problem being responsible for a lot nosocomial infections, it is therefore, most important and necessary for us to authenticate the relevance of this plant scientifically to give approval to or otherwise the claims of the locals on the efficacy of this plant in curing ailments associated with these organisms.

Leaves and an unripe fruit

Ripe and unripe fruits with leaves

**MATERIAL AND METHODS**

Clinical strains of the organisms *Escherichia coli* and *Staphylococcus aureus* were isolated from clinical specimens obtained from Ahmadu Bello University (ABU) teaching hospital Chika, Zaria. Isolates were identified using their physical as well as chemical characteristics (Buchanan and Gibbons, 1974).

**Sample collection:** Plant materials, (*Mormodica charantia*) were collected from Bida, Niger State. Identification was carried out by local people and confirmed by a Botanist and Taxonomist (S. Ayodele, Head, Herbarium, University of Ilorin, Nigeria).

**Extraction and preparation of plants' materials**: Ethanol was used as solvent for the extraction of the plant materials. The method of Silva *et al* (1997) was adopted. Twenty (20) grammes of ground sample was suspended in 100ml of 95% ethanol for a period of about 120 hours. The extract was decanted and filtered and the filtrate evaporated in vacuo at $45^0$C. The residue was reconstituted in 95% ethanol and reserved as stock concentration then stored.

**Antibacterial assay**

Each of the microorganisms was subjected to the action of the extracts using the agar cup plate technique as described by Silva *et al* (1997) and Abalaka (2003). Using cork borer No.4, three holes were bored on the surface of the agar medium equidistant from one another. The bottom of each hole was sealed with molten agar to avoid seepage. When solidified, each of the cups or holes made was filled with known volume and concentration (1ml of 5mg, 10mg, 15mg……40mg respectively) of the pre-prepared extract solution and allowed to fully diffuse. The surface of the agar was streaked for confluent growth with an 18 hour culture of the test organism which has been previously standardized to $10^6$ and incubated at the temperature of $37^o$C in the incubator for 24 hours.

**Minimum inhibitory concentration (MIC)**

Using tube dilution method, the least concentration of fractions from the plant extract in which there was no turbidity was taken as the minimum inhibitory concentration (MIC) (Hugo and Rusell, 2003). The MIC of the plant extracts was determined by serially diluting extract from $10^1$ to $10^{10}$. 1ml of each of the extracts (for example from the dilution of 5mg, 10mg, 15mg etc) was introduced into 9ml of nutrient broth in the test tube. This mixture was then inoculated with 0.1ml culture of the test organism previously standardized to $10^6$cfu/ml. This was then incubated at $37^o$C for 24 hours. The least concentration of plant extract in the test tube with no turbidity was taken as the Minimum Inhibitory Concentration (MIC).

**Minimum bactericidal concentration (MBC)**

This was an offshoot of the previously determined MIC. The least concentration of plant extract in the test tube with no turbidity was taken as the Minimum Inhibitory Concentration (MIC). Subsequently, those tubes that showed no turbidity were plated out on nutrient agar plates and absence of growth on incubation for 24 hours was confirmatory for Minimum Bactericidal Concentration (MBC).

**Phytochemical analysis of Plant extracts for Active Components**

Phytochemical screening of the extracts was carried out according to the methods described by Odebiyi and Sofowora (1978) and Trease and Evans (1989) for the detection of active components like saponins, tannins, alkaloids, phlobatanins, glycosides and e.t.c

a. **Alkaloids-** 1ml of 1%HCl was added to 3ml of the extract in a test tube. The mixture was then heated for 20 minutes, cooled and filtered. About 2 drops of Mayer's reagent to1ml of the extract. A creamy precipitate was an indication of the presence of alkaloids.
b. **Tannins-** 1ml of freshly prepared 10%KOH was added to 1ml of the extract. A dirty white precipitate showed the presence of tannins.
c. **Glycosides-** 10ml of 50% $H_2SO_4$ was added to 1ml of the extract and the mixture heated in boiling water for about 15 minutes. 10ml of Fehling's solution was then added and the mixture boiled. A brick-red precipitate was confirmatory for the presence of glycosides.
d. **Saponins-** (i) Frothing test: 2ml of the extract was vigorously shaken in the test tube for 2 minutes. No frothing was observed.

(ii) Emulsion test: 5 drops of olive oil was added to 3ml of the extract in the test tube and vigorously shaken. Absence of stable emulsion formed showed absence of saponins.

e. **Flavonoids-** 1ml of 10% NaOH was added 3ml of the extract. There was no yellow colouration which is indicative of the absence of flavonoids.

f. **Steroids-** *Salkowski test:* 5 drops of concentrated $H_2SO_4$ was added to 1ml of the extract in a test tube. Red colouration was observed which is indicative for the presence of steroids.

g. **Phlobatanins-** 1ml of the extract was added to 1%HCl. No red precipitate observed which means negative result.

h. **Triterpenes-** 1ml of the extract was added to 5 drops of Acetic anhydride and a drop of concentrated $H_2SO_4$ added. The mixture was then steamed for 1 hour and neutralized with NaOH followed by addition of chloroform. Absence of blue-green colour indicates the absence of triterpenes.

