Phytochemical Analysis Of *Momordica Dioica* Root Extracts

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Abstract

A lot of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health Care system (Ayurveda) named Rasayana proposed for their interesting Antioxidant activity and potential therapeutic properties. The present paper reports the qualitative and quantitative phytochemical studies of *Momordica dioica* which is a perennial, dioceous climbing creeper belonging to family Cucurbitaceae. This is climbing creeper generally found in India, Pakistan, Bangladesh, Himalayas to Ceylon, Brazil, China, Africa etc. The phytochemical study in root extract of *Momordica dioica* which belongs to the Cucurbitaceae family is a valuable in all nutritional parameters. The qualitative phytochemical analysis showed the presence of pytochemicals such as Phenols, Saponins, Alkaloids, Flavonoids, Cardiac glycosides, Tannins, Carbohydrates, Terpenoids and Steroids in petroleum ether and acetone root extracts of *Momordica dioica.*
Keywords- Phytochemical analysis, *Momordica dioica*, Root extract

1. Introduction

The use of medicinal plants in industrial sectors has been traced to the extraction and development of several drugs from the medicinal plants [1]. *Momordica dioica* is a perennial, dioecious climber belonging to Cucurbitaceae family, which is commonly known as teasel gourd, spiny gourd, or small bitter gourd worldwide whereas in India, it is known as, Kartoli, Kantola or Janglee karela. It is used not only as preventive and curative agent for various diseases but also considered as an underutilized vegetable, although containing a significant nutritional value than many frequently consumed vegetables over thousands of years. *Momordica dioica* has been known to have many medicinal properties namely anti-diabetic, anti-inflammatory, antitumorogenic, analgesic and anti-allergic activity [2-5]. Extraction and characterization of various phytochemicals from these green plants have given birth to some high activity profile drugs [6].

In India traditional medicines are widely spreaded [7]. Plant secondary metabolites play a crucial role in human health and are nutritionally important. Plant derived medicines has become popular in the treatment of many diseases due to the belief that they are safe, easily available and have lesser side effects [8].
According to Indian system of traditional medicine, roots are used in head trouble, for treating urinary calculi. The plant fruits and leaves are used as a medicinal agent in asthma, bronchitis, fever, leprosy, aphrodisiac and antihelmintic properties. The fruit are used in treatment of constipation [9]. *Momordica dioica* fruit are effective in controlling curing renal damages and drug-induced nephrotoxicity [10]. The fruits cooked with little amount of oil, consumed in treating diabetes [11]. The leaves are also strong antioxidant and show hepatoprotective action [12].

The dried root extract of this plant was used for its estrogenic activity and abortifacient [13], as well as they have anti-inflammatory, hepatoprotective [14], antiallergic [15] and antibacterial activity [16]. Phytochemical analysis states the presence of the traces of alkaloids, tannins, triterpenoids, saponins, sitosterol, saponin, glycosides, long chain aliphatic hydrocarbons and fixed oil [17-18].

2. Material and Methods

2.1 Sample collection

The roots of the *Momordica dioica* plant were collected from the field nearby Nanded district. The roots were cleaned by washing thoroughly 2-3 times with running tap water and once sterile distilled water to remove impurities and cut into small pieces and shade dried. After this they were coarsely powdered separately and stored in well closed containers for further laboratory use [19].

2.2 Preparation of extracts:

The dried powdered plant material of *Momordica dioica* roots (400 g) was extracted in a Soxhlet apparatus on heating mantle, with two different solvents petroleum ether and acetone. The extracts were concentrated by allowing for evaporation at room temperature and stored in a closed vial for further use. Concentrated extracts were preserved in refrigerator at 4ºC until required for further use [20].

2.3 Qualitative phytochemical tests:

The crude extracts were used for the phytochemical investigation of secondary metabolites study [21]. The tests were carried out in triplicate.

2.3.1 Test for identification of Alkaloids: About 0.5 gm of extract was taken in a test tube, diluted and homogenized with 10 ml distilled water, dissolved in 20 ml dilute Hcl solution and clarified by filtration. The filtrate was tested with Drangendroff's and Mayer's reagent. The treated solution was observed for precipitation of white or creamy colour.

