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Studies on phytochemical analysis and biological activities in *Momordica dioica* Roxb through Fruit

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Abstract

Momordica dioica is a perennial, dioecious, cucurbitaceous climbing creeper (commonly known as kakrol, spiny gourd or teasle gourd). It is native to Asia with extensive distribution in India. It is used not only as preventive and curative agent for various diseases but also as vegetable with a significant nutritional value over thousands of years. Kakrol is considered as an underutilized vegetable, although having significant presence of certain compounds containing higher nutritional value than many frequently consumed vegetables. Moreover, as a traditional medicinal plant, it is still potential for its phytochemical components that increase the demand of further extensive evaluation to justify its other therapeutical roles. Therefore, this effort will be helpful to researchers who interested to disclose the unjustified phyto therapeutical role of *Momordica dioica* through fruit. The Methanolic and ethanolic extract of fruits of *Momordica dioica* was investigated for its Phytochemical properties and analysis for its active chemical ingredients.

Keywords: *Momordica dioica* chemical constituents, Phytochemical screening, Methonol and ethanol extract.

Introduction

Momordica dioica Roxb belongs to the family Cucurbitaceae (and under the genus *Momordica*, a genus of annual or perennial climbers that contains about 80 species (Raj *et al.*, 1993) [5] *Momordica dioica* climber plant commonly known as Teasle Gourd, or Small bitter-gourd is a relatively small oval to ovoid vegetable. It is also called as janglee karela (Harish, 2008). It is often cultivated for its fruits, which are used as vegetable (Sastri, 1962) [9]. Teasle gourd is a cucurbitaceous popular summer vegetable. The fruits, young twigs and leaves of this crop are used as vegetable or cooked as a vegetable (Bandyopadhyay and Mukherjee, 2009). It has two different types of varieties male & female as well as fruited variety and fruitless variety. *Momordica dioica* Roxb. (chromosome number 28) dioecious and propagated vegetatively through tuberous root. It is dioecious, perennial in nature having tuberous roots. The green fruit is extensively used as vegetable by cooking or frying. Leaves 1.5-4 inches long, cordate, acute more or less 3-5 lobed; Flowers large, dioecious and yellow in colour; Fruit 1-3 inches long, shortly beaked, densely covered with soft spines (Rasul *et al.*, 2004). This is climbing creeper generally found throughout India, Pakistan, Bangladesh, Himalayas to Ceylon. Reported up to an altitude of 1500 m in Assam and Garo hills of Meghalaya (Ram *et al.*, 2002) [6]. Kakrol is a Cucurbitaceous crop originated in the Indo -Malayan region (Rashid, 1976 and Singh, 1990).

Phytochemical Studies: It contains Lectins, proteins, triterpenes and vitamins (Naik, 1951). The fruit contains a high amount of vitamin C (Bhuiya, 1977). The fruit is rich in ascorbic acid and contain iodine (Rao, 2001). The fruit also contain alkaloid, flavonoids, glycosides and amino acids (Kushwaha *et al.*, 2005) *Momordica dioica* also contains an alkaloid, a fragrant extractive matter and ash 3 to 4 p.c. Ash contains a trace of manganese (Data, 2010).

Momordica dioica as the average nutritional value per 100 g edible fruit was found to contain 84.1% moisture, 7.7 g carbohydrate, 3.1 g protein, 3.1 g fat, 3.0 g fiber and 1.1 g minerals. It also contained small quantities of essential vitamins like ascorbic acid, carotene, thiamin, riboflavin and niacin (Singh, 2006) [12] It also content protein in the leaves and dry weight of aerial plant parts remained higher in male as compared to female defruited, and monoecious plants (Ghosh,2005) [3] From *Momordica dioica* fruit isolated 6-methyl tritriacont-50on-28-of and 8-methyl hentracont-3-ene along with the known sterol pleuchiol. Momodicaursenol, an unknown pentacyclic triterpene isolated from the seeds, had been identified as urs-12,

18 (19)-dien-3 beta-ol on. Phytochemical investigations have revealed the presence of traces of alkaloids and ascorbic acid in fruits. Lectins, b-sitosterol, saponins, glycosides, triterpenes of ursolic acid, hederagenin, oleanolic acid, a-spiranosterol, stearic acid, gypsogenin, two novel aliphatic constituents (Ali and Srivastava, 1998, Sadyojatha and Vaidya, 1996, Ghosh *et al.*, 1981) ^[1, 8, 4]. From the dry root of

Momordica dioica isolated three triterpenes and two steroidal compounds. These were alphaspinasterol octadecanate (I), alphaspinasterol-3-O-beta-D-glucopyranoside (II), 3-O-beta-D-glucuronopyranosyl gypsogenin (III), 3-O-beta-D-glucopyranosyl gypsogenin (IV) and 3-O-beta-D-glucopyranosyl hederagenin (V). Constituent III was a new compound.

