

Full Length Research Paper

Antimicrobial activity and phytochemical screening of aqueous and ethanol extracts of *Momordica charantia* L. leaves

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The aim of the present study was to investigate the antimicrobial activity and to carryout phytochemical screening of aqueous and ethanol extracts of the leaves of *Momordica charantia* L. The result of the phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides. However, the antimicrobial activity was investigated by agar-well diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The result showed that at a concentration of 100 mg/ml, both aqueous and ethanol extracts inhibited the growth of all the tested pathogenic bacteria, though with varying degrees of susceptibility of the bacterium. The diameter of zones of inhibition obtained ranged from 17 to 14 and 15 to 11 mm for ethanol and aqueous extracts, respectively. The minimum inhibitory concentration (MIC) values ranged from 6.5 to 12.5 mg/ml for the ethanol extract and 12.5 to 50 mg/ml for the aqueous extract. Similarly, the minimum bactericidal concentration (MBC) values ranged from 12.5 to 25 mg/ml for the ethanol extract and 50 to 100 mg/ml for the aqueous extract. Also, the antimicrobial activity of both extracts was more under acidic conditions and at elevated temperatures. The results obtained in this study support the use of *M. charantia* in herbal medicine and it could be used as a source of broad spectrum oral antimicrobial agent for the treatment of diseases associated with these pathogenic bacteria investigated.

Key words: *Momordica charantia* L., antimicrobial, phytochemical screening, microorganisms, herbal medicine.

INTRODUCTION

Plants are used medicinally in different countries and are source of many potent and powerful drugs (Srivastava et al., 1996). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). The urgent need to discover new antimicrobial compounds with diverse chemical properties and novel mechanisms of action is on the increase, because of the alarming incidence of new and re-emerging infectious diseases. In addition, bacterial adaptations to antibiotic resistance over the past decades have generated a considerable worldwide public health problem

(Anderson, 2003). Plants contain numerous biologically active compounds, many of which have antimicrobial activity (Cowan, 1999). Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds (Chah et al., 2006; Nair and Chanda, 2006; Parekh and Chanda, 2007a). *Momordica charantia* (Karela) commonly known as bitter gourd, bitter melon or balsam pear is an economically important medicinal plant belonging to the family Cucurbitaceae. Its fruit extract act as anti-diabetic agent in normal and alloxan-diabetic rats (Kolawole et al., 2011). It is indigenous to tropical areas including India, Asia, South America and Nigeria and cultivated throughout South America as food and medicine. Various preparations of *M. charantia* extracts from fruit juice to dried fruit bits

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have been employed traditionally worldwide, particularly for blood-sugar lowering effects (Welihinda et al., 1986; Raman and Lau, 1996). In addition, it has been reported to exhibit diverse biological activities such as antioxidant, antimicrobial, antiviral, antihepatotoxic and antiulcerogenic activities which are attributed to an array of biologically active plant chemicals including triterpenes, proteins and steroids (Grover and Yadav, 2004). Analyses of phytochemicals from *M. charantia* revealed the presence of active components like momorcharins, momordenol, momordicin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins and multiflorenol (Murakami et al., 2001; Parkash et al., 2002).

M. charantia was also reported to contain ribosome inactivating protein which could be used for antiviral therapy (Puri et al., 2009). It also has antimicrobial, antioxidant activity (Leelaprakash et al., 2011), and antitumor activity toward human nasopharyngeal carcinoma cells *in vitro* and *in vivo* (Fang et al., 2011). *Cucumis sativa* (Cucumber) is a widely cultivated plant of gourd family which is eaten in the unripe, green form. Its fruit extract has shown free radical scavenging and analgesic activities in mice (Kumar et al., 2010). *M. charantia* was also reported to possess carminative and antacid properties (Sharma et al., 2012). The plant is being cultivated in Western Anatolia. In the view of ethno-medical reports, *M. charantia* is being used in folkloric medicine on various ulcers, diabetes and infections (Gurbuz et al., 2000; Scartezzini and Speroni, 2000; Beloin et al., 2005). Although, hundreds of plant species have been screened and tested for antimicrobial properties, the vast majority of the plants have not been adequately screened and evaluated (Balandrin et al., 1985). Considering the vast potentiality of plants as sources for antimicrobial drugs, the present research aimed to carryout phytochemical screening and evaluates the leaves of *M. charantia* for antimicrobial activity at elevated temperature and under acidic conditions. Also, these investigations aimed at verify the claims made by herbalists and local communities on the use of this plant in treating different ailments associated with the microorganisms investigated.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were of analytical grade and purchased from Sigma Chemicals Co. St. Louis, England.

