Determination of Antibacterial, Antifungal and Cytotoxic activities of n-Hexane, Chloroform and Ethyl Acetate extracts of Momordica charantia leaves

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ABSTRACT

A study was conducted to determine the antibacterial and antifungal activities with minimum inhibitory concentration and cytotoxic activity of Momordica charantia (Family: Cucurbitaceae) leaves. In our present study, the antimicroial activity of n-hexane, chloroform and ethyl acetate fractions of the plant were investigated against a number of pathogenic Gram-positive (Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus and Sarcina lutea), Gram- negative (Salmonella paratyphi, Salmonella typhi, Vibrio parahaemolyticus, Vibrio mimicus, Escherichia coli, Shigella dysenteriae, Shigella boydii and Pseudomonas aeruginosa) bacteria and three funguses (Candida albicans, Aspergillus niger and Saccharomyces cerevesiae). Here the zones of inhibitions for the test samples (500 µg /disc) were compared with that of reference standard (30 µg /disc) in determining antimicrobial activity. All the extracts showed significant antibacterial and antifungal activities against all the pathogenic bacteria except A. niger. The highest sensitivity for n-hexane, chloroform and ethyl acetate fractions was against gram positive bacteria B. cereus. Almost all the gram positive, gram negative bacteria and fungus were inhibited by ethyl acetate extract and showed better activity compared to n-hexane and chloroform extracts. All the three fractions were tested as antifungal against C. albicans and S. cerevesiae. They showed moderate activity against C. albicans whereas a very good activity against S. cerevesiae. But A. niger was not sensitive to the experimental extracts. Minimum inhibitory concentration (MIC) that is the lowest concentration at which the test sample shows its highest activity against microorganisms was tested by serial dilution method. The MIC for n-Hexane and chloroform extracts was against B. cereus (64 µg /ml). The ethyl acetate extract exhibited antibacterial activity with MIC of 64 µg /ml against S. aureus, S. luteae, S. boydii, S. dysentereae and V. mimicus. The Brine shrimp lethality bioassay method was used to determine the cytotoxic activity and vincristine sulphate was used as positive control. The LC50 values of standard vincristine sulphate, n-hexane, chloroform and ethyl acetate extract were 10.18 µg /ml, 24.71 µg /ml, 19.02 µg /ml and 30.38 µg/ml respectively which indicate the presence of bioactive compounds present in the plant extracts are promisingly cytotoxic.

Keywords: *M. charantia,* antibacterial, MIC, cytotoxic.

INTRODUCTION

Momordica charantia is called bitter melon or bitter gourd in English, is a tropical and subtropical vine of the family Cucurbitaceae, widely grown in Asia, Africa, and the Caribbean for its edible fruit, which is among the most bitter of all fruits. In folk medicine, M. charantia has significant role. The fruit and various parts of this plant have demonstrated wide range of medicinal properties in further researches. Bitter melon fruit contains triterpene lycosides, including the characteristic mormordin and charantin. Other triterpene glycosides (the momordicosides), vitamins, including beta carotene, ascorbic acid, niacin, and thiamin, elemental compounds (e.g. iron, iodine, magnesium, sodium, calcium), and fatty acids, including stearic, palmitic, and oleic, are also present. Bitter melon seeds and the pericarp contain the phenolics catechin and epicatechin, gallic, gentisic, and vanillic acids, as well as lutein, lycopene, carotenes, xanthins, momordicosides, and vicine [1] [2]. The essential oil obtained from the seeds contains sesquiterepene,

phenylpropanoids, and monoterpenes, including nerolidol, apiole, cis-dihydrocarveol and germacrene D [3]. Eight compounds have been isolated from the fruits of *M. charantia* were identified as momordicolide (10E)-3-hydroxyl-dodeca-10-en-9-olide, monordicophenoide A (4-hydroxyl-benzoic acid 4-O-beta-D-apiofuranosyl -O-beta-D-glucopyranoside, dihydrophaseic acid 3-O-beta-D-glucopyranoside , 6,9-dihydroxy-megastigman-4,7-dien-3-one (blumenol, 4), guanosine , adenosine , uracil and cytosine [4].

