

A REVIEW ON EXTRACTION AND EVALUATION OF IN VITRO ANTHELMINTIC ACTIVITY USING MOMORDICA CHARANTIA (BITTER GOURD)

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Abstract- Momordica charantia (Family: Cucurbitales), as known as bitter melon or gourd, is a daily consumption as food and traditional medicinal plant in Southeast Asia and Indo-China. It has been shown to possess anticancer, antidepressant, antidiabetic, anti-inflammatory, antimicrobial, anti-obesity, antioxidant, and anti-ulcer properties. It is also used for the treatment of many ailments such as abortifacient, contraceptive, dysmenorrhoea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney stone, laxative, leprosy, leucorrhoea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies. The present work aims at evaluation of anthelmintic property of M. charantia seeds extracts against Indian adult earthworm Pheretima posthuman, Stellantchasmus falcatus. Albendazole was used as the standard drug. Petroleum ether, chloroform, ethanol and aqueous extracts at concentration of 40 mg/mL each were evaluated for anthelmintic activity. The time taken for paralysis and death of each worm were determined. Among chloroform ethanol, aqueous, and petroleum ether extract the extracts chloroform extract showed best anthelmintic activity by inducing paralysis within 3 min and death within 8 min. This shows the better anthelmintic activity when compared to standard drug.

Keywords: Anthelmintic activity, Bitter gourd, Extraction, Earthworms.

INTRODUCTION:

Helminthic infection is one of the health problems that affect human and livestock in the world. This problem is seen in the children which affect child digestion leads to cause in the absorption of nutrition's from the gastrointestinal tract. The main helminths which infect the gastrointestinal system are nematodes, trematodes, cestodes. The synthetic drugs available have been shown to have side effects; moreover, resistance of the parasites to existing drugs is increasing.[1] Because of limited availability and affordability of modern medicines, most of the world's population depends to a greater extent on traditional medical remedies.[2,3] Helminthic infection could be prevented by maintaining environment sanitary and treatment as well as pharmacotherapy using synthetic drugs or traditional medicine as alternative; one of them is Momordica charantia. This review provides scientific evidence to provide updated information about the properties of M. charantia, one of the anthelmintic plants, which is being investigated for its mechanism.

COMMON NAME: Bitter melon, bitter gourd



Bioactive compounds present in bitter gourd:

The primary metabolites in bitter gourd are common sugars, proteins and chlorophyll while secondary metabolites are phenolics, carotenoids, cucurbitane triterpenoids, alkaloids, saponins etc. Secondary metabolites are responsible for the nutraceutical's properties of bitter gourd which scarcely contribute to the nutritional value but produce beneficial physiological effects in the body.[4] Around 228 different compounds were identified from different parts of M. charantia. [5] Aqueous extract of bitter gourd contained carbohydrates, proteins, amino acids, sterols, flavonoids, phlobatannins, terpenoids, cardiac glycosides and saponins. Qualitative tests found out the presence of carbohydrates, proteins, amino acids, phenolics, saponins, sterols, alkaloids, cardiac glycosides, cholesterol and phlobatannins in the ethanolic extract of bitter gourd. [6]

BROAD CATEGORY	COMPOUNDS IDENTIFIED	PLANTS PARTS
Phenolic compounds	Flavonoids such as catechin, epicatechin Non flavonoids such as gallic acid, gentisic acid, chlorogenic acid, tannic acid, tannins	Fruits Leaves Stem
Carotenoids	Lutein, α & β carotene, zeaxanthin, β cryptoxanthin, lycopene	Fruits
Phytosterols	Decortinone, clerosterol, ergosterol peroxide, stigmasterol, campesterol, β sitosterol	Fruits
Curcubitane Triterpenoids	Charantin, kuguacins A – S, momordicine I, II and III, Karavilagenin A, B, C, D, E, saponins (triterpenoid glycosides), goyasaponins, sapogenins such as diosgenin	Fruits Leaves

TABLE 1: - List of different bioactive compounds identified from different parts of bitter gourd plant [7]