Collection of plant

The leaves of *M. charantia* L. were collected in polythene bags in

the month of April, 2012 from Mada Village, Gussau, Zamfara State, and were identified by Mr. U. S. Gallah of the Herbarium Unit Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where a specimens was deposited and voucher number (VN/ 528/2012) was given.

Preparation of plant

Freshly collected leaves of *M. charantia* were cleaned and dried under the shade at normal room temperature. After drying, the plant material was ground using pestle and mortar into smaller particles and then blended to powder using an electric blender. 100 g of the powdered sample was then stored in airtight containers and kept under normal room temperature until required.

Collection of test organisms

The tested microorganisms were obtained from the Department of Bacteriology and Parasitology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. The microorganisms are Gram-positive: *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative: *Escherichia coli* and *Pseudomonas aeruginosa*. The microorganisms were grown in the nutrient broth and maintained on nutrient agar slants at 4°C.

Preparation of aqueous and ethanol extracts

Twenty grams of the dried powdered sample was soaked in 250 ml of distilled water contained in a 500 ml flask. The flask was covered with cotton plug and then wrapped with aluminium foil and shaken vigorously at 3 h interval for 48 h at room temperature (El-Mahmoud, 2009). After 48 h, the crude extract was shaken vigorously and filtered using a muslin cloth and then Whatman No. 1 filter paper. The filtered samples were sterilized by passing through Millipore filter and then evaporated to dryness using rotary evaporator set at 40°C temperature. The percentage yield of the extract obtained was 13.4%. The concentrated extract was stored in airtight sample bottle until required. For antimicrobial screening, a reconstituted aqueous extract was prepared by dissolving 50, 100 and 200 mg of the extract in 1 ml of distilled water to obtain a concentration of 50, 100 and 200 mg/ml, respectively. Similar procedure was repeated with ethanol for the preparation of ethanol extracts.

Phytochemical analysis of plant extracts

The extracts were subjected to phytochemical tests for plant secondary metabolites; tannins, saponins, steroid, alkaloids and glycosides were determined in accordance with the methods of Trease and Evans (1989), Harborne (1973), Baker and Thornsberg (1983) and AOAC (1990).

Preparation of microbial media

Nutrient agar was used for antimicrobial assay. This was prepared according to the manufacturer's specification. The nutrient agar was prepared by dissolving 7 g of the agar in 250 ml of distilled water contained in a 500 ml sterile conical flask. The media was then autoclaved at 121°C for 15 min. The sterilized media were allowed to cool to a temperature of 45°C and then approximately 20 ml was poured into sterile Petri-dish and allowed to gel.

Table 1. Phytochemical compounds of two leaves extracts of *M. charantia*.

Phytoconstituents	Water extract	Ethanol extract
Alkaloids	+	+
Tannins	+	+
Saponins	+	+
Flavonoids	+	+
Cardiac glycosides	+	+
Steroids	+	+

+: Presence; -: Not detected.

Antimicrobial activity

The antibacterial activity of the crude extracts was determined by agar-well diffusion method described by Irobi et al. (1994). The bacterial isolates were first grown in a nutrient broth for 18 h before used and standardized to 0.5 McFarland standards (10^6 cfu/ml). 500 μ l of aqueous and ethanol extracts at 100 mg/ml concentration were introduced into the wells and then incubated for 24 h at 37°C. Both positive (Streptomycin) at a concentration of 10 mg/ml and negative controls (water and ethanol) were also set up in parallel. The plates were observed for zones of inhibition after 24 h incubation period at 37°C.

Determination of minimum inhibitory concentration (MIC)

The estimation of MIC of the plant extracts was carried out using the method of Akinpelu and Kolawole (2004). Briefly, 6.25, 12.5, 25 and 50 mg/ml concentrations of the extracts were prepared and 1 ml was introduced into 9 ml of nutrient broth in test tubes. About 0.1 ml of the 18 h culture diluted to 10^6 cell/ml was added and incubated for 24 h at 37°C. The least concentration of the extract that did not permit any visible growth in the broth was taken as the MIC. The MIC of the extracts was done for each test organisms in triplicate.

