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SCREENING OF MEDICINAL PLANT MOMORDICA CHARANTIA LEAF FOR SECONDARY METABOLITES

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ABSTRACT

Plants have been known to relieve various diseases in traditional medicine and Ayurveda. Secondary metabolites are responsible for medicinal activity of plants. Hence in the present study petroleum ether extract of *Momordica charantia* were screened for the presence of phytochemicals by standard procedures and subjected to GC-MS analysis. Qualitative phytochemical analysis of these plants confirms the presence of various phytochemicals like sterols, flavonoids, Terpenoids, Proteins, alkaloids, quinones and anthocyanins. The GCMS analysis revealed that the major fragments of the extracts like COOH, methyl, Carbonyl and hydrocarbon group.

KEYWORDS : *Momordica charantia*, phytochemicals, petroleum ether and Secondary metabolites

INTRODUCTION

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.^[1] Nearly 80% of the world's population relies on traditional

medicines for primary health care, most of which involve the use of plant extracts.^[2]

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids.^[3] Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds.^[4]

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Momordica charantia is such an exemplary plant that is widely consumed in the tropics of Asia, the Caribbean, Africa, and the Amazon. It is in the Cucurbitaceae family, which also includes melons, squashes, and gourds. The plant's defining characteristic is its bitter taste, hence its common names like bitter melon, bitter gourd, balsam apple, balsam pear, sorosi, karela (Hindi), and ampalaya.^[5] ^[6] In the present work, qualitative phytochemical analysis were carried out in *Momordica charantia* leaf.

MATERIALS AND METHODS

Preparation of leaf and seed extracts

Fresh leaves of *Momordica charantia* leaf were collected (Figure – 1), washed in water and air dried under shade. Dried leaves were powdered using an electric pulverizer. 10g of the seed powder was weighed and subjected to extraction with 500 ml of petroleum ether solvent for 8h (60-80°C) using a Soxhlet apparatus. The leaf extract thus obtained was concentrated by distillation and dried by evaporation at 40°C.

Figure.1 Leaf of *Momordica charantia* (Cucurbitaceae)



Preliminary phytochemical analysis:

Preliminary screening of the extracts and identification was done by colour tests adapting standard methods.^[7]

Test for Alkaloids

Mayer's test

A fraction of extract was treated with Mayer's test reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water) and observed for the formation of cream coloured precipitate.

Wagner's test

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

Hager's test

A few ml of extract was treated with Hager's reagent (saturated aqueous solution of picric acid)

and observed for the formation of prominent yellow precipitate.

Test for Flavonoids

NaOH test

A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

H2SO4 test

A fraction of the extract was treated with concentrated H2SO4 and observed for the formation of orange colour.

Test for Sterols

Liebermann-Burchard test

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H2SO4 was added and observed for the formation of dark pink or red colour.

Test for Terpenoids

Liebermann-Burchard test

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H₂SO₄ was added

Proteins**Ninhydrin test (Aqueous)**

The extract was treated with aqueous ninhydrin and observed for the presence of blue colour, indicating the presence of amino acid or purple colour indicating the presence of protein.

Ninhydrin (acetone)

Ninhydrin was dissolved in acetone; the extract was treated with ninhydrin and observed for the formation of purple colour.

Test for Anthraquinones**Borntrager's test**

About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colourations of aqueous layer indicate the presence of anthraquinone.

The extract was treated with aqueous ninhydrin and observed for the presence of blue colour, indicating the presence of amino acid or purple colour indicating the presence of protein.

Ninhydrin (acetone)

Ninhydrin was dissolved in acetone; the extract was treated with ninhydrin and observed the formation of purple colour.

Biuret test

The extract was heated in distilled water and filtered. The filtrate is treated with 2% copper sulphate solution, to this added 95% ethanol and potassium hydroxide and observed the formation of pink ethanolic layer.

Test for Phenols**Ferric chloride test**

The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour

Liebermann's test

The extract was heated with sodium nitrite, added H₂SO₄ solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

