International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 6(1): January-February 2016

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences

Research Article……!!!

Received: 01-01-2016; Revised: 23-01-2016; Accepted: 24-01-2016

ANTIHELMINTHIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF *CUCURBITA MAXIMA* AND *MOMORDICA CHARANTIA* SEEDS

Bharthi.V[1]*, Hemalatha.G[1] and Shanthi.S[2]

1. Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli, Tamilnadu
2. Department of Microbiology, Shrimati Indira Gandhi College, Tiruchirappalli, Tamilnadu

Keywords:

Cucurbita maxima, anthelmintic activity

For Correspondence:

Bharthi.V

Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli, Tamilnadu

E-mail:

bharathi2679@gmail.com

ABSTRACT

The present study deals with phytochemical and anthelmintic evaluation of *Cucurbita Maxima (pumpkin) seeds* and *Momordica charantia* seeds.This evaluation revealed the presence of many phytochemical constituents. Crude tannins were isolated and identified using thin layer chromatography. All extracts were evaluated for anthelmintic activity. *Momordica charantia* Seeds extracts showed very good anthelmintic activity. Paralysis and death times of crude extracts were very close to the standard drug Albendazole.

INTRODUCTION

Heminthiasis refers to infections caused by parasites such as pin worms and wound worms. It is estimated that more than 3 billion people live with helminthes across the world (Dhar et al.,1982). Though these worms target the intestinal tract, when they increase in number they invade organs such as lungs and heart. Animals with parasitic worms in their bodies can experience a variety of symptoms related to the parasite including intestinal discomfort, weight loss abdominal bloating, and signs of malnutrition such as hair loss. Control of Helminthes is widely based on antihelminthic drugs. Antihelminthics are chemical compounds which expel parasitic worms from the body. However, current efficacy of these drugs has become a great question now because of the development of resistance among the parasites and drastic side effects caused to the host (Geert &Dorny, 1995; Coles, 1997. The development of microbial resistance to antibiotics has led the researches to investigate the alternative sources for the treatment of resistant strains. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value and has anthelmintic efficacy Akhtar et al., 2000; Iqbal et al., 2004). Currently, 80 percent of the world population relies on plant derived medicines and serves as first line of defense in maintaining health and combating many diseases.

The plant *Momordica charantia* belongs to the family cucuritaceae and is commonly known as bitter melon. Bitter melon grows in tropical and subtropic areas, where it is used as a food as well as a medicine. Although the seeds, leaves, and vines of bitter melon have all been used, the fruit is the safest and most prevalent part of the plant used medicinally. From ancient time, the juice of the leaves, fruits and seeds have been used to treat worm infection. Similarly, *Coccinia grandis* belongs to cucuritaceae family is also having the history of medicinal value. The juice of the roots and leaves is used in the treatment of diabetes. The juice of the stem is dripped into the eyes to treat cataracts. The leaves are used as a poultice in treating skin eruptions. The plant is laxative. It is used internally in the treatment of gonorrhoea. Aqueous and ethanolic extracts of the plant have shown hypoglycaemic principles. In the current investigation, aqueous and alcohol extract of the selected plants were assayed for their anti- helminthic activity. The active principles responsible for the activity were also screened.

MATERIALS AND METHODS

Collection and authentication of Plant Materials:

The seeds of *Coccinia grandis* and *Momordica charantia* were collected in the month of August from the local field of Lalgudi, Trichy, and South India. The plant material was taxonomically identified by RAPINAT Herbarium St Joseph College Trichy. A voucher specimen has been preserved in the same laboratory for future reference.

Preparation of Seed Powder

The collected *Coccinia grandis* and *Momordica charantia* seeds were surface sterilized with sterile distilled water to remove the dirt and soil particles adhered on them. Seeds were cut into small pieces and shade dried at room temperature (32^{o}C) for two weeks. The shade dried seeds were pulverized into a coarse powder and stored at room temperature for further use.