Determination of minimum bactericidal concentration (MBC)

The MBC of the plant extracts was determined by the method of Spencer and Spencer (2004). Briefly, 200 μ l of broth were taken from the plates with no visible growth in the MIC assay and sub-cultured on freshly prepared nutrient agar plates and later incubated at 37°C for 48 h. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

Effect of pH on the activity of extracts

The extract was reconstituted by dissolving 500 mg of the powdered plant material into 5 ml of water and ethanol separately, mixed very well so as to obtain a concentration of 100 mg/ml in separate sterile test tubes. The first test tube was adjusted to pH 2 by adding 1 N HCl drop wise until pH 2 was reached. The second test tube was adjusted to pH 10 by adding 1 N NaOH drop wise until pH 10 was reached as monitored by a pH meter. The third test tube was not treated with either acid or alkali and it served as positive control, while a solution of pure solvent served as negative control. Then, 1 ml bacterial culture suspension was added and

their antibacterial activity was determined using agar well diffusion method as described earlier.

Effect of temperature on the activity of extracts

The extract was reconstituted by dissolving 500 mg of the powdered plant material into 5 ml of solvent, mixed very well so as to obtain a concentration of 100 mg/ml in two separate sterile test tubes. One millilitre of the culture was incubated in a water bath at a temperature of 10°C for 30 min and the second tube was incubated in a water bath at a temperature of 100°C for 30 min. After treatment, the treated test tubes were left to stand for another 30 min at room temperature to stabilize. The third test tube was left untreated at room temperature and served as positive control, while a solution of the pure solvent served as negative control. Then 1 ml of the contents of each tube was introduced into wells bored on the Mueller Hinton agar plate and was incubated at 37°C for 24 h and the zones of inhibition produced were measured in diameter.

RESULTS AND DISCUSSION

The results of the extraction with water and ethanol showed that ethanol was more efficient solvent with a yield of 15.6% followed by water with 13.4%. This is a clear indication that the solvent system plays an important role in the solubility of the active principle and influences antimicrobial activity. Thus, phytochemical screening of the leaves extract of *M. charantia* revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids in both water and ethanol extracts (Table 1). These compounds are known to be biologically active and therefore aid the antimicrobial activities of *M. charantia*. These secondary metabolites exert antimicrobial activity through different mechanisms. For instance, tannins have been found to form irreversible complexes with proline rich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Dharmananda (2003) reported that tannins are used for treating disorders such as diarrhea and dysentery. These observations therefore support the use of the leaves of *M. charantia* in curing some ailments caused by the tested bacteria. Alkaloids were also detected in the leaves of *M. charantia*; alkaloids are toxic against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori et al., 1994). Just et al. (1998) reported the inhibitory effect of saponins on inflamed cells and this has supported the usefulness of this plant in managing inflammation. Quinlan et al. (2000) worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann et al. (2004) also confirmed the antiviral property of steroids. Similarly flavonoids were also reported to exhibit antimicrobial, anti-inflammatory, anti-angiogenic,