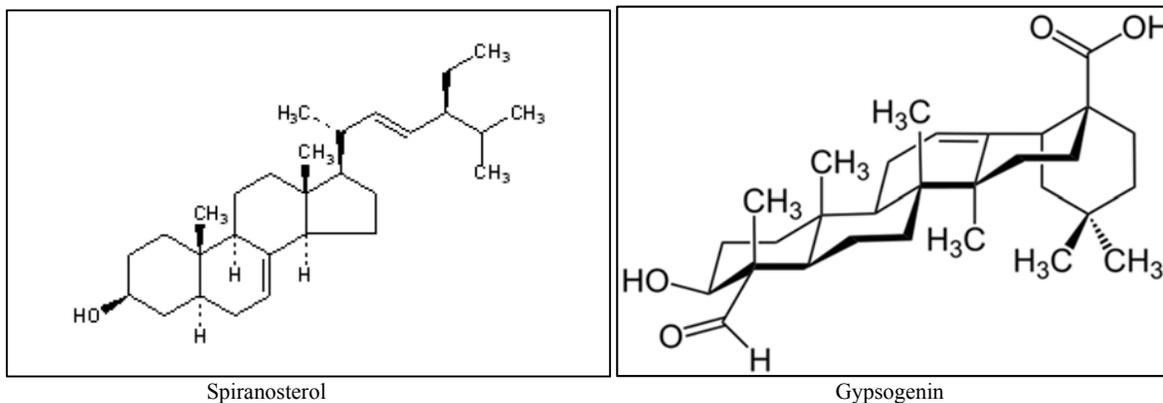


Fig 1: Structure of Spiranosterol and Gypsogenin

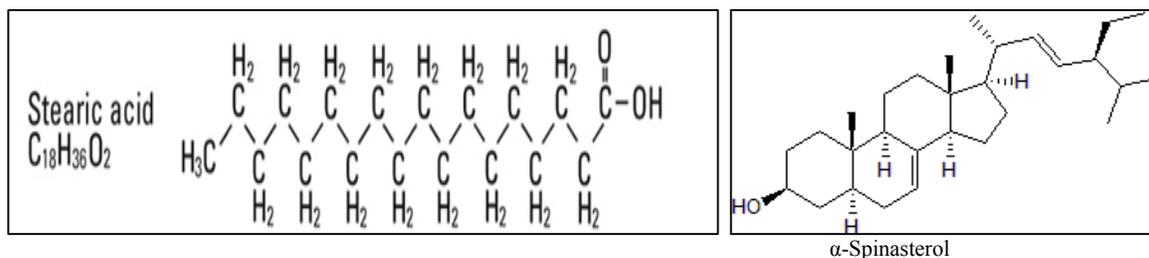


Fig 2: Structure of Stearic acid and α Spinasterol.

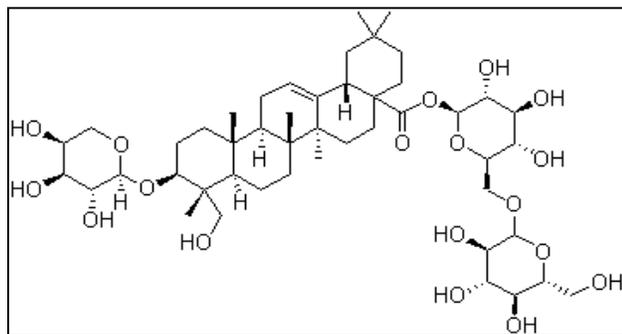


Fig 3: Structure of 3-O-beta-D-glucopyranosyl hederagenin (V)

The fruits have been used in the treatment of inflammation caused by lizard excretion (Sastri, 1962) ^[9] and mental and digestive disorders. The whole plant is known for its use in the treatment of eye disease and poisoning and fever (Satyavathi, 1987) ^[10]. The fruit pulps have been proved for its hypoglycemic, hepatoprotective, anti-inflammatory and analgesic activities and anti-feedant. The Leaves of the plant are antihelminthic and aphrodisiac. It is also used to cure tridosha, fever and alters pitta, jaundice, asthma, bronchitis, piles, hepatic damages, mental digestive disorders, bleeding piles bowel affection and urinary complaints. The juice of the leaves mixed with coconut, pepper, red sandalwood etc in order to form an ointment and applied to the head to relieve pain in the head. Leaf paste applied externally to skin and

orally two or three times daily for skin disease. The present study of *Momordica dioica* has been taken up to carry out qualitative phytochemical analysis for tannins, Phenols, Saponins, Alkaloids, Flavonoids, Anthraquinones, Cardiac glycosides, Carbohydrates, Triterpenoids and Steroids.

