A preliminary chemical study on secondary metabolites present in fruits of *Momordica dioica* (Thumbakariwila)

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Abastract

Results of isolation and identification of secondary metabolites present in fruits of Memordica dioica are reported in this paper.

M. dioica (Family Cucurbitaceae) is a dioecious perennial plant with tuberous roots and commonly available in the dry zone of Sri Lanka. The whole plant of M. dioica including fruits, leaves, tubers and seeds has been reported to possess potent healthcare properties and used in traditional medicine systems in many countries. The fruits of the plant are commonly consumed as a vegetable in Sri Lanka and they have therapeutic potential as hypoglycaemic and anti-ulcer drugs.

The chemical constituents present in fruits of M dioica were extracted into hexane, ethylacetate, methanol and water via sequential extraction method using Soxhlet extractor. The nature of the secondary metabolites present in each extracts was determined by phytochemical screening, chemical screening and spectroscopic methods. It was found that fruits of M. diocia contain alkaloids, steroids, triterpenoids and larger amount of saponins.

Keywords: *M. dioica*, perennial, dry zone, traditional system

Introduction

Momordica (Family *Cucurbitaceae*) is a herbaceous annual or perennial climber with 45 species native to tropical Asia and Africa. Some of the species of *Momordica* (i.e. *M Charantia* and *M. diocia)* are cultivated to get their fruits and to be used as a vegetable. Tenders shoots, leaves and root tubers of the plant are also used as a vegetable and the seeds as condiment. The species of *M. diocia* have been in use in indigenous medical system in various countries in Asia and Africa (http://www.indmedplants-Kr.org/Ayurveda).

M diocia (Thumbakarawila) grows in the dry zone of Sri Lanka. The whole plant of *M. diocia* possesses health care properties (Jayaweera, 1982). It has been reported that fruit extract of *M. dioica* has therapeutic potential both as hypoglycaemic and anti-ulcer drugs (Ratnasooriya et al., 1992). The result of studies on anti-diabetic effects of *M. dioica* have shown its hypoglycaemic effect both on acute and subacute administration (http://www.indmedplants-Kr.org/Ayurveda). It has been also reported that the mucosal protecting ability of fruit extract of *M. dioica* is similar to that of clinically useful cytoprotective drugs sucralfate (The Wealth of India 1962). *M. dioica* has shown acute cytoprotection against some of the well known ulcerogenic agents such as absolute ethanol, aspirin and indomethacin. It has been reported that the compounds responsible for this activity of *M. dioica* are triterpenoids (Ratnasooriya et al 1992). In addition to the above the fruits of *M. dioica* are given for asthma, leprosy, bronchitis, excessive salivation and heart ailments.

Not much study has been reported on isolation and characterization of chemical constituents present in fruits of *M. dioica*. Certain compounds present in fruits of *M. dioica* have been isolated and characterized (The Wealth of India 1962). It has been reported that ether extract of fruits of *M. dioica* has been found

to contain thiamine, riboflavin, niacin, ascorbic acid and I_2 whereas carbon tetrachloride extract of the fruits contain fatty acids.

Objective of this study was to carry out a preliminary study on isolaton and chracterization of secondary metabolites present in fruits of *M. dioica.*

Materials and Methods

Hexane (BDH), Ethylacetate (BDH) and Methanol (BDH) were used after distillation. Rotary evaporator (ROTAVAPOR RE 120) was used for evaporation of the solvents. For flash chromatography (FC) Silica Gel (BDH), analytical plates were prepared using Silica Gel F_{254} 30% $CaSO_4$. Analytical plates were checked by using either UV lamp or by a spraying agent (25 g of Phosphomolybdic acid, 10.0 g of Ce $(SO_4).4H_2O$, 60 ml of conc. H_2SO_4 and 940 ml of H_2O with subsequent heating). IR spectra were obtained using Hitachi 270– 50 IR spectrophotometer.

Preparation of reagents

All reagents including Mayer's , Wagner's, Dragondroff's, Kedde and $FeCl_3$ were prepared according to literature methods (Pathirana RN).