2.3.2 Test for identification of Flavonoids: About 0.5 gm of extract was added in 10 ml of ethyl acetate in a test tube and heated in boiling water for 1 min. The mixture was then filtered and about 4 ml of the filtrate was shaken with 1 ml 1% aluminum chloride solution and then incubated for 10 min. Formation of yellow colour in the presence of 1 ml dilute ammonia solution indicated the presence of flavonoids.

2.3.3 Test for identification of Terpenoids: 5 ml of the extract was mixed with 2 ml of chloroform and 2ml concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

2.3.4 Test for identification of Cardiac glycosides: in 1ml of plant extract glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid were added from the walls of the test tube. Appearance of the reddish brown at the junction of two layers and the bluish green colour in the upper layer indicates the presence of cardiac glycosides.

2.3.5 Test for identification of Phenols: About 0.5 gm of extract was taken in a test tube, mixed with 100ml distilled water and heated gently. Then, 2 ml of ferric chloride solution was added and observed for the formation of green or blue colour which indicates presence of phenols.

2.3.6 Test for identification of Saponins: About 0.5 gm of extract was taken in a test tube and 5 ml distilled water was added in it. The solution was then shaken vigorously and observed for 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 which indicates the presence of saponins

2.3.7 Test for identification of Tannins: 5 gm of the ground powder was extracted with 10 ml ammonical chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5 M sulphuric acid. The creamish white precipitate was observed for the presence of tannins.

2.3.8 Test for identification of carbohydrates: A few drops Molischs solution was added in 2 mL of aqueous solution of the extract, there after a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to from a layer without shaking. The interface was observed for a purple colour as indicates for carbohydrates.

2.3.9 Test for identification of Steroids: About 0.5 gm of extract was taken in a test tube, then 2 ml of acetic anhydride was added in it and 2 ml of sulphuric acid was added by the sides of the test tube and observed for the colour change to violet or blue green which indicates the presence of steroids.

2.4 Quantitative Determination of Secondary Metabolites:

2.4.1 Estimation of Alkaloids: the alkaloids were estimated by using Harborne method. 5 gm of the sample was taken into a 250 ml beaker in which 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one fourth of the original volume. Concentrated ammonium hydroxide was added drop by

drop to the extract until the precipitation was complete. The whole solution was allowed to settle down and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [22].

2.4.2 Estimation of Flavonoids: The total flavonoid content was estimated by the method of Chang. 0.25 ml of the sample was diluted to 1.25 ml with distilled water. 75 µl of 5% sodium nitrite was added and after 6 minutes, 0.15 ml of aluminium chloride solution was added. 0.5 ml of 0.1M NaOH was added after 5 min and made up to 2.5 ml with distilled water. The solution was mixed well and the absorbance was taken at 510 nm along with standard quercetin at 5-25µg concentration. The results are expressed as mg of flavonoids as quercetin equivalent/gm of dried sample [23].

2.4.3 Total Phenolic Content (TPC): Total phenolic content was estimated by Folin-Ciocalteau method. 0.1 ml of extract (200, 600 and 1000 µg/ml), 1.9 ml distilled water and 1 ml of Folin-Ciocalteau's reagent were seeded in a tube, and then 1 ml of sodium carbonate was added. The reaction mixture was incubated at 25 °C for 2 h and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate and a calibration curve with six data points for catechol was obtained. The results were compared with catechol calibration curve and the total phenolic content of sample was expressed as mg of catechol equivalents per gram of extract [24].

2.4.4 Total Tannins Content (TTC): Total tannins were estimated by Peri and Pompei method. 1 ml of the sample extracts, 1mg/ml was taken in a test tube. The volume was made up to 1ml with distilled water and 1 ml of water serves as the blank. To this 0.5 ml of Folin's phenol reagent (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5 min. Blue colour was formed and the colour intensity was read at 640 nm. A standard graph (gallic acid - 1 mg/ml) was plotted, from which the tannin content of the extract was determined. The total tannin content was expressed in mg/g of extract [25].

