

Mormodica charantia Linn. A Potential Antibiotic and Anti-Fungal Drug

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ABSTRACT : Resistant or multi resistant strains are continuously appearing in the treatment of ailments and this necessitates the synthesis of new drugs especially from naturally occurring plants. *Momordica charantia* is a very common indigenous plant of tropical and sub-tropical regions of the world. Phytochemical screening, antimicrobial analysis were conducted using recommended procedures. Ethanol and water were used for the extraction and Ultraviolet Visible and Infrared Spectroscopy were used to identify the functional groups. Alkaloids, flavonoids, saponins, tannins, anthraquinones were all present in both the ethanolic and aqueous extracts, terpenoids was only present in aqueous extract while cardiac glycosides was not present in both extracts. Concentration (200mg/ml) revealed the highest clear zone of inhibition in both aqueous and ethanol extracts. The zone of inhibition increases with increase in concentration of sample. The ethanol extract shows the higher clear zone of inhibition when compared to aqueous extract with a diameter of zones of inhibition of 28, 26, 24, 26, 26 and 24mm for *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae* respectively. The aqueous extract has no effect at concentration (6.25mg/ml) because no clear zone of inhibition was measured. The antimicrobial activity for aqueous extract was lower than and not as effective as ethanol extract because ethanol is a phenolic compound and non-polar solvent when compared with water that is a polar solvent. Infrared spectra showed OH stretch due to phenol was observed at 3702 cm^{-1} , - C - H stretch (alkane) at 2972 cm^{-1} , C - H bending vibration (alkane) at 1381 cm^{-1} , - C - N and - C = N at 2077 cm^{-1} , NH stretching at 3375 cm^{-1} . The results indicate that it contained a phenolic, amine and amide substitute. Therapeutic drugs with antibiotic and antifungal activities can be synthesized from *Mormodica charantia*.

Keywords: Infrared Spectroscopy, *Mormodica charantia*, Phytochemical screening, Ultraviolet Visible Spectroscopy

I. INTRODUCTION

Indigenous plants are the first source of therapy for most of the common ailments in developing countries like Nigeria because of availability, economic status of the users and incidence of resistant or multi resistant strains. Parekh *et al.*, 2006 was of the opinion that the revival of interest in plant derived drugs is mainly due to the current widespread belief that green medicine is safer and more dependable than the costly synthetic drugs, in which many have adverse side effects. Plants have naturally occurring chemicals that are non-nutritive and for their self protection. They have therapeutic values, protective or diseases preventive properties which can be synthesised into useful drugs. These are referred to as phytochemical. They are found in fruits, vegetables, grains, beans and other plants.

Momordica charantia commonly known as bitter melon, bitter gourd or balsam pear is a medicinal plant belonging to the family *Cucurbitaceae*. In Nigeria it is called by its local names which include *Ejirin* (Yoruba), *Daladdasu* (Hausa), *Okwunuolo* (Igbo). 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.



Figure 1: Showing *Momordica charantia* and the ripe fruit revealing the seeds

Momordica charantia extracts and juice have been found suitable for different diseases and problems. Several researchers had reported the effectiveness of its 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 (Plattel and Srinivasan 1997; Leatherdale *et al.*, 2001; 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.

It has been seen that it has strong antimicrobial activity against wide range of gram positive and gram negative bacterial (Taylor, 2000). It contained antibiotic and anti-tumor activities. In addition to these properties, leaf extracts of bitter melon have clinically demonstrated broad spectrum antimicrobial activity. Various extracts of water, ethanol and methanol of the leaves have demonstrated in vitro antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Bacillus subtilis*, an extract of the entire plant was shown to have antiprotozoal 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.

Several works on phytochemical screening and antimicrobial activities had been conducted to establish the potency of *Momordica charantia* but there is a dearth of research in instrumental analysis. Therefore the aims of this study were phytochemical screening, Infrared and Ultraviolet analysis and antimicrobial analysis in leaf of *Momordica charantia*.

II. MATERIALS AND METHOD

Sample Collection

The fresh leaves of bitter melon (*Momordica charantia*) were collected from Akufo village, Apete, Ibadan. The leaves were taken to Botany Department, University of Ibadan for identification and authentication.

Sampling procedure

Freshly collected leaves of bitter melon (*Momordica charantia*) were rinsed with water and air dried for 20 days. After drying, the leaves were pulverized with mortar and pestle after which was weighed.

Reagents

Ethanol, distilled water, chloroform, concentrated sulphuric acid, dilute ammonia, olive oil, ferric chloride, Mayer's reagent, Dragendorff's reagent, glacial acetic acid and nutrient agar.

