



Research Article

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PHYTOCHEMICAL AND *IN VITRO* ANTHELMINTIC ACTIVITY OF *MOMORDICA CHARANTIA* LINN FRUIT EXTRACTS

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ABSTRACT

Momordica charantia or bitter melon, also known as balsam pear or karela, is a tropical vegetable and common food in Indian cuisine that has been used extensively in folk medicine as a remedy for diabetes. As mentioned in Ayurveda it is also used as an abortifacient, anthelmintic, contraceptive, eczema, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies. It is mentioned as anthelmintic and used in krimiroga as per ayurveda although no more work done on extracts of *Momordica charantia*. In this work anthelmintic activity of methanolic and water extracts were checked on earth worms *Eisenia foetida*. Also the successive extraction petroleum ether, chloroform, ethyl acetate, methanol and hydroalcoholic extracts were checked at concentration 5mg/mL and 10mg/mL. Methanolic and water extract shows death time at 139 min and 152 min respectively at 10 mg/mL dose. While in successive extraction chloroform extract was showed more potent activities than any other fractions. Activity is probably due to presence of alkaloids and steroidal triterpenoids present in it. Hence the anthelmintic activities of *Momordica charantia* is proved as per mentioned in Ayurveda further need to isolate the compound which is responsible for it.

Keywords: *Momordica charantia* Linn, anthelmintic, *Eisenia foetida*, successive extraction.

INTRODUCTION

Helminth infections are one of the most prevalent diseases in developing and developed countries. Globally, 2 billion people are infected by intestinal nematodes. Most diseases caused by helminthes are chronic and debilitating in nature, they probably cause more morbidity and greater economic and social deprivation among humans and animals than any other single group of parasites. The parasitic gastroenteritis is caused by mixed infection with several species of stomach and intestinal worms, which results in weakness, loss of appetite, decreased feed efficiency, reduced weight and decreased productivity¹. Anthelmintics are drugs which may kill (vermicide) or expel (vermifuge) infesting helminthes. Chemotherapy is the only treatment and effective tool to cure and control helminthes infection, as effective vaccines have not been developed so far. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites². Thus the studies on the herbs and the phytochemicals focused in extracting anthelmintic constituents like alkaloids, steroids, triterpanoids and other phytoconstituents.

The use of medicinal plants plays an important role to cover basic health need in developing countries. *Momordica charantia* or bitter melon, also known as balsam pear is a tropical vegetable, is a common food in Indian cuisine and has been used extensively in folk medicine as a remedy for diabetes. It is a very common herb having various medicinal properties for the treatment of different kind of disease, viz. antidiabetic, abortifacient, anthelmintic, contraceptive, eczema, antimalarial, galactagogue, gout, Jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, Pneumonia, Psoriasis, Purgative, rheumatism, fever and scabies focused the investigator's attention on this plant³. *Momordica charantia* Methanol extract of fruit peel was proved for anthelmintic effect

against Indian adult earthworms *Eisenia foetida*⁴. The present study was designed to assess the anthelmintic potential of various fractions of *Momordica charantia* fruit against *Eisenia foetida*.

MATERIALS AND METHODS

Plant material

The fresh fruits of *Momordica charantia* Linn. were purchased from the local market of Rajkot, Gujarat in November 2014 and were authenticated by botanist from Department of Bioscience, Saurashtra University, Rajkot, Gujarat, India.

Worm collection

For the anthelmintic activity, earth worms *Eisenia foetida* were collected from Junagadh Agriculture University, Junagadh, Gujarat, India. They were transported to laboratory in a well ventilated bag with sufficient nutrition content.

Drying and Milling

The plant material was cut in the pieces and dried in sunlight for 2 weeks pulverized and dried materials were ground with the help of electric grinder and passed through sieve no. 40 and stored in air tight container for further extraction.