Extraction of Plant Material

Alcohol and aqueous extracts of *Coccinia grandis* and *Momordica charantia* seeds were obtained by standard hot and cold extraction methods. (Harborne *et al.*1999).

Aqueous extraction

50 g of coarsely powdered *Coccinia grandis* and *Momordica charantia* seeds were added with 150 ml of sterile distilled water separately and placed in a water bath at 80°C for 1.5 h. This solution was filtered through a 420µm stainless steel filter and dried into powder by flash evaporator under reduced pressure and controlled temperature (40-50°c) (Harborne *et al.,* 1999). The aqueous extract was placed in air tight containers, stored at 4^{o} C and utilized throughout the studies.

Alcohol extraction:

Seed powder was first defatted with petroleum ether. 100 g of *Coccinia grandis* and *Momordica charantia* seeds were mixed with 300 ml of alcohol, in a beaker. Beaker was closed with aluminum foil and left for 72 hours at room temperature. The extract was filtered through three layered muslin cloth and condensed into the powder by flash evaporator under reduced pressure and controlled temperature (40-50°c). The alcohol fraction was dried in to powder form and placed in air tight containers, stored at 4^{o} C and utilized throughout the studies (Harborne *et al.,* 1999).

Phytochemical Analysis:

Preliminary phytochemical analysis for biologically active phytoconstituents like quinones, flavonoids, tannin, coumarins , sugars, steroids, phenols, terpenoids, anthroquinones, of the plant extracts were carried out using standard methods described by Harborne *et al.,(*1999).

ASSAY OF ANTHELMINTIC ACTIVITY

Experimental Animals

The antihelmintic assay was carried out as per the method of Ajaiyeoba *et al*., (2001). The assay was performed in *in vitro* using Indian adult earthworm (*Pheretima posthuma*) as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings. For preliminary evaluation of anthelmintic activity adult *Pheretima posthuma* worms about 11 cm length and 0.3 to 0.4 cm width were collected from the moist garden. Worms were washed with saline water to remove the fecal matter and used throughout the experimental protocol.

Screening of Antihelmintic activity

Prepared extracts were evaluated for antihelmintic activity separately. Earthworms of nearly equal size were acclimatized to the laboratory condition before experimentation. The earth worms were divided into six groups of six earth worms in each. Albendazole was diluted with 5% DMF (Dimethyl Formamide) in normal saline solution to obtain 10, 25 and 50 mg per ml which served as positive control and poured into Petri dishes. The alcohol and aqueous extracts were dissolved in 5% DMF in normal saline solution and diluted to prepare concentrations such as 10, 20, 30, 40 and 50 mg/ml. 5% DMF in normal saline solution was taken as negative control. Earth worms were placed in Petri dishes containing 25 ml of different concentrations of standard and extracts at room temperature. All the test and standard drug solutions were prepared freshly before starting the experiments. The mean paralysis time and mean death time for each sample was calculated. The time taken for worms to become motionless was noted as paralysis time and to ascertain death, each worm was frequently applied with external stimuli which stimulates and induces movement in earth worm if alive. All the readings were taken in triplicate and analyzed statistically (Table.1).

RESULTS AND DISCUSSIONS

Table 1: Anthelmintic activity of seeds of *Coccinia grandis*

Treatment	Concentration(mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Aqueous extract	10	80±0.26	93 ±1.12
	50	40±0.86	80 ±0.65
	100	20 ±0.65	30 ±0.74
Ethanol Extract	10	75 ±0.65	83±0.58
	50	35±0.41	40±1.20
	100	20±0.98	25±0.68
Chloroform extract	10	35±0.98	40±0.41
	50	40±0.82	45±0.46
	100	18±1.07	20±1.15
Positive Control (Albendazole)	40	1.30 ±0.65	4.18±0.46
Negative Control (Normal saline)	-	-	-