Collection and preparation of plant material

The fruits of *Momordica dioica* were collected from Lunugamwehera and Udawalawa. The matured fruits were selected and cut into pieces.They were dried at room temperature for 24 hours.

Extraction of plant material

Sequential extraction with solvents of increasing polarity was carried out. Ground fruits (100 g) were extracted with hexane (250 mL) followed by ethyl acetate (250 mL) and then by water (250 mL) using soxhlet extractor for four hours. The extracts were concentrated using a rotary evaporator.

Phytochemical Screening for crude extracts of fruits of *Momordica dioica*

Screening for alkaloids

About 10 mg of crude extract was mixed with 2N HCl (2 mL) and heated on a steam bath for 5 minutes while stirring. It was then filtered and transferred into four test tubes in equal volumes.

Mayer's test

A few drops of Mayer's reagent were added to one of the test tubes and checked whether any turbidity or precipitate is formed.

Wagner's test

A few drops of Wagner's reagent were added to the second test tube and observations were made as in the Mayer's test. Alkaloids are assumed to be absent in the plant extract, if no precipitate or turbidity is observed in any of the above test tubes. If formation of a precipitate was observed then the fractions of the two remaining test tubes were combined, basified with concentrated ammonia and the resulting solution was extracted with chloroform. The combined extract was dried and the concentrated solution was subjected to a thin layer chromatography (TLC). The TLC plates were sprayed with iodoplatinate or Dragendorff reagent.

Screening for Saponins

Froth Test: The crude extract of fruits of 100 mg of *M. dioica* was added into a test tube and water (10 mL) was added. The mixture was shaken vigorously and formation of froth was monitored with

respect to its height above the surface of the liquid level. If the height of the froth remain at 3 cm or greater for more than 30 minutes it was considered to be a positive result.

Screening for Steroid and Triterpenoids

A volume of extract equivalent to 10 g of plant material was evaporated to dryness. The residue was stirred with light petroleum (10 mL) and the organic layer was discarded. The residue was dissolved in chloroform (10 mL) and was divided into three test tubes in equal volumes. One test tube was held at an angle of 45 ^{0} and concentrated H_2SO_4 acid (2 mL) was allowed to run down the inner wall of the test tube.

When the solution was gently mixed if a cherry colour is formed it indicates the presence of unsaturated steroids. The solution in the second test tube was used as a reference solution.

Liebermann-Buchardt Test

To the third test tube about three drops of acetic anhydride was added and mixed. Detection of the colour change of the mixture was started immediately after the addition of one drop of conc. H_2SO_4 into it. Colour change was monitored over a period of one hour.

In the above experiment if the solution turns bluish green in colour that is considered to be an indication for the presence of steroids while a red, pink or violet colour is considered to be an indication for the presence of triterpenoids. Formation of a pale yellow colour is considered to be an indication for the presence of saturated steroids or triterpenoids in the extract.

Screening for Cardiac Glycosides

If Salkowski and Leibermann-Buhardt tests gave positive results, then Kedd Test (to determine the presence of unsaturated lactones) and Keller-Killiani Test (to determine the presence of deoxy sugars) were performed. If the latter two tests give positive results then the plant extract is considered to contain cardiac glycosides.

Kedd Test

On the centre of a piece of chromatographic paper, the plant extract (0.2 mL) was spotted and developed using chloroform as the sprayed with Kedd reagent and air-dried.

Keller-Killiani Test

Plant extact (10 mL) was evaporated to dryness. The dried extract was defatted with light petroleum. Then the residue was treated with $FeCl_3$ reagent 3 mL). The test tube was held at an angle of 45° and conc. H_2SO_4 (2 mL) was added carefully along the inner wall of the test tube. Formation of a purple ring at the interphase is considered as a positive response to Keller-Killiani test.

Screening for Flavanoids

A volume of extract equivalent to 3 g of plant material was evaporated to dryness. The residue was defatted with light petroleum and was dissolved in rectified spirit (2 mL). The solution was divided into two test tubes in equal volumes. To one test tube conc. HCl acid (0.5 mL) and three magnesium turnings were added and it was shaken with octanol. If the colour of the solution turns orange, red or crimson within 10 minutes, it is considered as an indication for the presence of flavanoids in the extract.