2.4.5 Total Saponins: The fruit extract was ground and 20 g of extract put into a conical flask and 100 ml of 20% ethanol was added in it. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55 ºC. The mixture wass then filtered and the residue re-extracted with another 200 ml of 20% ethyl alcohol. The combined extracts were reduced to 40 ml over a water bath at about 90 ºC. The concentrate wass then transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added to the extract and vigorously shaken. The aqueous layer was recovered while the diethyl ether layer was discarded and the purification process was repeated. 60 ml of *n*-butanol was added and the combined *n*-butanol extracts was washed twice with 10 ml of 5% sodium chloride. The remaining solution was then heated in a water bath and after evaporation; the samples were dried in the oven to a constant weight and values were expressed as mg/g of extract [26].

3. Results

3.1 Qualitative phytochemical analysis of *Momordica dioica* root extracts:

Following the methods of qualitative analysis reveals the presence of phytochemicals in root extracts of *M. dioica* prepared in petroleum ether and acetone, used for variety of ethnic medicinal uses. The alkaloids, terpenoids, phenols, carbohydrates and steroids are present in both petroleum ether and acetone extract of *M. dioica.* The flavonoids and saponins are present only in the acetone extract of *M. dioica* root and absent in petroleum ether extract, while the cardiac glycosides and tannins are present only in the petroleum ether extract of *M. dioica* root and are absent in acetone.

3.2 Quantitative phytochemical analysis of *Momordica dioica* root extracts

Following the methods of quantitative analysis of root extract reveals that the total alkaloid content estimated were 3.43 mg/g and 1.89 mg/g in petroleum ether and acetone respectively. 2.67 mg/g of flavonoids were present in acetonic root extract. The total phenolic content present in petroleum ether and acetone root extract were 2.83 and 1.69 mg/g respectively. The total tannins estimated in petroleum ether root extract was 3.13 mg/g, while the total saponins estimated in acetonic root extract was 2.12 mg/g.

Sr. No.	Phytochemical constituents	Petroleum ether	Acetone
1.	Alkaloids	++	++
2.	Flavonoids	--	++
3.	Terpenoids	++	++
4.	Cardiac glycosides	++	--
5.	Phenols	++	++
6.	Saponin	--	++
7.	Tannin	++	--
8	Carbohydrates	++	++
9	Steroids	++	++

Table 1.-Qualitative phytochemical analysis of *Momordica dioica* root extracts

++Positive, --Negative

Sr. No.	Phytochemical constituents(mg/g)	Petroleum ether (mg/g)	Acetone (mg/g)
1.	Alkaloids	3.43	1.89
2.	Flavonoids	--	2.67
3.	Phenols	2.83	1.69
4.	Tannin	3.13	--
5.	Saponin	--	2.12

Table 2.-Quantitative phytochemical analysis of *Momordica dioica* root extracts

4. Discussion

The Phytochemical analysis of *Momordica dioica* root extract is carried out. The present study contributes the information of qualitative Phytochemicals present in root extracts of *M. dioica* plant. The plants possess numerous phytochemicals in the form of secondary metabolites like alkaloids, flavonoids, terpenoids, cardiac glycosides, phenols, tannins, sterols, and saponins etc. Phytochemicals of plants play an important role in defense mechanism against many microorganisms [27]. Similar kinds of results are reported phytochemical analysis and biological activities of *Momordica Dioica* through fruit. They reported that extracts contain bioactive compounds such as Carbohydrates, Alkaloids, Flavonoids, Phenols, Saponins, Anthraquinones, Cardiac glycosides and Triterpenoids [28].

5. Conclusion

The qualitative as well as quantitave phytochemical analysis showed the presence of pytochemicals such as alkaloids, terpenoids, cardiac glycosides, phenols, tannins, carbohydrates and steroids in root extracts of *M. dioica* prepared in petroleum ether. And the alkaloids, flavonoids, terpenoids, phenols, saponins, carbohydrates and steroids are present in extract of *M. dioica* prepared in acetone.

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