Leaves extracts showed the presence of different classes of secondary metabolites as flavonoids, alkaloids and tannins [5].

M. charantia fruit extract possesses the anti-oxidant activity [6]. It prevents alterations in lipid profile and lipogenic enzymes [7], prevents hyperglycemia and hyperinsulinemia [8], prevent carcinogenesis of colon [9]. Bitter melon is used experimentally in the treatment of HIV infection [10] and it also inhibits microsomal triglyceride transfer protein gene expression and apoB secretion in HepG2 cells [11].

As various parts of *M. charantia* are of great use in folklore medicine and previous investigations signifies that different parts are therapeutically effective, it may be possible that the leaves have therapeutic effects too. The present study was undertaken for performing different biological screening such as *in vitro* antibacterial activity, minimum inhibitory concentration and cytotoxic activity of crude extracts of *M. charantia* leaves.

MATERIALS AND METHODS

Plant material

The fresh leaves from the plant of *M. charantia* were collected in the month of January, 2011. This is a familiar plant and widely distributed in all over Bangladesh.

Plant materials extraction and fractionation

The fresh leaf was collected, sun dried for seven days and ground. The dried powder of *M. charantia* leaf (200 gm) was soaked in 600 ml of ethanol for 7 days and filtered through a cotton plug followed by Whatman filter paper number 1. The concentrated ethanolic extract of leaf was fractionated by the modified Kupchan partitioning method into n-hexane, chloroform and ethyl acetate. The subsequent evaporation of solvents afforded n-hexane (450 mg), chloroform (700 mg) and ethyl acetate (350 mg) from leaf extract.

Antibacterial assay

In our present study, the antibacterial activity of n-hexane, chloroform and ethyl acetate fractions of the plant were investigated against a number of pathogenic Gram-positive and Gram- negative bacteria and three funguses. The microorganisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. The sample solution of the material to be tested was prepared by dissolving a definite amount of material in methanol to attain a concentration of 50 mg/ml. 10 µl of such solution was applied on sterile disc (5 mm diameter, filter paper) and allowed to dry off the solvent in an aseptic hood. Thus, such discs contain 500 µg of crude extracts. To compare the activity with standard antibiotics, kanamycin (30 µg/disc) was used.

Minimum Inhibitory Concentration (MIC) measurements:

A current definition of the minimum inhibitory concentration (MIC) is the lowest concentration which resulted in maintenance or reduction of inoculums viability [12]. Serial tube dilution technique [13] [14] was used to determine of MIC of the extracts against four gram-positive and four gram-negative bacteria. The plant extract (0.512 mg) was dissolved in 2 ml distilled water (2 drops tween-80 was added to facilitate dissolution) to obtain stock solution. After preparation of suspensions of test organisms (107 organism per ml), 1 drop of suspension (0.02 ml) was added to each broth dilution. After 18 h incubation at 37°C, the tubes were then examined for the growth. The MIC of the extract was taken as the lowest concentration that showed no growth. Growth was observed in those tubes where the concentration of the extract was below the inhibitory level and the broth medium was observed turbid (cloudy). Distilled water with 2 drops of tween-80 and kanamycin were used as negative and positive control, respectively.

Cytotoxicity Screening

Brine shrimp Lethality Bioassay

Brine shrimp lethality bioassay [15] [16] was used for probable cytotoxic activity. The eggs of Brine Shrimp (*Artemia salina*) was collected from local pet shops and hatched in a tank at a temperature around 37 ºC with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO) to attain concentrations of 5, 10, 20, 40 and 80 µg/ml. With the help of a pasteur pipette nauplii were exposed to different concentrations of the extracts.