TAXONOMICAL CLASSIFICATION:

Kingdom : Plantae
 Subkingdom : Viridiplantae
 Infrakingdom : Streptophyta
 Super division : Embryophyta
 Division : Tracheophyta
 Subdivision : Spermatophytina
 Class : Magnoliopsida
 Superorder : Rosanae
 Order : Cucurbitales
 Family : Cucurbitaceae
 Genus : Momordica
 Species : Momordica charantia.[8]

The plant *Momordica charantia* is a small shrub which contains almost sixty species in its family distributed across tropical and subtropical regions.[9,10]

NOMENCLATURE:

The genus “*Momordica*” from Latin “*Mordeo*” means to bite and the species “*charantia*” from Greek means beautiful flower.[11] *M. charantia* is a native of the tropic’s areas including East Africa, South America, Asia, the Caribbean, India, and Southeast Asia.[12] The vernacular names of *M. charantia* include bitter melon, bitter gourd, balsam pear, or African cucumber (English); kyethinkhathee (Burmese); Lai pu Tao, Ku gua (Chinese); balsamagurk (Danish); margose, momordique amere (French); balsambirne (German); karela, Tita Kerala (Hindi); paria, pare (Indonesian); pomo meraviglia (Italian); niga Uri, Tsuru reishi (Japanese); Merah (Khmer); kaypa (Malayalam); Karli (Marathi); karelaa (Nepalese); kara Velli (Sanskrit); karavila, Pavaki (Sinhalese); balsam, *Momordica Amarga* (Spanish); bittergurka (Swedish); Kakara (Telugu); mara (Thai); and la khoqua (Vietnamese).[13,14]

DESCRIPTION OF MOMORDICA CHARANTIA FRUIT:

Fruit: The orange to yellow pendulous cylindrical fruit is egg shaped and 2–10 cm long, which covered with longitudinal ridges and warts. Seed: The seed which is 8–15 mm long black but covered with a soft, flesh white in unripe to red in ripe. The fruit is edible when still green, but poisonous once ripped. [15]

TRADITIONAL USES:

Traditional use of Bitter gourd has been used in various Asian traditional medicine or pharmaceutical systems for a longer period of time, as valuable for the prevention and curing of many diseases. The bitter gourd fruit can be used in the treatment of many diseases such as asthma, ulcers, constipation, fever, diabetes, cough, gout, helminthiases, inflammation, leprosy, skin diseases, burns and wounds. The hypoglycaemic properties are found in the both animals and as well as human studies. Diseases such as piles, cleanse blood and liver recompenses, dyspepsia, jaundice and cholera can be treated using the leave juices of bitter gourd.[16]