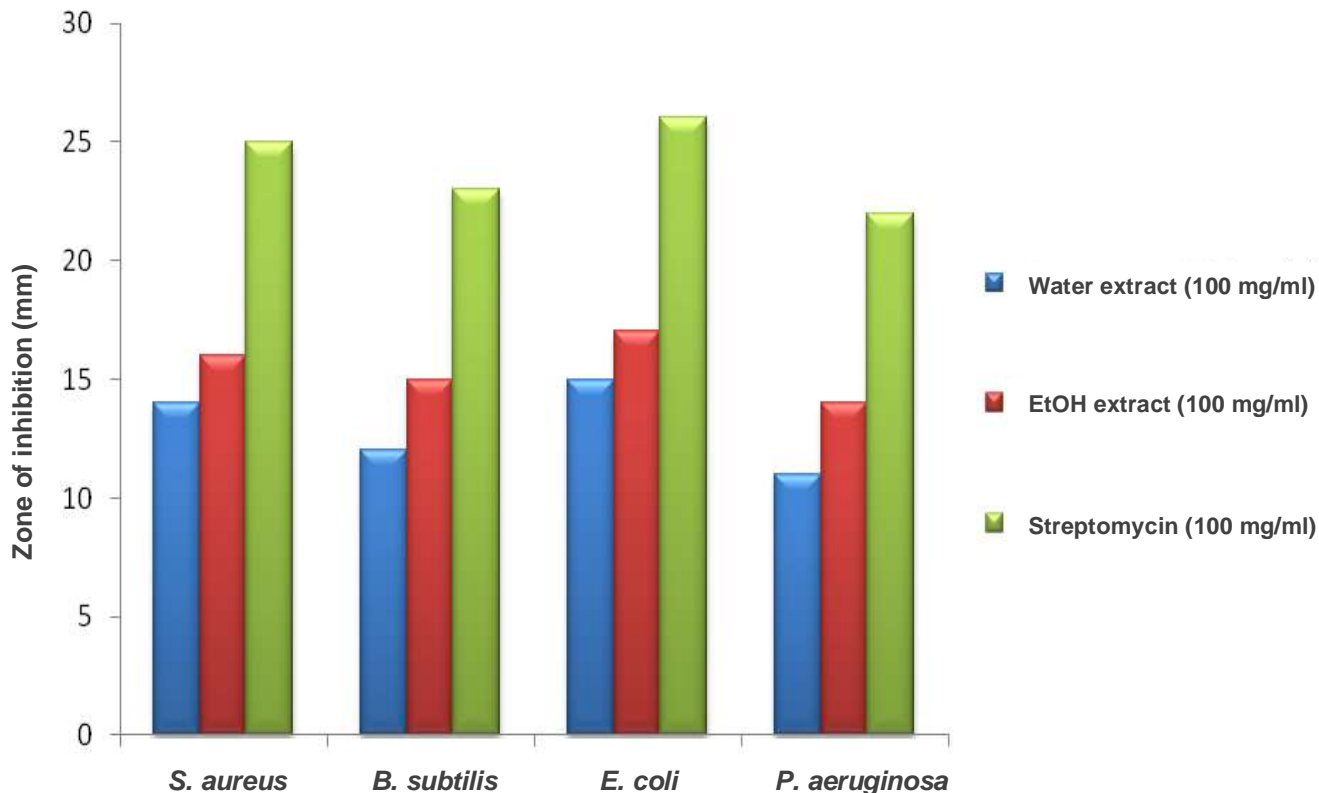


Figure 1. Antibacterial activity profile of two extracts from the leaves of *M. charantia*.

analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek et al., 2002). The presence of these secondary metabolites in the leaves of *M. charantia* has justified the claim by the local communities in Mada village for the use of this plant in the treatment various ailments.

The results for antimicrobial activity of the leaves of *M. charantia* revealed that both aqueous and ethanol extracts of the exhibited varying degree of antibacterial activities though with ethanol extracts demonstrating highest activity of 17 mm for *E. coli*, 16 mm for *S. aureus*, 15 mm for *B. subtilis* and 14 mm for *P. aeruginosa* at a concentration of 100 mg/ml (Figure 1). However, the zone of inhibition for aqueous extract was low when compared with both ethanol extract and standard drug (streptomycin) which serve as positive control. Streptomycin demonstrated the highest antimicrobial activity when compared with both ethanol and aqueous extracts, with a diameter of zones of inhibition of 26, 25, 23, and 22 mm for *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa*, respectively. The highest activity was recorded with streptomycin, because streptomycin is standard antibiotic drug and is in pure state.

The basic parameter for the determination of *in-vitro* activity of antimicrobial agents with antimicrobial potential is the MIC and MBC. The MIC of the extract for different pathogenic bacteria tested ranged between 6.5 to 12.5

and 12.5 to 50 mg/ml for the ethanol and aqueous extracts, respectively. While the minimum bactericidal activity (MBC) of the extract for different bacterial species tested ranged between 12.5 to 25 mg/ml for the ethanol extract and 50 to 100 mg/ml for the aqueous extract (Figures 2 and 3). The antimicrobial activity for aqueous extract was low and not as effective as ethanol extract. This may be attributed to the presence of soluble phenolic and polyphenolic compounds that are readily extracted with non-polar solvent such as ethanol when compared with polar solvent (water).

The inhibitory effect of the extract of the leaves of *M. charantia* against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by these pathogenic bacteria.

