



Research Article

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Phytochemical screening and bioactivity of *Momordica charantia* L

A. Immaculate Cynthia, L. Nivethini and T. Diana Victoria*

Department of biotechnology, Sathyabama University, Chennai

ABSTRACT

Plants have been used as alternative remedy for the treatment of various ailments since ancient times. *Momordica charantia* is a medicinal plant. Its common name is "bitter gourd" was widely used in treating diseases like diabetics. Secondary metabolites are responsible for the medicinal activities of plants. In the present study presence of alkaloids, flavonoids, terpenoids, tannins, phenol, saponin etc. are confirmed by phytochemical analysis. Various solvent extracts viz petroleum ether, acetone, and ethanol extract of *Momordica charantia* were showing anti oxidant activity using DPPH, H₂O₂ and NO methods. Among the different extracts used ethanol extract was having maximum activity. So ethanol extract was further analyzed using column chromatography and GC-MS.

Keywords: Antioxidant, *Momordica charantia*, H₂O₂, Nitric oxide, DPPH

INTRODUCTION

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Plant based drugs are considered to be an important source for identification of therapeutic agents due to ease in availability, considerably cheap in cost and non-toxic properties with respect to modern medicine [1].

Many of these medicinal herbs known to produce antioxidant compounds which play a vital role in shielding the cells from the harmful effect of reactive oxygen species. Reactive Oxygen Species (ROS) viz superoxide anion, hydroxyl radical and hydrogen peroxide are major reason for development of various ailments including arthritis, asthma, dementia, mongolism, carcinoma and parkinson's disease. These free radicals are created in the human body through aerobic respiration or from exogenous sources [2].

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies. [3].

Ajero and Mbagwu(4) reported how traditional herbalists use medicinal plants instead of pills, they use powered medicine instead of injection and they apply incision. Unlike the orthodox doctors, the traditional healers prepare their medicine from local herbs and administer them to their patients.

EXPERIMENTAL SECTION

PREPARATION OF EXTRACT

The shade dried and powdered whole plant of *Momordica charantia* was used for the extraction using cold extraction method. Powdered plant material was soaked in solvents such as petroleum ether, acetone, ethanol in the ratio of 1:10 in a conical flask and continuously shaken by using electrical shaker for 72hrs. Then the extract were

filtered and the filtrate was evaporated under reduced pressure to obtain crude extract which is used for further analysis.

PHYTOCHEMICAL SCREENING (5)

Bradford's Test:

To 1ml of extract add few drops of bradford's reagent (Coomassie Brilliant Blue G250) and formation of blue colour product indicates the presence of proteins.

Fehling's Test:

Add 1ml of fehling's reagent to the filtrate of extract in distilled water and heat in a water bath. Brick red precipitate indicates the presence of carbohydrates.

Ferric chloride Test:

To 2ml of extract, add 2ml of ferric chloride solution and the formation of deep bluish green solution indicates the presence of phenols.

Kellar-Killani Test:

Dissolve the extract in glacial acetic acid containing a trace of ferric chloride. Add same amount of ferric chloride dissolved in concentrated sulphuric acid along the side the test tube to settle at the bottom. Appearance of a reddish brown colour changing to bluish green colour at the junction of two reagents within 2-5 minutes spreading slowly into the layer of acetic acid confirms the presence of cardiac glycosides.

Tannin test:

0.5 g of plant extract was boiled in 20 ml of water in a test tube and filtered. A few drops of 0.1 %ferric chloride was added and observed for brownish green or a blue black colouration which indicates the presence of tannins.

Alkaloid test:

Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath. 1 ml of the filtrate was treated with drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

Saponins Test:

About 0.5 ml of extract was added with 5ml of distilled water in a test tube. The suspension is shaken vigorously and observed. The presence of froth indicates the presence of saponins.

volatile oils test:

Shake 2ml of extract solution with 0.1ml of dilute sodium hypochloride and a small quantity of dilute HCl. formation of white precipitate indicates the presence of volatile oils.

Terpenoids test:

Add 2ml of chloroform to 0.5 g of sample. To this add 5ml of concentrated sulphuric acid along the side of the test tube. A reddish brown colouration in the interphase indicates the presence of terpenoids.

Flavonoids test:

To 5 ml of diluted ammonia solution, a portion of the aqueous filtrate of each plant extract was added. Followed, the addition of concentrated sulphuric acid, A yellow colouration observed in each extract indicated the presence of Flavonoids. The yellow colouration disappears on standby.