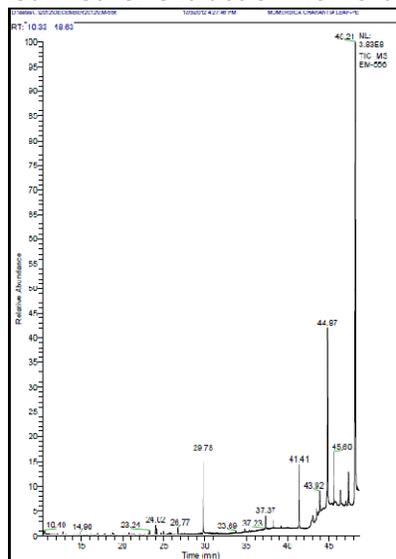
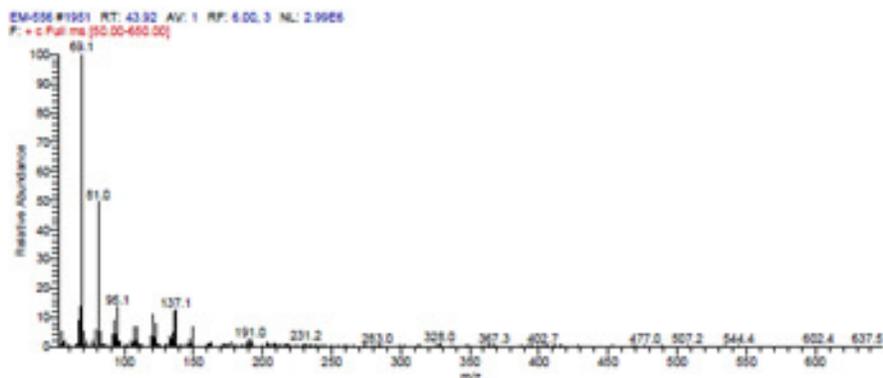
Gc – ms analysis

Mass experiments were performed on GC (T8000 Top CE) combined with Mass Spectrometer (Md 800 FIS ONS). Sample was dissolved in methanol and introduced into the column TR-5-MS capillary standard non-polar by splitless injection system. Ultra high purity helium was introduced as the buffered collision gas with flow rate of 1.0 ml/min. The source temperature for ionization was set at 2500C. All the experiments were performed on the positive ion mode.

RESULTS and DISCUSSION**Leaf of *Momordica charantia***

Petroleum ether extract:-

The GCMS analysis of the extract of *Momordica charantia* leaf showed 12 major peaks. The mass spectrum showed characteristic peaks for (M-12) (M-14) (M-15)(M-17)(M-18)(M-27)(M-28) and (M-44), which showed the presence of hydrocarbon fragments, methyl, hydroxyl, carbonyl and carboxylic acid group. (Figure 2 and 3).

Figure.2 GC-MS Chromatogram of petroleum ether extract of *Momordica charantia* leaf**Figure.3** Mass spectrum at (RT: 43.92)

The results of preliminary phytochemical study were tabulated in (Table-1). The phytochemical study revealed the presence of steroids, flavonoids, alkaloids, sterols, terpenoids, tannins, proteins and phenols. The extracts of *Plectranthus glandulosus* by using hexane, ethylacetate and ethanol as solvent were screened for secondary metabolites. The extracts revealed the presence of alkaloids, tannins, anthraquinones, and glycosides, reducing sugar, saponins, flavonoids, phlobatanins, steroids and terpenoids. Steroids were present in hexane and ethyl acetate extract but absent in ethanolic extract.^[7]

Table - 1. Phytochemical constituents of the *Momordica charantia* leaf extracts

S. No.	Fractions	<i>Momordica charantia</i> leaf
1	Alkaloid	+
2	Flavonoids	+
3	Sterols	+
4	Terpenoids	+
5	Anthraquinones	+
6	Proteins	+
7	Phenols	+
8	Quinones	-

+ Detected - Not detected

A preliminary absorbance survey scan of the methanolic extract of *R. nasutus* evidenced the presence of multiple components in the extract. Two peaks observed in the HPLC spectrum showed the presence of two components in the extract.

GC-MS profile revealed that the active components present in the leaf extract might be alkaloids or polyphenols.^[8]

Presence of alkaloids, phenols, steroids, tannins, triterpenoids the absence of flavonoids in the methanol extract of water hyacinth which was prepared by soxhlet extraction.^[9] *N. oleander* leaves exhibited the presence of few phytochemical groups. Results of phytochemical analysis are in agreement with the earlier studies where the aqueous extract of *N. oleander* leaves was reported to possess carbohydrates, proteins, amino acids, sterols, flavonoids, alkaloids, phlobatinins and terpenoids.^[10] In our present study involves a detailed phytochemical investigation of *Momordica charantia* leaf.

The ethanol fractionate of aqueous extract showed that flavonoids, terpenoids and proteins which were present in aqueous extract, after fractionation was found in petroleum ether fractionate.^[11] Various extracts of stem bark of *Bauhinia racemosa* L. (Caesalpinaceae) phytochemical study revealed the presence of steroids, flavonoids, alkaloids, coumarins, triterpenoids, tannins and carbohydrate.^[12]

In the present study chemical constituents have been identified from petroleum ether extract of *Momordica charantia* leaf by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the leaves for various ailments by traditional practitioners. It could be concluded that *Momordica charantia* leaf contains various bioactive compounds. However, further studies will need to find its bioactivity, phytoremediation and toxicity effect on the pests. Isolation of individual phytochemical constituents and subjecting it to drug designing will definitely give fruitful results in phyto pharmaceutical.

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