Figures 4 and 5 showed the effect of pH 2 and 10 on the antimicrobial efficacy of aqueous and ethanol extracts of *M. charantia* leaves. Result showed that adjustment towards alkalinity had slight diminishing effect on the antimicrobial activities of the extracts. For instance at pH2, the ethanol extracts demonstrated antimicrobial activity with a diameter of zones of inhibition of 14, 13, 11 and 10 mm for *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa*, respectively. But at pH 10, the activities decreased to 10, 9, 8 and 7 mm for *S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa*, respectively. However, for

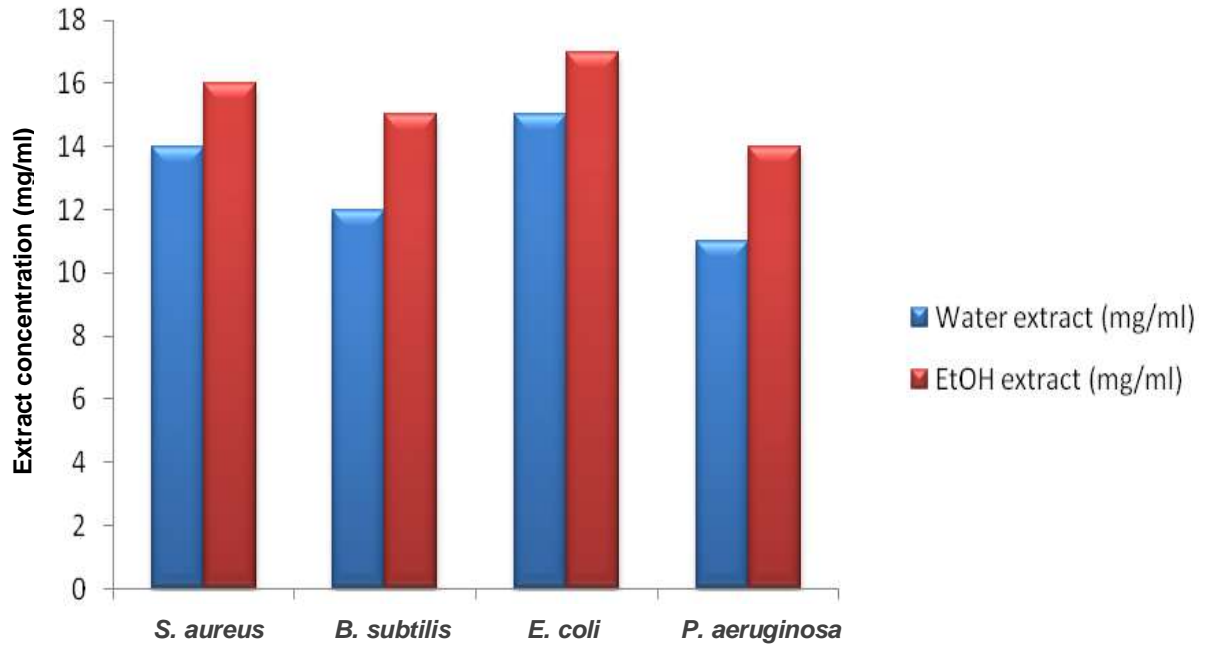


Figure 2. The MIC of leaves extracts of *M. charantia*.