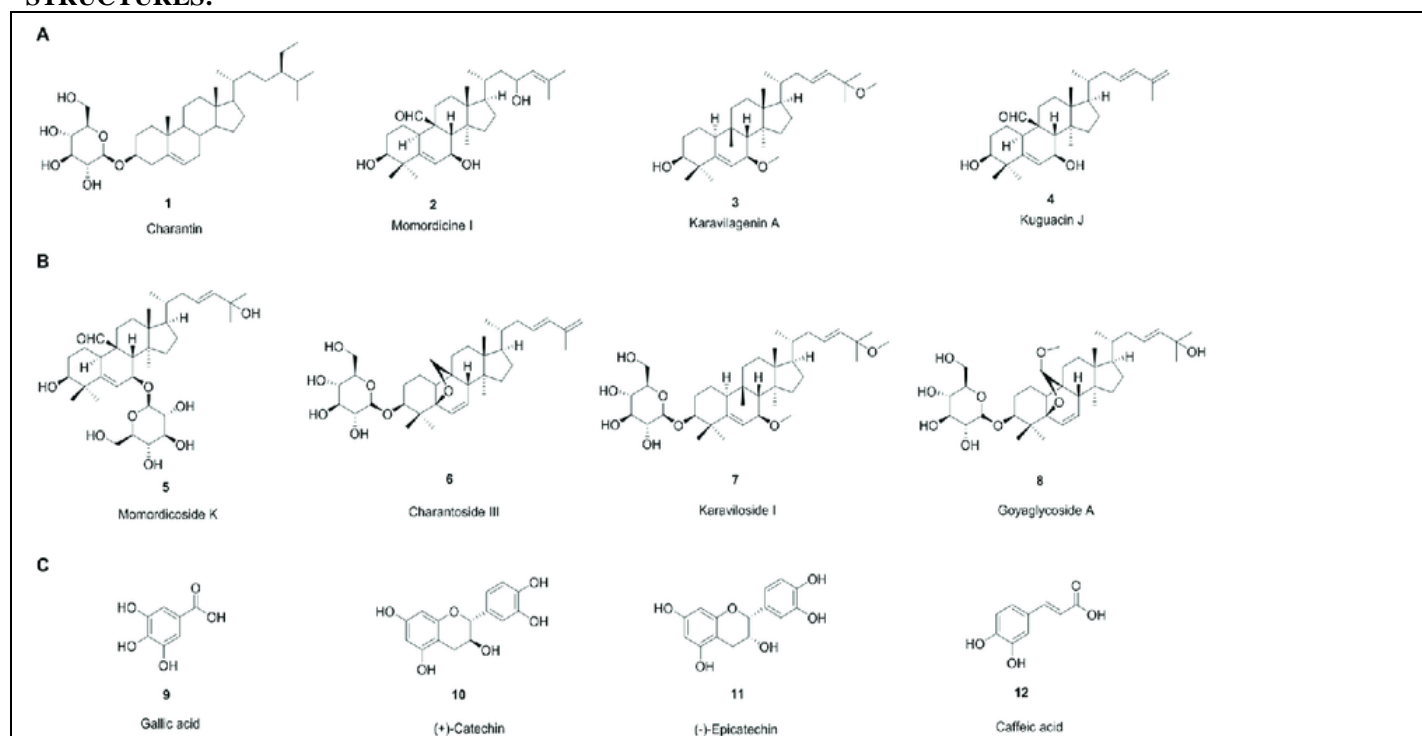
PHYSICO - CHEMICAL PROPERTIES OF BITTER GOURD:

The moisture content in the bitter gourd was found high that is 92.2%. The moisture free content of protein and fat in bitter gourd powder was found to be 0.90 and 1.02% respectively, ash content of bitter gourd powder was 1.4%. Fibre content was found to be in good amount 2.08%. Carbohydrate content was found that 2.4%. [17]

TABLE 2: Proximate composition of bitter gourd

S NO	PARAMETERS	OBSERVATION
1	Colour	Light to dark green
2	Appearance	Bumpy skin and oblong in shape
3	Moisture	92.2±0.1
4	Ash	1.4±0.2
5	Fats	1.02±0.41
6	Carbohydrate	2.4±0.12
8	Protein	0.90±0.01
9	Fibre	2.08±0.03

STRUCTURES:



Phytochemical screening:

Phytochemical screening of the petroleum ether, ethyl acetate and ethanol extracts of two varieties of *M. charantia* fruits were carried out. The presence of alkaloids, tannins, flavonoids, saponins, and anthraquinones were carried out according to the methods of Sofowora (2006), Harbone (1991), Trease and Evans (2002) and Edeoga et al. (2005). [18]

Alkaloid Screening:

Determination of alkaloid was performed using the procedure put forward by Harborne (1973) as described by Edeoga et al. (2005). Briefly, five grams (5 g) of the powdered sample were weighed into 250 ml beaker. Acetic acid (10%) in ethanol was then added. The mixture was covered and allowed to incubate for 4 h. This was then filtered, and the extract concentrated on a water bath to $\frac{1}{4}$ of the original volume. Thereafter, concentrated ammonium hydroxide added drop wise until precipitation was completed. The solution was then allowed to settle and the precipitate was collected, then washed with diluted ammonium hydroxide and filtered. The residue was dried and weighed was alkaloid.