**RESULTS AND DISCUSSION**

The ethanol extracts from leaves and stem of *Momordica charantia* revealed strong activities against clinical strains of the bacterial isolates. The high activities of the leaves extracts against *Escherichia coli* and *Staphylococcus aureus* even at concentration as low as 10mg/ml is quite encouraging (Table1). *E. coli* is known to cause opportunistic infection of the unary tract (UTI) and is also responsible for infantile gastroenteritis as well as travelers' diarrhoea wound infection e.t.c (Dilip 1985). The test organism *Staphylococcus aureus* is also known to be responsible for urinary tract infection (UTI), rare cause of meningitis, wounds infection e.t.c (Dilip 1985).

The rate of emergence of antibiotics resistant bacteria is not matched by the rate of developments of new antibiotics to combat them (Prescott and Klein, 2002). The above reason makes the findings in this work so important and relevant to achieving good health care delivery in our modern world where organisms are fast developing resistance to the antibiotics that were once upon a time "miracle cure all" in the control of infectious disease, while *E. coli* is a Gram negative rod, *S. aureus* is a Gram positive coccal shaped organism. That the extracts have activity against the organisms is an indication of broad spectrum of activity of the extracts which is one of the characteristics of any plant material useful in chemotherapy. The results show that the extract could be used to treat diseases caused by both Gram positive and Gram negative bacteria.

The minimum inhibitory concentration (MIC) ranges from 20-25mg/ml for stem extract and 10-15mg/ml for leaf extract while MBC ranges from 35-45mglml and 30-40mg/ml for stem and leaf extracts respectively (tables 2 and 3). The results showed that leaves extracts have more activity which is indicated by the low MIC concentration of 10mg/ml against *E. coli* as compared with those of stem extracts of 20mg/ml as the lowest against the same organisms (*E. coli*). This is not surprising as the leaves are involved majorly in photosynthesis thereby being the reservoir of manufactured materials (Roberts, 1986). Various phytochemicals where identified which include tannins, alkaloids, cardiac glycosides and steroids. The leaves contain more chemicals than the stem (Table 4). Some of the phytochemicals have been reported to have resistance modulating activities against strains of microorganisms which means that the herbal materials can act as antibiotic resistant inhibitors (Gibbons *et al*., 2003).

Activities of this plant extracts expressed *invitro* against these organisms (though preliminary) could be a pointer to the fact that the solution to human health care delivery may lie with nature and nature has abundance supply of these plants.

**Table 1: Results of susceptibility testing of the Stem and Leaves extracts of *M. charantia* against *E. coli* and *S. aureus***

| | Mean Diameter of zone of inhibition (mm)* | | | |
|---|---|---|---|---|
| | Stem | | Leaves | |
| Conc (mg/ml) | *E. coli* | *S. aureus* | *E. coli* | *S. aureus* |
| 5 | - | - | - | - |
| 10 | - | - | 10±0.00 | - |
| 15 | - | - | 18±0.01 | 12±0.03 |
| 20 | 16±0.02 | 12±0.03 | 21±0.02 | 15±0.01 |
| 25 | 17±0.01 | 14±0.04 | 23±0.01 | 16±0.02 |
| 30 | 19±0.01 | 15±0.02 | 26±0.01 | 19±0.03 |
| 35 | 21±0.02 | 17±0.01 | 30±0.03 | 24±0.04 |
| 40 | 22±0.02 | 19±0.01 | 33±0.04 | 25±0.03 |
| Tetra (0.33mg/ml) | 30±0.01 | 35±0.02 | 30±0.01 | 35±0.02 |
| Amp (10µg/ml) | 27±0.00 | 30±0.01 | 27±0.00 | 30±0.01 |

*Results are mean of triplicate trials

**Table 2 Minimum Inhibitory Concentration (MIC) of extracts from stem and leaf against test organisms**

| | | Concentration of extract (mg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Plant parts** | **Organisms** | **30** | **25** | **20** | **15** | **10** | **5** | **0** | **MIC** |
| Stem | *E. coli* | - | - | - | + | + | + | + | 20 |
| | *S. aureus* | - | - | + | + | + | + | + | 25 |
| Leaf | *E. coli* | - | - | - | - | - | + | + | 10 |
| | *S. aureus* | - | - | - | - | + | + | + | 15 |

Key: + = Activity, - = No activity

**Table 3 Minimum Bactericidal Concentration (MBC) of stem and leaf extracts against test organisms**

| | | Concentration of extract (mg/ml) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Plant parts** | **Organisms** | **50** | **45** | **40** | **35** | **30** | **25** | **20** | **15** | **10** | **5** | **0** | **MBC** |
| Stem | *E. coli* | - | - | - | - | + | + | + | + | + | + | + | 35 |
| | *S. aureus* | - | - | + | + | + | + | + | + | + | + | + | 45 |
| Leaf | *E. coli* | - | - | - | + | + | + | + | + | + | + | + | 40 |
| | *S. aureus* | - | - | - | - | - | + | + | + | + | + | + | 30 |

**Key:** + = Activity, - = No activity

**Table 4 Results of the phytochemical screening of the extracts**

| **Phytochemicals** | **Stem** | **Leaves** |
|---|---|---|
| Saponins | - | - |
| Tannins | + | + |
| Alkaloids | + | + |
| Cardiac Glycosides | + | + |
| Steroids | - | + |
| Flavonoids | - | - |
| Athroquinones | - | - |
| Triterpenes | - | - |

**Key:**
+ = Present, - = Absent

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