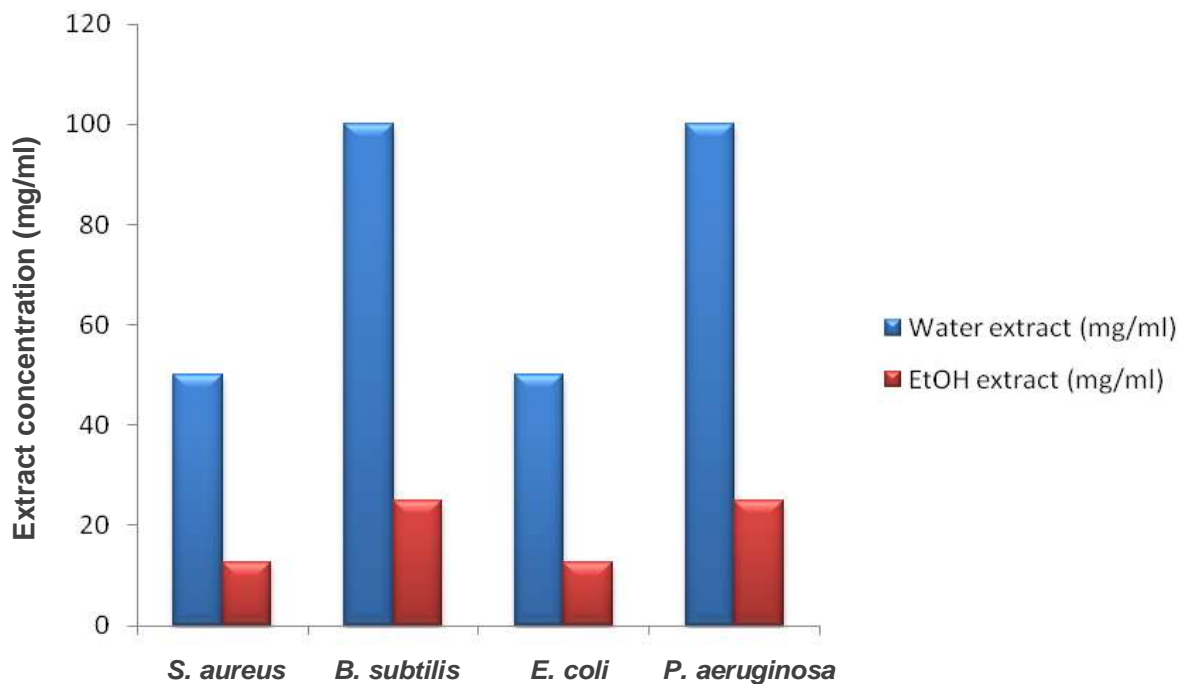


Figure 3. The MBC of leaves extracts of *M. charantia*.

aqueous extract, similar pattern of diminishing effect on the antimicrobial activity was also observed at pH 10, but at pH 2 the antimicrobial activity was more effective. Though, the antimicrobial activity was low when compared with the ethanol extract. Therefore, the effect of pH on

the activity indicated that the tested organisms were more susceptible to acidic pH of the plant extracts than to the alkaline pH of the same extracts. This is an indication that leaves extracts of *M. charantia* could be stable under acidic conditions of the stomach and can be taken orally

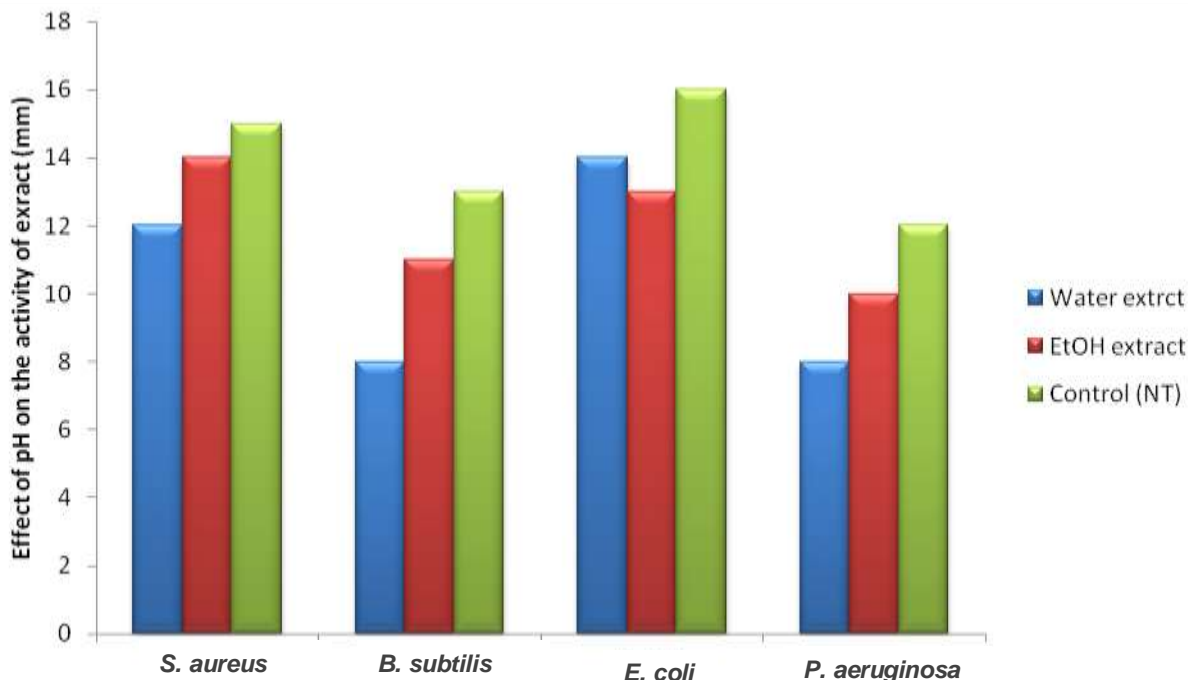


Figure 4. Effect of pH 2 on the activity of leaves extracts of *M. charantia*.