Table 2: Anthelmintic activity of seeds of *Momordica Charantia*

Treatment	Concentration(mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Aqueous extract	10 50 100	100 ±0.65 90 ±0.41 85 ±0.46	93 ±0.82 80 ±0.86 90 ±0.98
Ethanol Extract	10 50 100	30 ±0.58 20 ±1.15 19 ±1.20	34 ±1.24 25 ±0.68 30 ±0.60
Chloroform extract	10 50 100	40 ±1.18 30 ±1.36 26 ±0.88	43 ±1.07 33 ±0.74 30 ±0.65
Positive Control (Albendazole)	40	1.30±0.65	4.18±0.46
Negative Control (Normal saline)	-	-	-

TABLE-3: Preliminary Phytochemical Screening Of *Coccinia grandis*

S.NO	TESTS	*Aqueous*	*Chloroform*	*Ethanol*
1	Alkaloids	+	+	-
2	Terpenoids	-	-	-
3	Glycosides	-	+	+
4	Coumarins	+	+	+
5	Tannins	+	+	+
6	Flavonoids	+	+	+
7	Phenols	+	+	+
8	Volatile oils	-	-	-
9	Quinone	+	-	+
10	Saponin	+	+	+

TABLE-4: Preliminary Phytochemical Screening Of *Momordica Charantia*

S.NO	TESTS	*Aqueous*	*Chloroform*	*Ethanol*
1	Alkaloids	+	+	+
2	Terpenoids	+	+	-
3	Steroids	-	-	-
4	Coumarins	+	+	+
5	Tannins	+	+	+
6	Flavonoids	-	+	-
7	Phenols	+	+	+
8	Volatile oils	-	-	-
9	Quinone	+	+	+
10	Saponin	+	+	+

Table 5: FT -IR Results of Seed extract of *Coccinia grandis*

S.no	Frequency range	Bond	Type and group
1	3407.59	=C–H stretch	alkenes
2	2171.46	–C(triple bond)C– stretch	alkynes
3	1573.84	C–C stretch (in–ring)	aromatics
4	1398.25	C–H bend	alkanes
5	1128.31	C–N stretch	aliphatic amines
6	831.20	C–Cl stretch	alkyl halides
7	762.25	C–Cl stretch	alkyl halides
8	704.22	C–H rock	alkanes
9	620.09	C–Br stretch	alkyl halides

Table 6: FT IR Result of seed extract of *Momordica charantia*

S.no	Frequency range	Bond	Type and group
1	3434.10	O–H stretch, H–bonded	alcohols, phenols
2	2397.35	H–C=O: C–H stretch	aldehydes
3	2104.28	–C(triple bond)C– stretch	alkynes
4	1572.95	C–C stretch (in–ring)	aromatics
5	1400.78	C–H bend	alkanes
6	1126.11	C–N stretch	aliphatic amines
7	826.11	C–Cl stretch	alkyl halides
8	828.64	C–Cl stretch	alkyl halides
9	761.91	C–Cl stretch	alkyl halides
10	697.97	–C(triple bond)C–H: C–H bend	alkynes
11	652.02	C–Br stretch	alkyl halides
12	620.39	C–Br stretch	alkyl halides

Anthelmintic activity

Anthelmintic activity of *Coccinia grandis* and *Momordica charantia* seed extracts were analysed against *Pheretima posthuma* an analogue of human and animal intestinal worms. Seed extracts of *Coccinia grandis* exhibited anthelmlnic activity in dose dependent manner at

10, 50 and 100mg/ml. All the extracts showed the anthelmentic activity. The Chloroform extract demonstrated shortest time of paralysis and death of worms in a less time as compared to other extract at 100 mg/ml . (18 min &20 min respectively). Ethanol and aqueous extracts were also effective at 100 mg/ml (20 &25, 20 &30 min respectively). (Table: 1)

Aqueous extract of *Momordica charantia* was least effective than other two extracts. In this plant ethanol extract showed shortest paralysis time (19 min). Both ethanol and chloroform extracts showed equal death time (30 min). (Table: 2).