Leucoanthocyanidines test:

Add few ml of concentrated HCl to the extract and then heat until it boils. Appearance of reddish colour indicates the presence of leucoanthocyanidines.

ANTI-OXIDANT ACTIVITY

DPPH Free radical scavenging activity

Radical scavenging activity of *Momordica charantia* extracts against stable 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) free radical was studied with the slightly modified method of Brand-Williams et al 1995. [6] 1ml of different concentration(1000µg-62.5µg) of plant extracts in methanol was added with 0.004% solution of DPPH in methanol. Then it was incubated in dark for 30 minutes. The colour change from violet to yellow due to the DPPH reduction can be read as the absorbance was measured at 517 nm in UV-Vis spectrophotometer. Ascorbic acid is used as standard and scavenging activity was read in same way. Lower absorbance of the reaction mixture indicates

higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenging Effect (\% Inhibition)} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100$$

Where

Ablank is the absorbance of DPPH radical + methanol

Asample is the absorbance of DPPH radical + sample extract/ standard.

Scavenging of nitric oxide radicals

Plant extract was dissolved in distilled water, Sodium Nitroprusside (5mM) in standard phosphate buffer saline (0.025M, pH 7.4) was incubated with different concentration (1000µg-62.5µg) of plant extracts and tubes were incubated at 29°C for 3 hours. Control experiment without the test samples but with equivalent amount of buffer was taken in an identical manner. After 3 hours incubated samples were diluted with 1 ml of Griess reagents. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Naphthyl ethylene diaminehydro- chloride was observed at 550nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to plant extract. Calculated the % inhibition by formula and plot graph in compared to standard. (7)

$$\text{Formula: \% inhibition} = \frac{[\text{O.D. of control} - \text{O.D. of Test}]/\text{O.D. of control}}{1} \times 100$$

Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extracts (1000µg-62.5µg) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Same procedure was done with ascorbic acid which was standard in comparison to plant extract. Calculated the % inhibition by formula and plot graph in compared to standard. The percentage of hydrogen peroxide scavenging by the extracts and standard compounds was calculated as follows: % Scavenged [H₂O₂] = [(A_o - A₁)/A_o] × 100 where A_o was the absorbance of the control and A₁ was the absorbance in the presence of the sample of extract and standard.(8)

COLUMN CHROMATOGRAPHY

Initially the glass column was cleansed and one third of the column was filled with solvent (petroleum ether). In a beaker, the required amount of silica was measured. In a separate flask, measure the solvent approximately one and a half times the volume of silica. Then the slurry was prepared by adding the silica to the solvent. Some of the slurry was poured into the column and the solvent was allowed to drain to prevent overflowing. The column was tapped gently to encourage bubbles to rise and the silica to settle. Transfer the slurry to the column until all the silica is added. Rinse the inside of the column by pipetting solvent down the inside edge. Drain the solvent until the solvent level is just even with the surface of the stationary phase. Transfer the crude sample (0.5 ml) into the solvent layer above the silica gel in the packed column and carry out elution. Fractions were collected on the basis of colour.

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY

The GC-MS analysis of the sample was performed using a GC model (451_GC) and Autosampler 8410 equipped with a Bruker column(0.25mm) and FID detector is used. Helium was the carrier gas at a flow rate of 1ml/min. The injector and the oven temperature was programmed as follows, 40°C for 3mins, then gradually increased to 280°C and the total time taken was 30 mins. Then the identification of components was based on comparison of their mass spectra with those of NIST Library (Version 12) (9).

RESULTS AND DISCUSSION

Phytochemical test:

Different phytochemical test indicated the presence and absence of phytochemicals. The tests revealed the presence of alkaloids, terpenoids, glycosides, saponins, tannins, phenols and flavanoids. (Table1)The extracts showed immediate and late responses according to the amount of secondary metabolites present in the plant.

Table 1

Bio active compound	Appearance of colour	Petroleum ether	acetone	ethanol
Bradford's test	blue colour	++	++	++
Fehlings test	brick red precipitate	-	-	-
Phenol ferric chloride	Deep bluish green	+	+	+
Glucosides	Bluish green	+	+	+
Tannin	Blue black	+	+	+
Alkaloids	turbidity	+	+	+
Saponins	Foam	+	+	+
Volatile oils	White	-	-	-
Flavonoids	yellow	+	+	+
Terpenoids	Reddish brown	+	+	+
Leucoanthocyanides	Reddish colour	-	-	-

Where, ++ indicate immediate response of presence, + is late response of presence and - is absence.