Preparation of extracts

First methanol and water extracts were prepared and evaluated for anthelmintic activity. After that the successive solvent extraction procedure was adopted for the preparation of various extracts of *Momordica charantia* Linn. fruit powder. The materials were subjected to successive extraction with solvents their ascending order of polarity. In this process the substance, which is soluble in a solvent with particular range of polarity was extracted in the solvent and remaining marc further

extracted with next solvent. The constituents which were soluble in both polar and non-polar solvent can be extracted separately by adopting this approach. The powder of air dried drug weighing about 200 gm was extracted in Soxhlet apparatus with solvent of increasing polarity like petroleum ether, Chloroform, ethyl acetate, methanol and hydroalcoholic (50:50). The extracts were concentrated using rotary evaporator and then after dried in oven at 50 °C for complete dry. All the extracts mentioned above were subjected to preliminary phytochemical study and anthelmintic activities.

In vitro anthelmintic activity^{5,6}

The anthelmintic activity was performed by estimating the survival time of the earth worms when treated with the extracts⁷. This study was conducted by exposing the worms to different concentrations of methanol and water extracts (Table 1). After that successive fraction of petroleum ether, chloroform, ethyl acetate, methanol and hydro-alcoholic extract of the fruit powder were evaluated⁸. All the worms were washed in normal saline solution and their length measured before exposing to the extracts. Worms of approximately equal sizes were taken in petriplates (3 worms /plate) and various concentrations 5 mg/mL and 10 mg/mL of extracts were added. The extracts were given

in form of suspension for that 0.5% acacia is used as suspending agent. Albendazole (5 mg/mL & 10 mg/mL respectively) was used as the positive control⁹ and it was also suspending in 0.5% acacia and distilled water was use as the negative control for the study. Time taken for paralysis and death were observed for each individual worm in each plate. For all the above samples, the experiments were performed in triplet (Table 2).

Statistical analysis

The results were analysed for statically significance using one-way ANOVA followed by Dennett’s ‘t’ test and are presented in respective tables.

Phytochemical evaluation

Preliminary phytochemical screening was employed to detect various secondary metabolites in the extracts of *Momordica charantia*. The extracts were tested for the presence of alkaloid, phenolic and tannins, glycosides, flavonoids, phlobatannins, carbohydrates, steroids and terpenoids. Phytochemical analysis of various secondary phytoconstituents were carried out with the various extract using standard chemical test protocols (Table 3)^{10,11,12}.

Table 1: Comparative in vitro anthelmintic activity various extracts of *Momordica charantia* Linn

Groups	Concentration (mg/mL)	Paralysis time (min)	Death time (min)
Control	---	No effect	No effect
Standard (albendazole)	10	72±0.57	106.7±1.453
Methanol extract	10	100.3±1.82***	140.2±1.195**
Water extract	10	117.3±2.011**	150.5±1.33**

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at *** P<0.001, ** P<0.01, *P<0.05, ns> 0.05 vs. control group respectively. (One-way ANOVA followed by Tukey’s post hoc test

Table 2: Comparative in vitro anthelmintic activity various successive fractions of *Momordica charantia* Linn

Groups	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
Control (distill water)	---	No effect	No effect
	---	No effect	No effect
Standard (albendazole)	5	112.0±0.57	141.0±2.73
	10	72±0.57	106.7±1.453
Petroleum ether	5	No effect	No effect
	10	No effect	No effect
Chloroform	5	82.50±0.76***	114.7±1.202***
	10	55.17±95.50***	90.67±0.88***
Ethyl acetate	5	137.2±0.703***	152.3±4.716*
	10	95.50±0.76***	114.3±1.54**
Methanol	5	No effect	No effect
	10	No effect	No effect
Hydro alcoholic	5	No effect	No effect
	10	No effect	No effect

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at *** P<0.001, ** P<0.01, *P<0.05, ns> 0.05 vs. control group respectively. (One-way ANOVA followed by Tukey’s post hoc test.