Preparation of Aqueous and ethanol extracts

Twenty grams (20g) of the dried powdered samples was soaked in 250ml of distilled water contained in a 500ml flask. The flask was covered with cotton plug and then wrapped with aluminum foil and shaken vigorously at 3hours interval for 48hours at room temperature. After 48hours, the crude extract was shaken vigorously and filtered using filter paper. The filtrates were concentrated using rotary evaporator. Similar procedure was repeated with ethanol for the preparation of ethanol extracts. The concentrated extracts were stored in airtight sample bottle.

III. METHODS OF ANALYSIS

Phytochemicals Analysis

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 two portions. Mayer's reagent was added to one portion and Dragendortf's reagent to the other. The formation of a cream (with Meyer's reagent) or reddish brown precipitate (with Dragendortf'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 sulphuric 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 vigorously 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

ANTIMICROBIAL ANALYSIS

Preparation of microbial media

Nutrient agar was used for antimicrobial assay. The nutrient agar was prepared by dissolving 2.8g of the agar in 100ml of distilled water contained in a conical flask. The media was autoclaved at 121°C for 15minutes. The sterilized media was allowed to cool to a temperature of 45°C and was poured into the sterile Petri dishes.

Preparation of graded concentration of the sample

1g of the sample was weighed and dissolved into 5mls of the solvent of extraction for proper dissolution, from which 2.5mls taken in to another 2.5mls of the solvent, this was taken to the 6th test tube which was the last tube for the extract. The 7th test tube contains the blank which has solvent alone but no sample. The 8th test tube is the standard which has gentamicin in place of the sample.

Pour plate Method (Bacterial)

An overnight culture of each organism was prepared by taken a loop full of the organism from stock and inoculated each in to the sterile nutrient broth of 5mls each incubated for 18-24hrs at 37°C. From overnight culture 0.1ml of each organism was taken and put into 9.9mls of sterile distilled water to get 1:100 (10⁻²) of the organism.

From the diluted organism (10^{-2}) 0.2ml was taken in to the prepared sterile nutrient agar which was at 45°C, then aseptically poured into sterile Petri dishes, allowed to solidify for about 45-60 minutes. Using a sterile cork borer of 8mm diameter, the wells were made according to the number of graded concentration of sample. In each well, the different graded concentrations of the sample were produced, this was done in duplicates. The plates were allowed to stay on the bench for 2hrs to allow pre-diffusion. The plates were incubated uprightly in the incubator for 18-24hrs at 37°C.

Isolation of Alkaloids

The extracts were concentrated and acidified with 20ml of 1M HCl and basified with 50ml 1M NaOH. The mixture was mixed thoroughly; precipitate was formed and filtered out by the uses of filter paper. The filtrate was then poured into separating funnel and equal amount of dichloromethane was added to it and shaken thoroughly and left for 15 minutes. The mixture separate into two distinct layers, the lower layer which contains alkaloids was run into a clean and dry conical flask and the process was repeated until the dichloromethane became colourless. The extract was distilled to separate dichloromethane and the alkaloids. The extract was taken for instrumental analysis using Ultraviolet and Infrared Spectrometer.

IV. RESULTS AND DISCUSSION

Results

Table I., Phytochemical Screening Result for Momordica charantia leaves

Phytochemicals	Aqueous extract	Ethanol extract
Alkaloids	+ve	+ve
Flavonoids	+ve	+ve
Saponins	+ve	+ve
Tannins	+ve	+ve
Anthraquinones	+ve	+ve
Terpenoids	+ve	-ve
Cardiac glycosides	-ve	-ve

Keys used: (+ve) = Positive ; (-ve) = Negative

The result of the phytochemical screening of the leaves extract of *Momordica charantia* revealed the presence of alkaloids, flavonoids, saponins, tannins, anthraquinone and terpenoids in aqueous extract while in ethanol extract all observed phytochemicals were present except tannins. In both aqueous and ethanol extracts, cardiac glycosides was not detected as shown in the table 1. The presences of these secondary metabolites that are biologically active are responsible for the antimicrobial activities of *Momordica charantia*. The presence of saponins support the fact that bitter melon 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 leaves showed that this plant can be effectively used as anti-malaria drug, since alkaloid consists of quinine which is antimalaria. Prarthna *et al.*, 2014 confirmed the presence of phytochemical like flavanoids, saponins, terpenoids, coumarins, emodins, alkaloids, proteins, cardiac glycosides, anthraquinones, anthocyanins, steroids etc in the qualitative phytochemical analysis of *Momordica charantia*. In Nigeria water and ethanol were two major solvent that are used in preparation of herbal remedies.