Flavonoids Screening:

Flavonoids were determined by the methods developed by Boham and Kocipaiabyazan (1994). Briefly, 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was then filtered using Whatman No.42 (125 mm) filter paper. The filtrate was later transferred into crucible and evaporated to dryness over a water bath and weighed to a constant weight. The weighed was flavonoids.

Saponins Screening:

Saponins were determined according to the method described by Obadoni and Ochuko (2001). Based on this method, 10 g of the powdered sample for each plant species was transferred into a conical flask, and 50 ml of 20% aqueous ethanol was added. This was heated over a hot water bath for 4 h while stirring continuously at 55° C. Thereafter, the mixture was filtered and the residue re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml using water bath at 90° C. Then,

the concentrate was transferred into a 250 ml separatory funnel. Diethyl ether 10 ml was added to the funnel, and the mixture was shaken vigorously. The aqueous layer was recovered after the ether layer was discarded. The purification process was repeated. In addition, 30 ml of n-butanol was added. The combined n-butanol extract was washed twice using 5 ml of 5% aqueous sodium chloride, and the remaining solution was then heated in a water bath.[19]

Phenolic content analysis in different extracts:

The total phenolic content of the *M. charantia* extracts was determined using the Folin-Ciocalteu reagent (Mills, 1981). The reaction mixture was contained: 1 ml of diluted extract (200mg/ml), 5ml of freshly prepared diluted Folin Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. Mixtures were kept in dark at ambient conditions for 1h to complete the reaction. The absorbance at 765 nm was measured using a spectrophotometer (Shimadzu UV PC-1600). Gallic acid was used as standard, and the results were presented as dry weight basis with μg gallic acid equivalent (GAE)/mg of sample.[20]

PHARMACOLOGY:

MATERIALS AND METHODS:

Materials:

Raw materials:

Raw materials fresh bitter melon was cultivated and collected from the local farms.

Chemicals and glassware:

Chemicals, reagents, glassware and processing equipment required.

STEPS IN PREPARATION OF BITTER MELON POWDER

Pre-treatment to bitter melon:

Fresh bitter melons were washed using water to remove adhere material then cut by knife into 3mm thickness. Then 2% NaCl was sprinkled and kept that for 15 minutes. Then by using muslin cloth bitter melon slices were squeezed to remove the excess water and reduce the bitterness.

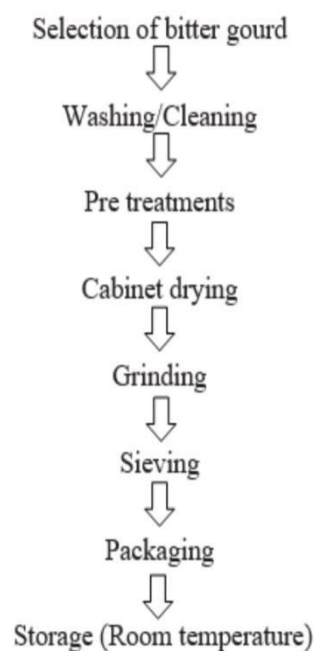
Drying:

The treated bitter melon slice of 3mm were uniformly spread in a single layer on steel trays and dried at 60°C for 6hrs by maintaining air velocity at 1.2 m/s in cabinet tray dryer.

Preparation of bitter melon powder:

Bitter melon powder was prepared by grinding the dried slices of bitter melon and pulverized continuously till the whole sample passed through 160-micron sieves. Obtained powder was weighed and packed in HDPE pouch. [21]

1: Preparation of bitter melon Powder



DIFFERENT EXTRACTION METHODS:

Method 1: EXTRACTION USING SOXHLET APPPARATUS

Soxhlet extraction was carried out using a Soxhlet apparatus. A 20g of grounded bitter melon was added into round bottom flask with 200ml of different solvents each. The Soxhlet extraction takes about 6hours to complete. The solvent was added distilled water, ethanol, dichloromethane & petroleum ether with boiling point 100c ,78.37c ,39.6c & 42-62c at 1atm respectively. The next step is solvents were removed via rotatory evaporator at a temperature. slightly above the boiling point of the solvent and remaining oil yield then were stored in freezer(-20c) for further analysis.[22]

Method 2: EXTRACTION USING SONICATOR

The dried powder was extracted with methanol using a sonicator at 25 °C for 30 min and then kept in a shaking incubator for 24 h at 250 rpm and 30 °C. The extract was centrifuged at 12000 rpm for 10 min. The solvent was then evaporated using a rotary evaporator at 45 °C, and the extract was weighed and kept at – 80 °C until use. Crude seed extract was also prepared in a similar manner.