Materials and methods

Momordica dioica fruits used as experimental material were collected from farm lands in around Ramagiri fort South region Mahadevapur Reserve Forest Karimnagar India, in October 2015. The collected plant material was placed in a polyethylene bag to prevent loss of moisture during transportation to the laboratory.

Preparation of plant extract

The fruit of the collected plant material was washed thoroughly in tap water, shade dried in open air separately. Powder of the fruit is obtained by grinding them mechanically. About 100 gm of each dried powder of the plant were soaked separately in 100 ml of different solvents like methanol, ethanol, chloroform, pet ether and hot water in conical flasks and then subjected to agitation on a rotary magnetic shaker for about 72 hours. After three days the plant extracts were subjected to filtration, filtered with No 42 whatman filter paper separately. Concentrated extracts was preserved in sterilized air tight labeled bottles and preserved in refrigerator at 4°C until required for further use. The extract was filtered under reduced pressure using rotary flash

evaporator and subjected for further preliminary phytochemical tests. Different tests conducted for the identification of phytochemicals is adopted by using the methods described by (Mohan *et al.*, 2015).

Test for identification of Alkaloids: About 0.5 gm of methanol extract was taken in a test tube and was 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.

Test for identification of Flavonoids: About 0.5 gm of extract was introduced into 10 ml of ethyl acetate in a test tube and heated in boiling water for 1 min. The mixture was then filtered. About 4 ml of the filtrate was shaken with 1 ml 1% aluminium chloride solution and incubated

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. To this, 2 ml of ferric chloride solution was added and observed for the formation of green or blue colour.

Test for identification of Saponins: About 0.5 gm of methanol extract was taken in a test tube and 5 ml distilled water was added to it. The solution was 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.

Test for identification of Steroids: About 0.5 gm of methanol extract was taken in a test tube and 2 ml of acetic anhydride was added to 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.

Test for identification of Tannins: Five grams 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. Creamish white precipitate was observed for the presence of tannins.

Table 1: Photochemical screening test of fruit extracts of *Momordica dioica* Roxb.

Sl No	Chemical components	Methanol	Ethanol	Chloroform	Pet Ether	Water
1	Alkaloids	+	+	+	+	+
2	Terpenoids	+	+	+	+	+
3	Flavonoids	-	+	+	-	-
4	Anthraquinones	-	-	-	-	+
5	Tannins	+	-	-	-	-
6	Saponins	+	+	+	-	+
7	Glycosides	-	+	+	-	+
8	Reducing sugars	+	-	+	+	+
9	Steroids	+	+	+	+	+
10	Cardiac glycosides	+	+	+	+	+
11	Phenol	-	-	-	-	-