Result of antimicrobial Effect

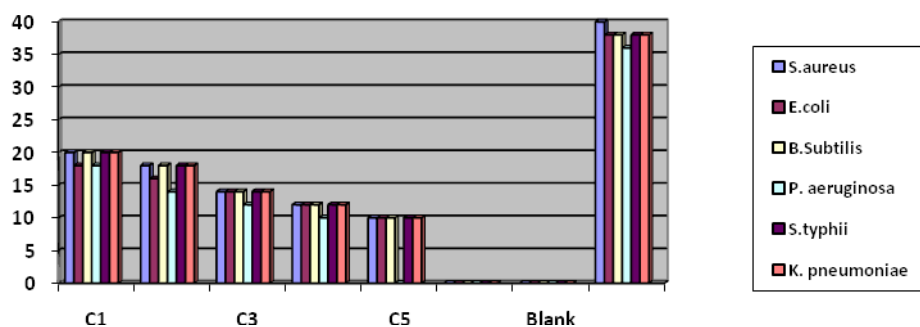


Figure 2: Zone of Inhibition of aqueous extract

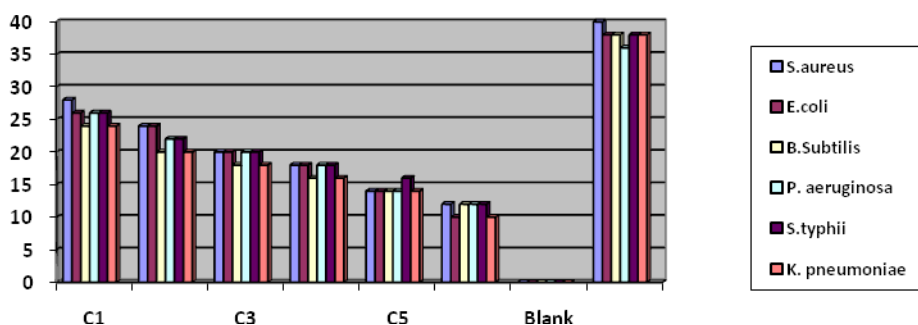


Figure 3: Zone of Inhibition for ethanol extract

Key: Used in Figure 1 and Figure 2: C₁ = Concentration One (200mg/ml); C₂= Concentration Two (100mg/ml); C₃ = Concentration Three (50mg/ml); C₄ = Concentration Four (25mg/ml); C₅ = Concentration Five (12.5mg/ml); C₆ = Concentration Six (6.25mg/ml); Standard = Gentamycin (10µg/ml); Blank = Negative control

In the antimicrobial analysis, graded concentration of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml were done respectively. The first concentration (200mg/ml) revealed the highest clear zone of inhibition in both aqueous and ethanol extracts. The zone of inhibition increases with increase in concentration of sample.

The ethanol extract shows the higher clear zone of inhibition when compared to aqueous extract with a diameter of zones of inhibition of 28, 26, 24, 26, 26 and 24mm for *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae* respectively as shown in Table 3. This is in agreement with the result of Jagessar *et al.*, 2008 showing that ethanolic extract was more potent than other solvents against microorganisms that were studied. It is clearly seen that *Momordica charantia* has antimicrobial properties. Thus, the ethanol extract of *Momordica charantia* can be used as the active constituent of antimicrobial and antifungal natural products. The aqueous extract has no effect at 6th Concentration (6.25mg/ml) because no clear zone of inhibition was measured. The antimicrobial activity for aqueous extract was lower than and not as effective as ethanol extract because ethanol is a phenolic compound and non-polar solvent when compared with water that is a polar solvent. The highest activity was shown by standard (Gentamicin) because it is standard antibiotic drug and is in pure state. Lutein and lycopene are chemicals responsible for antibiotic and anti-tumor activities. In addition to these properties, leaf extracts of bitter melon have clinically demonstrated broad spectrum antimicrobial activity. Various extracts of water, ethanol and methanol of the leaves have demonstrated in vitro antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Bacillus subtilis*, an extract of the entire plant was shown to have anti-protozoal activity against *Entamoeba histolytica*.

Grover *et al.*, 2004 reported the ability to inhibit enzymes “*guanylate cyclase*” that is thought to be associated with psoriasis, Leukemia and tumor 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.

ULTRAVIOLET VISIBLE AND INFRARED ANALYSIS

Ultraviolet visible analysis

Result of Ultraviolet visible analysis showed that the alkaloid extract was conjugated.