Phytochemical Analysis

The preliminary phytochemical analysis of three different extracts of the seeds of *Coccinia grandis* and *Momordica charantia* was carried out. The Chloroform fraction of the seeds contain more phytocompounds when compared to other fractions. Alkaloids, coumarins, flavonoids, phenols and saponins were present in all the three fractions of *Coccinia grandis* . Terpenoids and volatile oils were absent in all the three fractions. (Table:3). Alkaloids, coumarins ,phenols, tannins, quinones and saponins were present in all the three fractions of *Momordica charantia*. Flavonoids available only in Chloroform fraction. Steroids and volatile oils were absent in all the three fractions. (Table:4). The presence of these phytoconstituents were confirmed by suitable chemical tests.

Tannins are polyphenolic compounds. Some synthetic phenolic anthelmintics, e.g. niclosamide, oxyclozanide, bithionol, nitroxynil, etc, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation Martin et al.,(1997). In another study, polyphenols from bryophytes were shown to have anthelmintic activity against *Nippostrongylus*

brasiliensis. The hydroalcoholic and chloroform extracts showed better paralytic and death at 10 mg/ml than the standards . The presence of glycosides and tannins may be the responsible chemical constituents Martin et al., (1997)

Athnasiadou et al., (2001) reported that, indigenous system of medicine has anthelmintic efficacy. However, their scientific evaluation as compared to commercial anthelmintics is limited. Many plants have proven to possess anthelmintic activity *in vitro* and *in vivo*. In their study, tannins were detected in methanol extract. Tannins were found to possess anthelmintic activities and they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death.

The phytochemical constituents of medicinal plants exert a definite physiological action in the human body. The medicinal value of these plants lies in some chemical substances which

are inherent to these plants. The most important bioactive constituents are alkaloids, tannins, flavonoids, and phenolic compounds (Kritikar, and Basu ,1933). Vidyadhar *et al.*,2010 reported that, in particular the ethanol extract exhibited an increased paralytic as well as anthelmintic effect over albendazole at the given experimental concentrations. This may be due to the increased level of extraction of tannins in ethanol and the different degree of helminthiasis of the different extracts are due to the level of tannins present in the fractions.

Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997) or, binds to the glycoprotein on the cuticle of parasite (Thompson and Geary, 1995), and cause death. Coming to the chemistry of nematode surface, it is a collagen rich extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, and is critical for viability, the collagen is a class of proteins that are modified by a range co-and post –translational modification prior to assembly into higher order complexes (or) CMS (Page and Winter 2003). The mammalian skin also consists largely of collagen in the form of fibrous bundles. In leather making industry, vegetable tannins are commonly used in the tanning operation of leather processing that imparts stability to collagen of skin matrix through its reactivity and hence make the collagen molecule aggregate into fibres. This results in the loss of flexibility in the collagen matrix and gain of mechanical property with improved resistance to the thermal (or) microbial/enzymatic attack. Similar kind of reaction is expected to take place between the nematode cuticle (the earth worm) and the tannin of selected medicinal plants of the current study. This form of reactivity brings toughness in the skin and hence the worms become immobile and non-functional leading to paralysis followed by death (Vidyadhar *et al.*,2010). Hence further investigation and proper isolation of the active principles might help in the findings of new lead compounds, which will be effective against various parasitic infections.

Many of these indigenous medicinal plants are used as spices. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes (Okwu, 1999).In the current study, the phytochemical analysis revealed the presence of flavonoids in the selected herbal plants.According to Middleton and McLanghlin, (1992), the flavonoids have long been recognized to possess anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities and also affect some aspects of mammalian metabolism. Flavonoids are also responsible for the protection against free radicals, platelet aggregation, microbes, ulcers and hepatoxins.