Table 3: Preliminary phytochemical analysis

Phytochemicals	Methanol	Water	Successive Solvent Extraction				
			PE	CH	EA	ME	HA
Alkaloids			-	+	+	+	-
Glycosides	+	+	-	+	+	+	-
Flavonoids	-	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-
Phenolics	+	+	-	-	-	+	+
Saponins	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	-
Amino acids	+	+	-	-	-	+	-
Fixed oils	+	+	+	-	-	-	-

PE-petroleum ether, CH-chloroform, EA-ethyl acetate, ME-methanol, HA-hydroalcoholic
+ present - absent

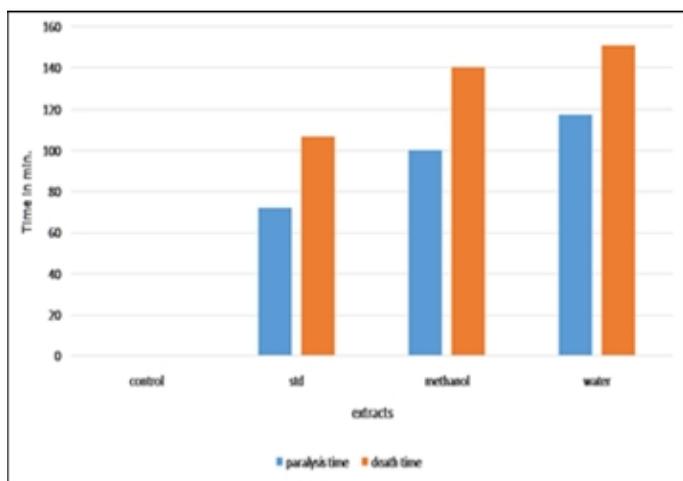


Figure 1 *In vitro* anthelmintic activity of methanol and water extracts of *Momordica chirantia* fruits

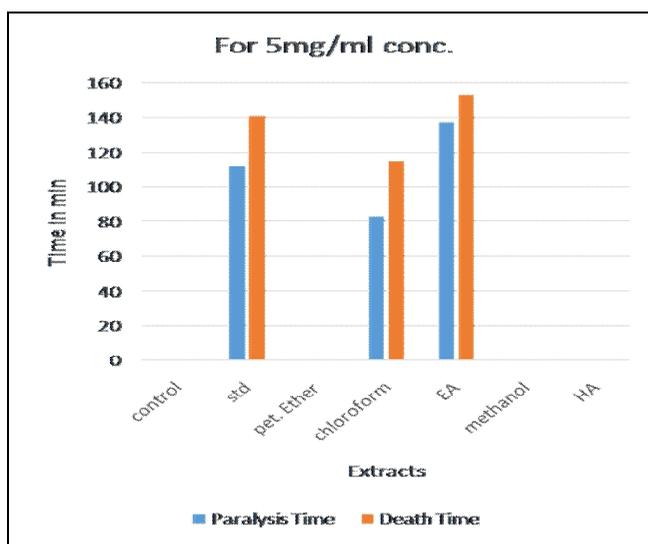


Figure 2 *In vitro* anthelmintic activity of extracts (5 mg/mL)

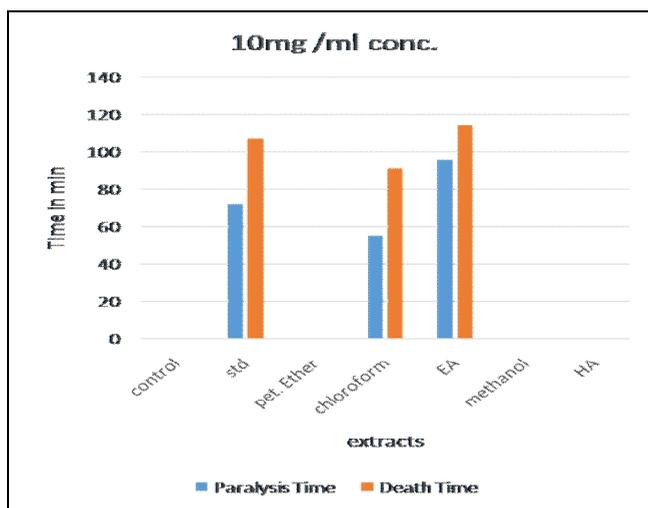


Figure 3 *In vitro* anthelmintic activity of extracts (10 mg/mL)