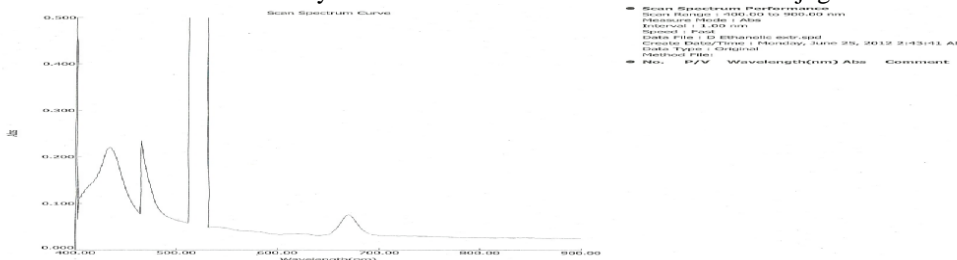


Figure 4: Ethanolic Extract Ultraviolet Visible Spectrum

Infrared for the ethanolic extract from Figure 4 and 5.

Absorbance	Ethanolic extract in the leaf
OH stretch due to phenol	3702 cm^{-1}
C - H stretch (alkane)	2972 cm^{-1}
C - H bending vibration (alkane)	1381 cm^{-1}
C - N and - C = N	2077 cm^{-1}
NH stretching	3375 cm^{-1}

OH stretch due to phenol was observed at 3702 cm^{-1} , - C - H stretch (alkane) at 2972, cm^{-1} , C - H bending vibration (alkane) at 1381 cm^{-1} , - C - N and - C = N at 2077 cm^{-1} , NH stretching at 3375 cm^{-1} .

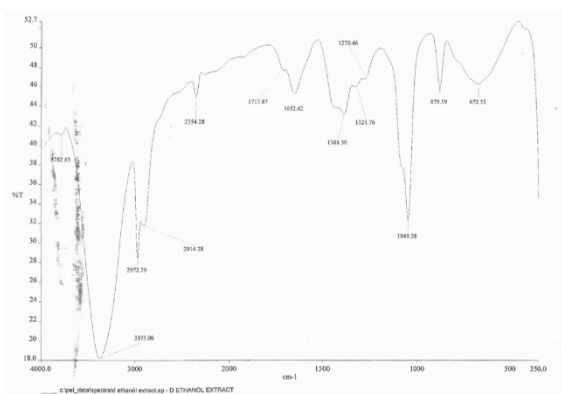


Figure 5: Ethanolic Extract Infrared Spectrum

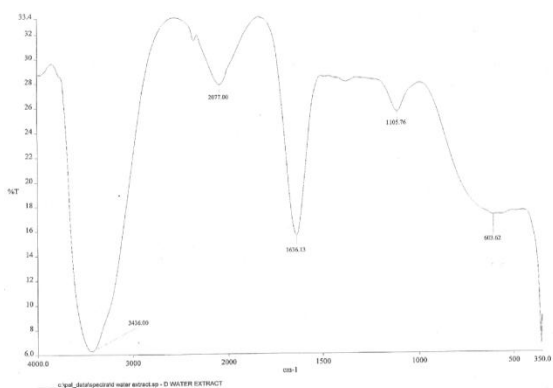


Figure 6: Aqueous Extract Infrared Spectrum

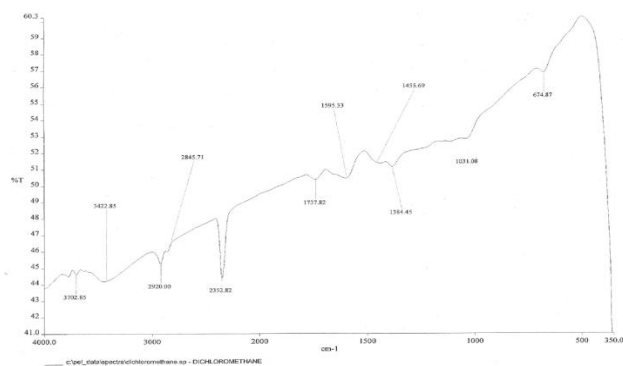


Figure 7: Blank Infrared Spectrum

The Infrared spectral showed that the active constituent contained phenolic, amine and amide substitute which are responsible for the antimicrobial activity of the plant.

Conclusion

The leaves extracts from *Momordica charantia* contained the following phytochemical: alkaloids, flavonoids, saponins, tannins, anthraquinones and terpenoids which are responsible for the antimicrobial activity observed. The antimicrobial analysis showed that *Momordica charantia* inhibit the growth of tested organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*. Both aqueous and ethanol extracts were resistant to the growth of microorganisms tested. The antimicrobial activity is solvent dependent, with ethanol extract being most potent than aqueous extract.

Recommendation

Momordica charantia 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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