Seperation of components in crude extracts of fruits of *Momordica dioica* by Column chromatography

Each crude extract (hexane, ethylacetate and methanol extracts) was separated into components by flash chromatography (2 g of the extract and 60 g of Silica gel). Fractions were collected and analysed by TLC.

Phytochemical screening and Chemical tests for isolated compounds

Phytochemical screening was carried out for each isolated component as done for crude extracts. The preliminary chemical tests and functional group analyses were done for each pure compound. Presence of carbonyl group was tested using Brady's reagent. If orange colour precipitate is formed with the Brady's reagent, it indicates the presence of carbonyl group in the compound. Presence of phenolic group was tested using $FeCl_3$ reagent.

Infra-red Spectrophotometric analysis of isolated compounds

Infra-red spectra of the isolated compounds were taken in liquid film state. Anhydrous CH_2Cl_2 was used as the reference.

Results and Discussion

Results of phytochemical screening of crude extracts.

Table 1: Phytochemicals of crude extracts

Extract	Alkaloid		Confirm test # of spots	Steroid tritepenoid			Quaternary alkaloid			
	Wagner	Mayer		Saponin	Salwoski	Liberman burchardt	Wagner	Mayer	Cardiac glycoside	Flavonoid
Hexane	-	-	-	?	✔	-	?	?	-	✔
Ethyl acetate	++	++	3	-	✔	✔	-	+	-	-
Methanol	+++	++	2	✔	✔	✔	-	+	-	✔
Water	+	++	?	✔ ✔ ✔	✔	✔	-	+	-	-

\+ : Slight turbidity ✔ : Present +++ : Heavy precipitate
++ : Definite turbidity ✔ ✔ ✔ : Large amount - Completely absent

According to the above results, fruit extract of *M. dioica* contains alkaloids, saponins, quaternary bases, flavonoids, steroids and triterpenoids.

Results of components isolated from crude extracts of fruits of *M. dioica* by Column chromatography.

Table 2:Number of components in crude extracts

Extract	Eluent	Number of components
Ethyl acetate	EtOAc:CH_2Cl_2 (1:1)	4
Methanol	EtOAc:CH_2Cl_2 (7:1)	5

Results of Phytochemical screening and chemical tests of isolated compounds from fruits of *M. dioica.*

Table 3: Phytochemicals and functional groups present in isolated compounds

Extract	Alkaloids		Flavonoids	Quaternary base	Brady's test	Ferric chloride
Ethylacetate	Mayer's	Wagner's				
1	-	++	√	-	-	-
2	-	++	√	-	√	-
3	-	-	√	-	-	-
4	-	-	√	-	√	-
Methanol						
1	?	?	?	?	?	?
2	-	+++	-	-	-	-
3	-	-	√	-	√	-
4	-	+++	-	-	-	-
5	-	-	√	-	√	-

According to the above results the ethyl acetate extract and methanol extract of fruits of *M. dioica* coantain alkaloids and flavonoids having carbonyl functionality.

Results of Infra-red spectroscopic analysis of isolated components of fruits of *M. dioica.*

Table 4.0 : IR data of isolated compounds

ν/cm^{-1}	Group	Hexane	Aqueous	Ethyl acetate			Methanol				
				1	2	4	1	2	3	4	5
3200-4000	N-H	√	√	√	√	√	-	-	√	√	√
2900-3050	Sp^3 C-H	√	√	√	√	√	√	√	√	√	√
1600-1700	C=O	√	-	-	√	√	-	-	√	-	-
1390-1440	CH bend	√	√	√	√	√	√	√	√	√	√

According to the above results certain compounds present in hexane, ethylacetate and methanol extracts of *M. dioica* fruits contain both N-H and C=O functional groups whereas compounds present in aqueous extract contains N-H functionality.

Conclusion

According to the results of chemical analyses of fruits of *M. dioica,* it contains secondary metabolites including alkaloids, steroids, triterpenoids and saponins. Of these compounds, four compounds isolated from ethyl acetate extract and five compounds isolated from methanol extract of *M. dioca* consist of alkaloids and flavonoids with NH and C=O functional groups respectively as revealed by the Infra-red spectroscopic data.

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