RESULTS

Result of Antimicrobial activity

The results representing antibacterial and antifungal activity of the n-hexane, chloroform and ethyl acetate fraction of *M. charantia* are presented in Table: 1. Among all the extracts ethyl acetate extract of *M. charantia* leaves showed very good (average zone 14-20 mm) antimicrobial activity against most of the gram positive and gram negative bacteria and fungus at a concentration of 500µg/disc. In the comparison to reference standard the ethyl acetate extract of *M. charantia* leaves showed significant antibacterial activity. In the present experiment, we found that the ethyl acetate extract showed comparatively better antimicrobial activity than that of n-hexane and chloroform extracts. The chloroform and the n-hexane extracts were also found as good antimicrobial with an average zone 11-20 and 8-19 mm. The chloroform fraction inhibited most of the organisms that is significant to reference standard. The highest zones of inhibition for n-hexane and chloroform extracts were 19 mm and 20 mm respectively against *B. cereus* whereas maximum inhibition (20 mm) for ethyl acetate extracts against *B. cereus, S. lutea* and *S. dysenteriae*. All the extracts showed antifungal activity and are active against *C. albicans* and *S. cerevesiae* but not sensitive to *A. niger*.

Result of Minimum Inhibitory Concentration

The Minimum inhibitory concentration (MIC) of all the extracts was 64µg /ml against *B. cereus*. Chloroform extract showed very good activity against *S. luteae* where the MIC was 64µg /ml. The ethyl acetate extract showed very good activity against *S. aureus, S. luteae, S. boydii, S. dysenteriae and V. mimicus* with MIC value 64 µg/ml. Ethyl acetate extract showed good activity against *S. paratyphi, V. parahaemolyticus, P. aeruginosa, S. cerevisiae and E. coli*. with MIC value 128µg /ml.

Result of Brine Shrimp Lethality Bioassay

The Brine shrimp lethality bioassay method was used to determine the cytotoxic activity and vincristine sulphate was used as positive control. The LC_{50} values of standard vincristine sulphate, n-hexane, chloroform and ethyl acetate extract were 10.18µg/ml, 24.71 µg/ml, 19.02 µg/ml and 30.38 µg/ml respectively.

DISCUSSION

Finding healing powers in plants is an ancient idea. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives [17]. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total [18]. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Some such phytochemicals include terpenoids and essential Oils, quinones and tannins, flavones, flavonoids, and flavonols, coumarins, alkaloids, lectins and polypeptides and others.

The presence of as flavonoids, alkaloids and tannins [5] confirm its activity as antimicrobials. So, antimicrobial activity of the studied plant *M. charantia* is probably due to the ability of i) flavones to complex with extracellular and soluble proteins and to complex with bacterial cell walls, more lipophilic flavonoids may also disrupt microbial membranes [19], ii) tannins to inactivate microbial adhesins, enzymes, cell envelope transport proteins [20].

Brine shrimp lethality bioassay is a recent development in the bioassay for bioactive compounds which indicates toxicity as well as a wide range of pharmacological activities (i.e. anticancer,

antiviral, insecticidal and pesticidal etc) of the compounds. Bioactive compounds are almost always toxic in high doses. Here, *in vivo* lethality in a simple zoological organism (Brine Shrimp nauplii) is used as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. The mortality rate of brine shrimp was found to be increased with increase in concentration of samples and a plot of log of concentration versus percent mortality on the graph produced an approximate linear correlation between them. From this graph the concentration at which 50% mortality (LC_{50}) of the brine shrimp nauplii occurred was determined for most of the samples. In this assay, the extracts showed positive results indicating that the compounds are biologically active. This experiment revealed that each of the test samples showed different mortality rates at different concentrations.