Antioxidant assays:

Phenols and polyphenolic compounds, such as flavonoids, are commonly found in plant sources are responsible for significant antioxidant activities (10)

DPPH radical scavenging activity:

Evaluation of free radical scavenging ability of various sample can be carried out using scavenging the stable DPPH radical as a model. (11). Change in colour from violet to yellow was due to the reduction of DPPH, a stable nitrogen-centered free radical by either the hydrogen- or electron- donation. Antioxidants are the substance which are capable of carry out the same reaction thus radical scavengers (12). It was found that the radical-scavenging activities was concentration dependent there was linear increase in the activity with increase in the concentration of the extracts Fig 1. Among the three extracts used ethanol extracts showed comparatively more scavenging activity than the other extracts.

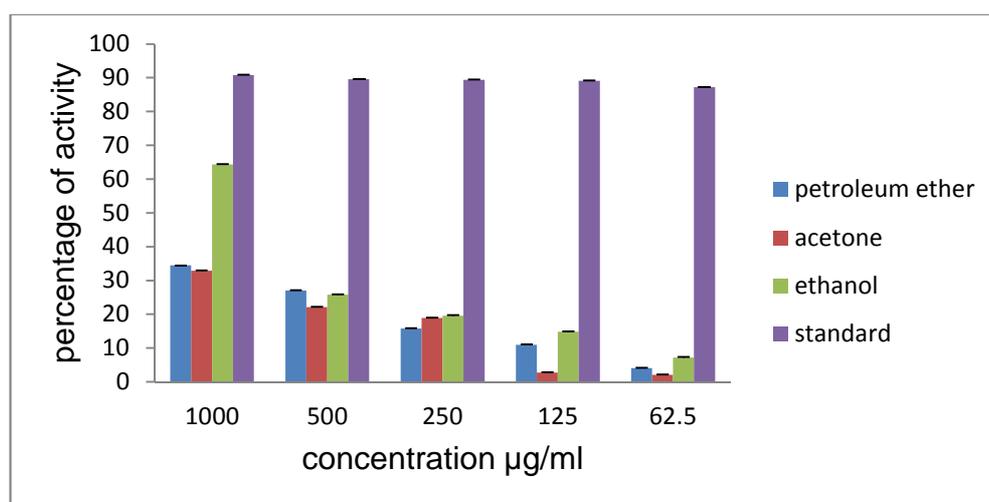


Fig 1: Free radical scavenging activity of plant extract and Ascorbic acid

Hydrogen peroxide scavenging activity:

Hydrogen peroxide rapidly cross the cell membrane and enter the inside of cell. Even though they are considered to be a weak oxidizing agent, H_2O_2 are capable of inactivating a few enzymes directly, by essential thiol (-SH) groups oxidation. Toxic effect of H_2O_2 is due to the formation of hydroxyl radical by the reaction of Fe^{2+} and Cu^{2+} ions with H_2O_2 (13). So that the cell tend to control the accumulation of H_2O_2 inside the cell. The scavenging activity of the *Momordica charantia* extract is shown in Fig2.

Nitric Oxide radical scavenging assay:

Nitric oxide is a radical which contributes to various inflammatory processes and are toxic and contribute to vascular collapse in prolong production. Chronic level of Nitric oxide leads to various disease such as carcinoma, juvenile diabetes.(14)When reacted with super oxide radical, toxicity of Nitric oxide increase greatly by forming peroxy nitrite anion ($ONOO^-$)(15) Nitric oxide scavenging activity was performed in different concentration ranging from 1000µg-62.5µg. The % inhibition was increased with increasing concentration of the extract. Sodium nitro prusside release nitric oxide reacts with oxygen to form nitrite. The ethanolic extract of *Momordica charantia* leaves

inhibits the nitrite formation by directly competing with oxygen in the reaction with NO. In this assay ethanol extract shows higher scavenging activity than acetone and petroleum ether extracts. Fig 3.