FTIR ANALYSIS:

FT-IR analysis of two selected medicinal plants were carried out for the extracts which showed maximum anthelmintic activity to identify the possible biomolecules responsible for antheliminic activity. This spectrum showed lot of absorption bands. (Table: 4 & 5). FT-IR results proved that bioactive compounds such as alkene, alkynes, aromatic amines, alkanes, aliphatic amines , Alkyl halides alcohols, phenols were found in the fractions which could be responsible for anthelmentic activities.

CONCLUSION

From the above preliminary study, we conclude that the ethanolic seed extract of *Coccinia grandis* and *Momordica charantia* was proved to be one of the effective herbal remedies for Helminthic infections. Moreover, it is necessary to isolate and identify the possible active phytoconstituents responsible for the anthelmintic activity and study its pharmacological actions. After sufficient scientific validations, these could be recommended as alternative safe, economic anthelmintic drug candidate.

REFERENCE

1. Ajaiyeoba EO, Onocha P A, Olarenwaju OT. In vitro anthelmintic properties of Buchholzia coriaceae and Gynandropsis gynandra extract. Pharm Biol. 2001; 39: 217-20.
2. Akhtar, M.S., Z. Iqbal, M.N. Khan and M. Lateef, (2000). Anthelmintic activity of medicinal plants with particular reference to their use in animals in Indo–Pakistan subcontinent. Small Rumin. Res., 38: 99–107.
3. Athnasiadou S, Kyriazakis I, Jackson F and Coop RL: Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: In vitro and in vivo studies. Vet. Parasitol.2001; 99:205-219.
4. Coles, G.C., (1997).Nematode control practices and anthelmintic resistance on British sheep farms.Vet.Rec, 141:91-3.
5. Dhar D.N., R.L. Sharma and G.C.Bansal, (1982).Gastrointestinal nematodes in sheep in Kashmir.Vet.parasitol, 11:271-7.
6. Geert, S.and P.Dorny, (1995).Anthelmintic resistance in helminthes of animals of man in the tropics.Bulletin-des-Seances, Academic-Royale-des-Sciencesd.DutreMer, 3:401-23.
7. Harborne, J.B., Baxter, H., Moss, G.P., 1999. In: Phytochemical dictionary: A handbook of bioactive compounds from plants. Tylor and Francis Ltd., London, p. 773.
8. Iqbal, Z., Lateef, M., Ashraf, M., Jabbar, A., (2004). Anthelmintic activity of Artemisia brevifolia in sheep. Journal of ethaopharmacology 93,265-268
9. Kritikar, K.R and B.D Basu (1933). Indian Medicinal plants 2nd E d., Basu, Allahabad, India, pp.231-236.
10. Martin RJ., Mode of action of anthelmintic drugs. Vet J, 154:11-34, (1997).
11. McLanghlin, J. L., Ratanyake, S. Rupprecht, J. K. and, Potter, W. M. (1992). Evaluation of various parts of the pawpaw tree, Asimina triloba (Annonaceae), as commercial source of the pesticidal annonaceous acetogenins. J. Econ. Entomol. 85: 2353-2356.
12. Okwu, D. E, and Okwu, M.E., (2004). Chemical composition of Spondia mombin plants. J. Sustain Agric. Environ. 6: 140-147.
13. Page A.P., winter.A.D (2003).Enzymes involved in the biogensis of the nematode cuticle.53:85-148.
14. Nargund L.V.G.,(1999).Anthelmintic activity of 8-fluoro-9-substituted(1,3)-1,3,4-triazoles on pheretima pothuma,Indian drugs,Vol.36(2),137-139.
15. Thompson, D.P., T.G.Geary .(1995). The structure and function of helminth surfaces in Biochemistry and Molecular Biology of parasites (j.j.marr, ed.), 1st ed. Academic press, New York, pp.203-232
16. Vidyadhar S, M.Saidulu, T.K.Gopal, D. Chamundeeswari, Umamaheswara rao and David Banji. (2010). In vitro anthelmintic activity of the whole plant of enicostemma littorale by using various extracts. International Journal of Applied Biology and Pharmaceutical Technology .I (3): 1119 – 1125.