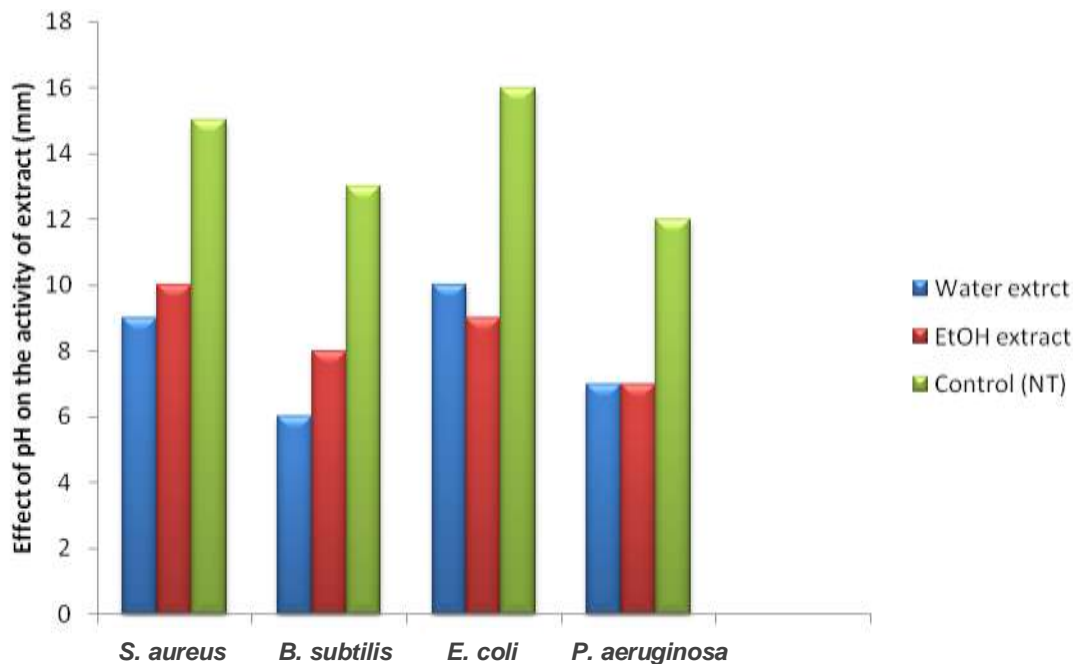


Figure 5. Effect of pH 10 on the activity of leaves extracts of *M. charantia*.

for the treatment of diseases associated with these bacterial species investigated. The broad spectrum antimicrobial activity obtained from the aqueous and ethanol leaves extract of *M. charantia* was in agreement with the work of Jagessar et al. (2008). Ankita et al. (2012) also reported broad spectrum antibacterial activity against

some pathogenic bacteria by *M. charantia* (Cucumber) and *Praecitrullus fistulosus* (Tinda).

The effect of temperature on the activity of the extracts of leaves is as shown in Figures 6 and 7. Ethanolic extracts at temperature of 10°C inhibited the growth of *S. aureus* by producing a zone diameter of 13 mm at 10°C