CONCLUSION

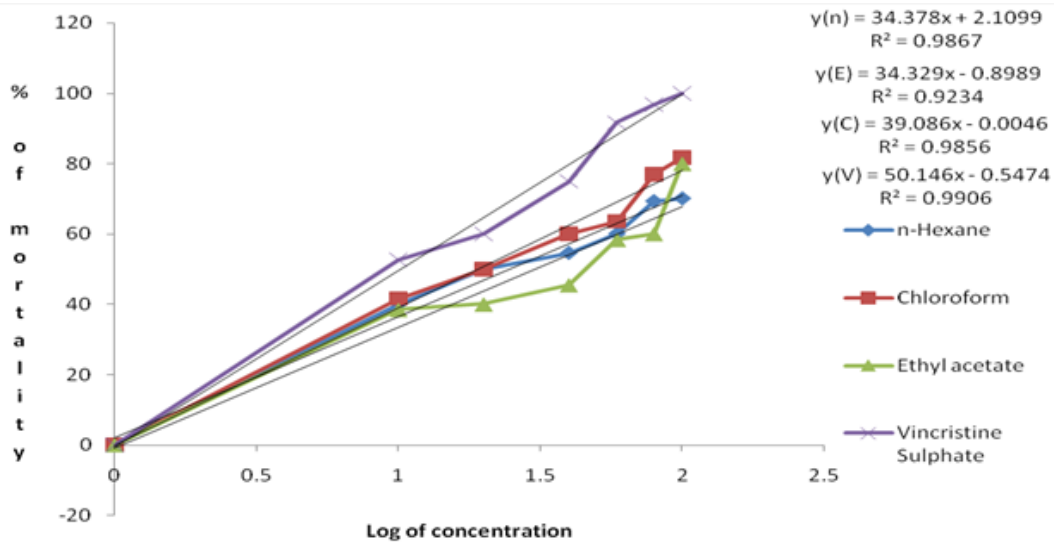
The present study indicates that the n-hexane, chloroform and ethyl acetate extracts of the different parts of *M. charantia* exhibited good to excellent antimicrobial activity. The different extracts of leaf of the plant have most potential antimicrobial properties. So the plants may be considered as good sources of natural antimicrobial activity for medicinal uses in various infections. Now the future study of *M. charantia* will be directed to explore the bioactive compounds responsible for antibacterial activity. Therefore, further investigation should be necessary for the development of novel lead compound.

Table 1: *In vitro* antimicrobial activity of the extracts of *M. charantia* (leaves)

Name of the Organism	Type of the Exract			Standard
	n-Hexane	Chloroform	Ethyl acetate	Kanamycin(30 µg/disc)
Gram positive	Zone of Inhibition (mm)			
B. subtilis	14	11	15	30
B. megaterium	8	12	14	29
B. cereus	19	20	20	32
S. aureus	15	12	18	30
S. Lutea	14	16	20	32
Gram negative				
S. paratyphi	16	13	15	29
V. parahaemolyticus	-	15	17	31
V. mimicus	13	15	17	30
S. boydii	-	13	15	29
S. dysenteriae	14	14	20	32
E. coli	10	14	18	30
P. aeruginosa	14	13	18	31
Fungus				
C. albicans	9	11	14	29
A. niger	-	-	-	30
S. cerevesiae	16	13	17	30

(-)=No significant antibacterial activity.

Figure 1: Determination of LC_{50} values for standard and crude n-hexane, chloroform and ethyl acetate extracts of *M. charantia* leaves from linear correlation between logarithms of concentration versus percentage of mortality.



y(n)= n-Hexane, Y(C)= Chloroform, Y(E)= Ethyl acetate, Y(V)= Vincristine sulphate

Table 2: The minimum inhibitory concentrations (MIC) of the leaf extract of n-Hexane, chloroform and ethyl acetate of *M. charantia*

Name of the Organism	**Minimum Inhibitory Concentrations (MIC)**		
	n-Hexane (µg /ml)	**Chloroform (µg /ml)**	**E. acetate (µg /ml)**
Staphylococcus aureus	128	256	64
Bacillus cereus	64	64	64
Sarcina luteae	128	64	64
Salmonella paratyphi	64	256	128
Vibrio parahaemolyticus	-	128	128
Shigella boydii	-	256	64
Shigella dysenteriae	256	128	64
Eshcheria coli	512	128	128
Pseudomonus aeruginosa	256	256	128
Vibrio mimicus	256	128	64
Sacaromyces cereveceae	128	256	128

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