RESULTS AND DISCUSSION

Anthelmintic activity

From the results of the anthelmintic activity of the methanolic and water extract it was found that the time taken for paralysis of *Eisenia foetida* was 100.3 ± 1.82 min and 117.3 ± 2.011 min respectively. While time taken for death is 140.2 ± 1.195 min and 150.5 ± 1.33 min respectively. This proves that plant *Momordica charantia* fruits has good anthelmintic activities while in further successive extraction chloroform fraction shows 55.17 ± 95.50 min for paralysis and 90.67 ± 0.88 min for death time as compare to albendazole for that paralysis time is 72 ± 0.57 min and 106.7 ± 1.453 min for death of worms at concentration 10 mg/ml. for each. So it proves that chloroform fraction has good anthelmintic activities as compare to marketed product albendazole. The paralysed earth worms had not showed any movement and remains scattered in the media whereas earth worms which did not showed paralysis remained mobilized and aggregates many times. This activity may be due to presence of alkaloids, glycosides and steroids present in chloroform fraction. Further need to isolate a compound which is responsible for anthelmintic activity. The time taken for paralysis and death showed an orderly decline with the increasing concentration of the test extracts. The mean \pm SEM values were calculated for each extract. Result of anthelmintic activity on *E foetida* against each concentration of each extract was compared with that of corresponding concentration of albendazole as reference drug. Albendazole acts by inhibiting the polymerization of helminthic β -tubulin, and thus interferes with microtubule dependent functions like glucose uptake and glycogen depletion¹³. Several mechanisms serve anthelmintic activity like steroids are known to have an effect on membrane permeability and pore formation of parasites thereby leads to mortality of parasites¹⁴.

Phytochemical evaluation

The phytochemical evaluation showed the presence of alkaloids, flavonoids, starch, saponins, steroids, glycosides, proteins and tannins in the methanol and water extracts. Alkaloids, glycosides, saponins and steroids are present in chloroform fraction. Steroidal saponin also produces disruption of monogenea teguments. microvilli that acts as an absorptive surface in the earth worms¹⁵. Alkaloids in parasite reduces nitrate generation thereby decreases ribosomal and mitochondrial protein synthesis¹⁶ and interferes with the synthesis and activities of DNA and RNA, inhibits glucose supply and causes paralysis of worms by acting on central nervous system¹⁷. Anthelmintic activity of chloroform extract was more significant it may be due to alkaloid or steroidal or bitter glycoside.

CONCLUSION

The *in vitro* studies carried out using water and methanol extract of *Momordica charantia* Linn. revealed a perfect anthelmintic activity on *Eisenia foetida*. Further in successive fractions chloroform fraction shows more potent activity than any other fractions and a standard drug albendazole. The results of this study provide an evidence to conclude that the chloroform extract of *Momordica charantia* linn. can be used for the effective treatment of helminthes. The experimental evidence obtained in the laboratory model could provide a rationale for the folkloric use of *M. Chirantia* as "krimiroga" means anthelmintic drug. Hence, the anthelmintic potential of this plant strongly signifies its use as a dietary supplement with an additional advantage of chemotherapeutic prevention from helminthiasis. Further *in vivo* anthelmintic activity to

substantiate the folk claim, standardization of the plant extract and development of the best herbal formulation to replace synthetic drugs could be carried out. It would be also interesting to find out any novel or existing chemical entities responsible for the anthelmintic activity and their mechanism of action.

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