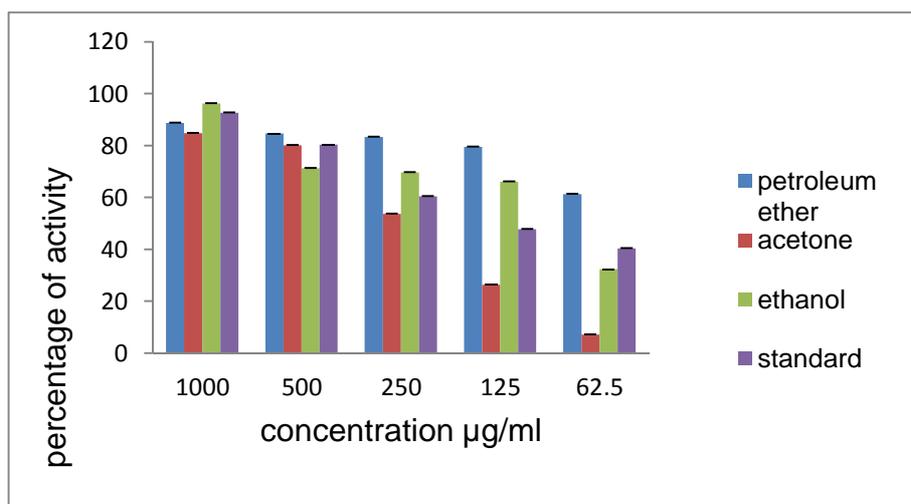


Fig 2: Hydrogen peroxide radical scavenging of various plant extracts

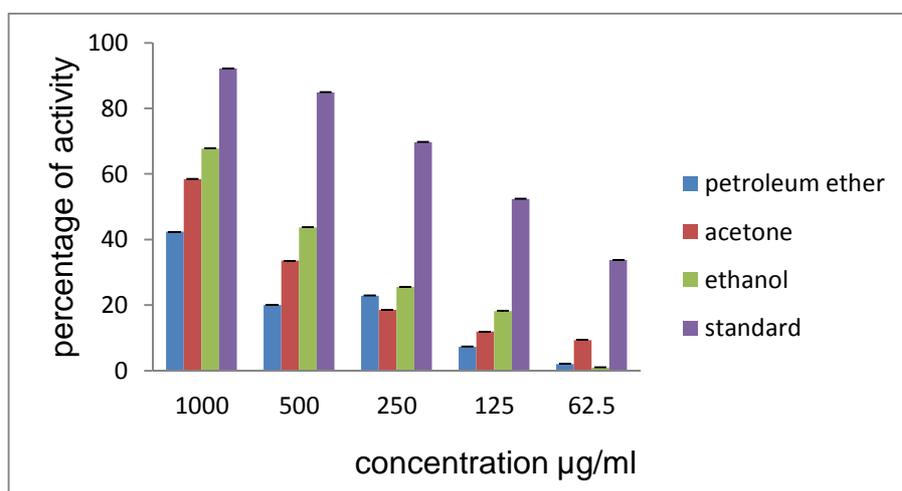


Fig 3 : Nitric oxide radical scavenging efficacy of various plant extracts

Column chromatography

The crude ethanol extract of *Momordica charantia* sample (0.5 ml) which showed good antioxidant activity was fractionated by silica gel column chromatography, on the basis of colour 2 fractions were collected and designated as Fraction 1 and 2.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis:

To characterize the fractions both fraction 1 and 2 was analyzed using GCMS. Peak 13 and 17 of the fraction 1 (Fig4) and 2 (Fig5) respectively are found to be major peak. The mass spectrum shows peak 13 of 1st fraction was found to be Di-N-butylphthalate and peak 17 from 2nd fraction was found to be Di-ethylphthalate. The compound diethyl phthalate is used medicinally as a component in insecticide sprays, mosquito repellents and camphor Substitute (16)

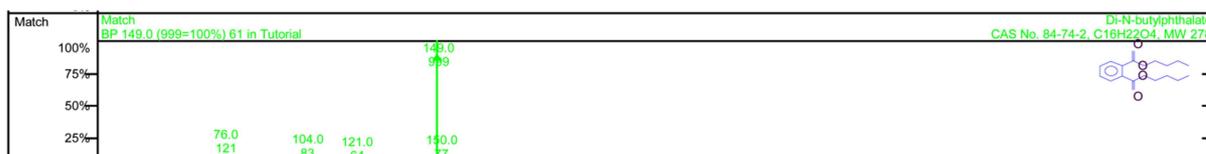


Fig 4: Characteristic peak (13) from GC-MS analysis



Fig 5: Characteristic peak (17) from GC-MS analysis

CONCLUSION

Momordica charantia are the source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, and tannin. From the antioxidant activity of DPPH, H₂O₂, and nitric oxide method, we conclude the activity of ethanol extract of *Momordica charantia* is greater than the activity of petroleum ether and acetone extract. GC-MS analysis showed the presence of Di-n-butyl phthalate and Di ethyl phthalate in ethanolic extract of *M. charantia* leaves.

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