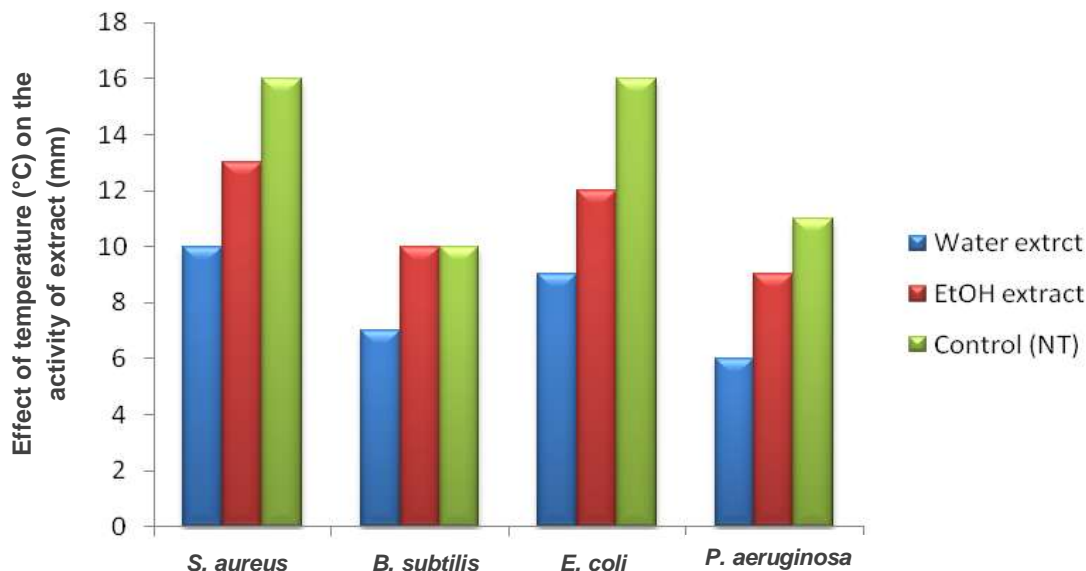


Figure 6. Effect of temperature (10°C) on the activity of leaves extracts of *M. charantia*

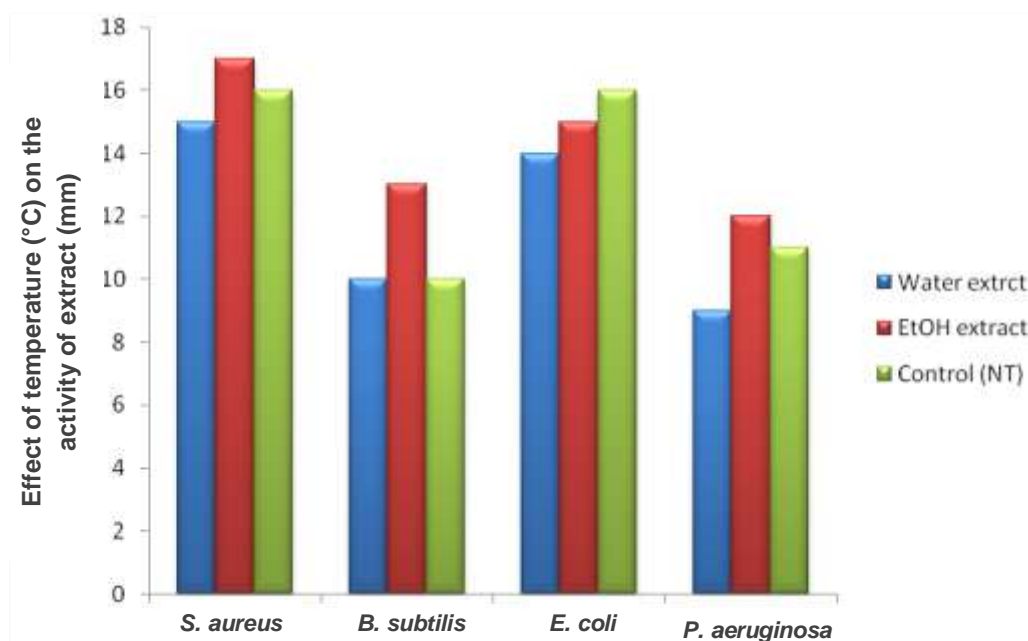


Figure 7. Effect of temperature (100°C) on the activity of leaves extracts of *M. charantia*

and 17 mm at 100°C; for *E. coli*, the zone diameter was 12 mm at 10°C and 15 mm at 100°C; for *B. subtilis*, the zone diameter was 10 mm at 10°C and 13 mm at 100°C; for *P. aeruginosa*, the zone diameter was 9 mm at 10°C and 12 mm at 100°C. However, the effects of temperature of aqueous extract revealed low antimicrobial activity when compared with ethanol extract. Therefore, the present investigation found that increase in temperature of the extracts leads to increase in the antimicrobial activity of the extracts. This could suggest

the reason why traditional healers and local communities boil plant extract before they are taken by the patients.

Conclusion

This investigation found that *M. charantia* leaves contain secondary metabolites such as alkaloids, tannins, saponins, flavonoids and glycosides which could be responsible for the antimicrobial activity observed. The

antimicrobial activity is solvent dependent with ethanol extract being most potent than aqueous extract. Also, the plant demonstrated low MIC and MBC values for both aqueous and ethanol extracts which are very important for evaluation of antimicrobial activity. Therefore, plant could be used as a potential source for the development of an effective antimicrobial agent. Similarly, the extracts demonstrated high activity at elevated temperature and under acidic conditions. Hence, the plant could be stable to the acidic content of the stomach and can be formulated as drug for used in the treatment of infections such as respiratory tract, urinary tract and diarrheal diseases.

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