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

Results and Discussion

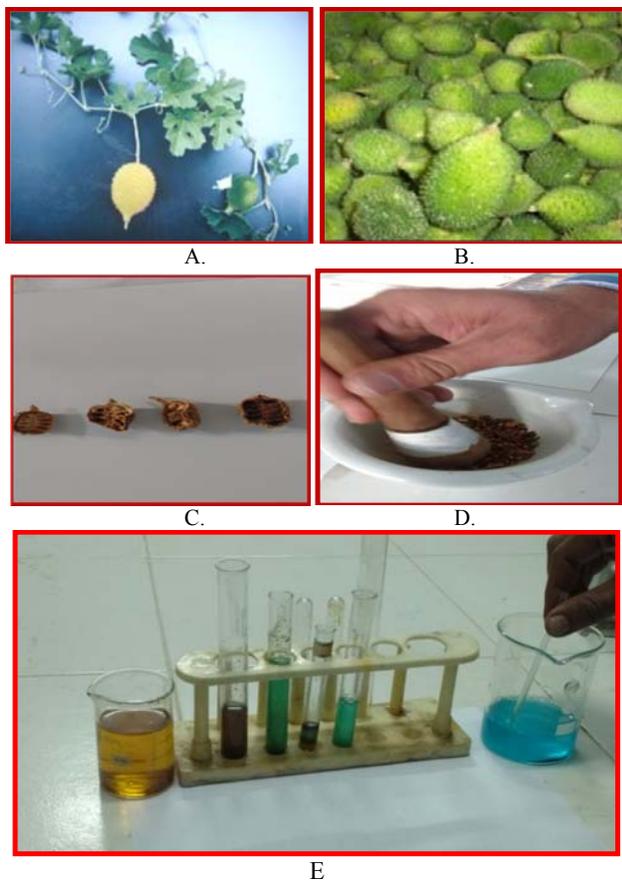
The present study contributes valuable information of bioactive compounds in *M. dioica*, which is a plant with a variety of ethnic medicinal uses. The qualitative analysis of *M. dioica* shows Methanol, Ethanol, Chloroform, Pet Ether and Water extracts contain bioactive compounds such as Phenols, Saponins, Alkaloids, Flavonoids, Anthraquinones, Cardiac glycosides, Carbohydrates, Triterpenoids and Steroids except Tannins and Anthraquinones (Table-4.1 Plate-IV). The present study confirms the wild relationship of *M. dioica*, which can be further explored to the comparative biological activity for further confirmation (Plate-III) Steroids and flavonoids are known to be biologically active. These compounds are known to be toxic to microorganisms. The site and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to the microorganisms. The probable mechanism responsible for phenolic toxicity to microorganisms include enzyme inhibition by oxidized compounds possibly through reaction with sulfhydryl groups or through more nonspecific interaction with proteins. Similarly in our study, the phytochemical analysis of the extracts had shown the presence of bioactive compounds like steroids, fatty acids,

saponin glycosides and triterpenes. Thus, the antimicrobial activity might be due to the presence of these compounds.

Biological activities: The plant is used, for the treatment of eye diseases, against fever, snake bite, inflammation caused by lizard; is also used as medicine for diabetes. While investigating the spermatogenic properties of the ethanolic extract of the fruit extract of *M. dioica* on the animal, the behavioral observations led us for sedative activity of the extract. Not much work is available about the pharmacological and other activities of the plant. *Passiflora actinia* possesses anxiolytic and sedative activity. Likewise, many plants have been reported to have anxiolytic and sedative activity such as *Aloysia polystachya*, *Euphorbia hirta*, *Kigelia Africana* and *Coriandrum sativum*. Juice of root of *Momordica dioica* Roxb. *ex* is immunostimulant and antiseptic. Its fruit are used as vegetable (Sadyojatha and Vaidya, 1996)^[8].

The fruit leaves, and tuberous roots of *Momordica dioica* have long been used in India as a folk remedy for diabetes and other health problems. The juices appear toxic and abortificant. The whole plant is used for treatment of eye disease and against fever Mishra (1991) and Gupta (1993) reported antimalarial and antiallergic activity, respectively, in *M. dioica* Roxb. *ex*. Thirupathi *et al.* (2006) reported protective effect of *Momordica dioica* Roxb. *ex* against hepatic damage caused by carbon tetrachloride in rats. Ali and Deokul have found some important nutrient in different plants

and provided the concentration of important nutrients.



Studies on Phytochemical and Biological Activities of *Momordica Dioica*. Roxb Fig a: Showing the plant with fruit Fig. b: Separated large numbers of fruits. Fig. c: Keeping on dried washed fruits on the filter paper Fig. d: Air dried fruits are grinding by Mortar and pistil Fig. e: Showing the results of Phytochemical Analysis is kept in the test tubes with stand.

Momordica dioica also possess many essential nutrient compound which are essential for proper functioning of the body. It contains Calcium -0.5 mg/g, Sodium -1.5 mg/g, Potassium -8.3 mg/g, Iron -0.14mg/g, Zinc-1.34 mg/g, Protein-19.38%, Fat-4.7%, Total phenolic compound 3.7 mg/g, Phytic acid -2.8 mg/g, 4.1calories and ash value was 6.7%. Jain *et al* reported the antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica*. From both the extracts, ethanolic extract was found to be more potent hepatoprotective.

The antioxidant and free radical scavenging activities were positive for both ethanolic and aqueous extract. This activity may be due to free radical scavenging and antioxidant activities which may be due to presence of flavonoids in extracts. Mishra *et al.* reported that the solvent extracted from vegetable seed oil of Small bitter gourd (*Momordica dioica*), evaluated as grain protectant against *Callosobruchus chinensis* on the stored legume-pulse grains. *Momordica dioica* roots show antiallergic activity for alcoholic extract.

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