METHOD 3: USING COLUMN CHROMATOGRAPHY

M. charantia fruit extract (2 g) was subjected to column chromatography using silica gel 60 silanized (0.063–0.200 mesh; Merck, Darmstadt, Germany). The sample was prepared by adsorbing 2 g of the extract to 20 g of silica and then left to dry. The dry powder was applied on top of the column (5 × 25 cm) and then eluted using trichloromethane, ethyl acetate, and methanol-water (50:50) with pressure. Each solvent at a volume of 500 ml was collected in a beaker.

METHOD 4: C₁₈ CARTRIDGES

C₁₈ cartridges were used to further fractionate the ethyl acetate active fraction isolated from *M. charantia* fruit. The active fraction (2 mL) was diluted with 8 mL of distilled water. The SPE cartridge used was Chroma bond C18 cartridge (Macherey & Nagel). The cartridge was attached to a vacuum and sequentially conditioned by passing 10 mL of methanol, 10 mL of Milli-Q water, and 10 mL of methanol-water (2:8 v/v). Diluted active fraction (10 mL) was loaded onto the preconditioned cartridge and eluted at a drop-wise to ensure efficient adsorption of the compounds. Elution of C₁₈ cartridge-bound compounds was achieved by adding 10 mL of methanol-water (2:8), followed by methanol drop-wise. Finally, two fractions were collected and concentrated using a rotary evaporator at 45 °C. The fractions were dried, reconstituted, and stored at – 80 °C until use.[23]

ANTHELMINTIC ACTIVITY:

The extracts of various plant parts of *M. charantia* including the leaf, fruit, and seeds have been investigated and found to be pharmacologically active against helminths.

EARTHWORM:

Sen et al. [24] from India studied in vitro anthelmintic activity of methanolic extract of 150 mg/ml of whole fruit, fruit peel, seed, whole fruit juice, and peel juice of *M. charantia* against Indian adult earthworms (*Eisenia foetida*). They reported the fruit peel showed paralysis time at 8.5 min and death time at 14.5 min like those of 8.2 min and 16.3 min of 40 mg/ml of albendazole, standard drug treatment. Veda Murthy et al. [25] from India studied the *M. charantia* seed extract against *Pheretima posthuma*, Indian adult earthworm. The chloroform extract exhibited the best anthelmintic activity by inducing paralysis within 3 min and death within 8 min, followed by ethanol, aqueous, and petroleum ether extract. Vinay et al. [26] from India studied the effect of *M. charantia* fruit on *E. foetida*. They reported that the 10 mg/ml of aqueous and methanolic extracts exhibited paralysis time at 117 min and 100 min and death time at 151 min and 140 min, respectively.

Stellantchasmus falcatus Buddhachat et al. [27] (2012) from Thailand studied the effect of aqueous extract of *M. charantia* fruit on the mortality and tegmental surface change on *Stellantchasmus falcatus*, a gastrointestinal trematode of fresh fish, birds, and mammals. They reported that the 12.5%, 50%, and 100% concentrations of plant extracts were able to kill all the worms at 280, 270, and 80 min, respectively. The tegmental surface of worm exhibited bleeding, rupturing, and curving of the spine.

CONCLUSION:

Momordica Charantia[Bitter Gourd] edible fruit which existing various biological activities among that one of the important activity is anthelmintic activity. In this present review have given that information regarding the extract of the fruit and various evaluation methods for anthelmintic activity of bitter gourd.

CONFLICT OF INTEREST:

The authors have no conflict interest regarding this investigation.

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