Research Article

IN VITRO ANTHELMINTIC ACTIVITY OF AERIAL PARTS OF *MIRABILIS JALAPA* LINN

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ABSTRACT

Helminth infections are among the widest spread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health[1]. The gastrointestinal helminthes becomes resistant to currently available anthelmintic drugs. Hence there is an increasing demand towards natural anthelmintics. Hundreds of millions if not billions of human infections by helminthes exist worldwide and increased world travel and immigration from the developing countries[2]. *Mirabilis jalapa* Linn is a perennial herb of family Nyctaginaceae as a long history of traditional use as an anthelmintic. All parts of the plant are a rich source of medicinally useful componentsThe total phenolic content varied from 1.36 ± 0.02 mg / gm of dried extract equivalent to Gallic acid. To our knowledge, this is the first report on the anthelmintic activity of aerial parts using *in vitro* models using Pheretimaposthuma as test worms. Various concentrations (20%, 40%, 60%, 80%) of *M.jalapa* extracts were tested in the assay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Albendazole is the first drug of choice for the treatment of worm infections. It is also first reported anthelmintic which promises to have useful activity against all the types of helminth. The methanolic extracts of *M.jalapa* Linn were more potent as anthelmintic probably because of flavonoids, glycosides and tannins in dose-dependent manner giving shortest time of paralysis and death with 80% w/v concentration. The methanolic extract of *M.jalapa* Linn caused paralysis in 12.6 min and death in 13.5 min. The reference drug albendazole showed the same at 2.3 min and 3.24 min. The results shows that methanolic extract possess vermicidal activity and found to be effective as an anthelmintic.

Keywords: *Mirabilis jalapa*, Pheretima posthuma, Albendazole, Anthelmintic, Phenolic content.

INTRODUCTION

The herbal drugs form the backbone of all traditional systems of medicine around the world due to their endless therapeutic diversity. From the time immemorial, plants have been used in medicinal practice because they fitted the immediate personal needs and are easily accessible and inexpensive. The chemical constituents present in them are a part of the physiological functions of a living flora and is believed to have better compatibility with the human body. Antihelminthics are those agents that expel parasitic worms from the body, by either stunning or killing them. Intestinal infections with worms can more easily treated than those the infections occurs in other locations in the body, because the worms need to be killed by the drug and the drug need not be absorbed when given by oral route. The various crude extracts of *Mirabilis jalapa* Linn of family Nyctaginaceae is widely used in traditional medicine. It is a large herbaceous plant grown in gardens throughout India. This plant is 50-100 cm high.The plant has shown specific antiviral actions. It is also found to posses antispasmodic and antinociceptive properties. In traditional medicine *Mirabilis jalapa* Linn is widely used as antidysenteric, antiparasitic, carminative digestive stimulant, diuretic, purgative tonic, vermifuge, wound healer etc. *Mirabilis jalapa* is rich in many active compounds of which, particular interest to researchers is a group of amino acid-based proteins, called mirabilis antiviral proteins[3, 4]. This plant contains alanine, alpha-amyrins, arabinose, beta amyrins, brassicasterol, beta-sitosterols, campesterol, C-methylabronisoflavone, stigmasterol, tartaric acid, trigonelline, and is used to treat conjunctivitis, edema, fungal infections, inflammation. A number of active compounds were extracted from different organs of *M. jalapa*, including ribosome-inactivating protein (RIP) associated with anti-viral activity, antifungal phenolic compound[5], antimicrobial peptides[6] and rotenoids showing inhibition of HIV-1 reverse transcriptase,[7] further isolation of active components is under progress.

Figure 1: Aerial part of *Mirabilis jalapa*

Collection and Identification of Plant material

The fresh plant of *Mirabilis jalapa Linn* were collected in the months of July-August from the local areas of Kochi and authenticated by the authority of the botany department. A voucher specimen was submitted at Institute's herbarium department for future reference. The aerial parts were washed with water, shade dried powdered in a mechanical grinder and kept in air tight container till use at room temperature.

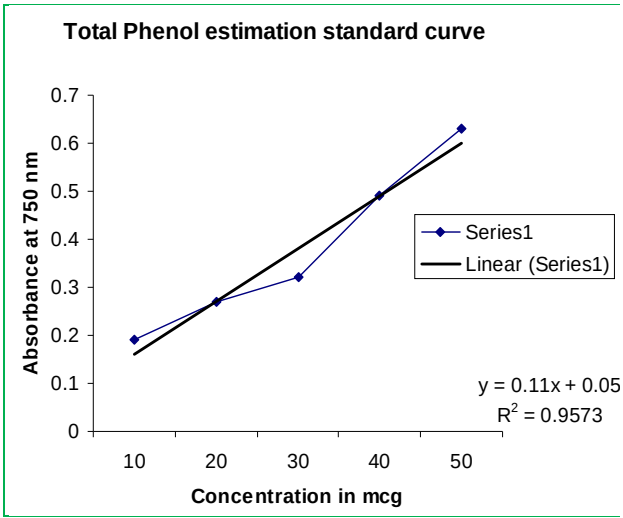
Preparation of the Plant Extract

The extraction of the *Mirabilis jalapa* aerial parts were carried out by known standard procedures. The powder (100 gm) was initially de-fatted with petroleum ether (60-80°C) for 24 h. The extract was filtered through Whatman No.1 filter paper in a Buchner funnel. The filtrate was evaporated to dryness under reduced pressure in a rotatory vacuum evaporator followed by 500ml methanol by Soxhlet extraction method for 72 hrs separately. The extract was dried in a vacuum desiccator to obtained constant weight. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts were obtained by the solvent evaporation and used to determine concentration in mg/ml. The dried sample of each extract was weighed and the yield of soluble constituents was determined. The dried extracts were kept in dark at +4°C until further analyses. The phytochemical screening was carried out as described by Norman. The methanolic extract yield a dark drown residue (2.5 %) respectively. Present work was undertaken to evaluate traditional anthelmintic property of the aerial parts of *Mirabilis jalapa*.

Estimation of total phenolic content

Total phenol content of the extracts was determined by using the Folin-Ciocalteu[8] Method. This test is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation the green–blue complex formed was measured at 750 nm.

Figure 2: Results from the quantitative estimation of total phenolic content



In a test tube, 200 µl of the extract (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu reagent and 800 µl of sodium carbonate. After shaking, it was kept for 2 h for reaction. The absorbance was measured at 750 nm. Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 10-50 µg/ml (figure 2). Using the standard curve the total phenol content of the extract was determined and expressed as gallic acid equivalent in mg/g of the extract. TPC was expressed as mg gallic acid equivalents (GAE) per gram extract. Values presented are the average of three measurements.

MATERIALS AND METHODS

Biological Study

Indian adult earthworms *Pheretima posthuma* collected form moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro*. The earthworms of 8±1 cm in length and 0.3-0.4 cm in width were used for all experimental protocol due to its anatomical and physiological resemblance with intestinal roundworms parasite of human beings intestinal roundworm parasites of human being and also in intestinal roundworms.[9-11]

Screening for phytochemicals:

Qualitative assay of the extracts for the presence of phytoconstituents such as alkaloids, glycosides, tannins, flavanoids, carbohydrates and proteins etc were performed following Standard procedure.[12, 13]

Drugs and Chemicals

The following drugs and chemicals were used. Drugs: Albenzadole (Glaxo Smithkline) Chemicals: Methanol A.R, Petroleum ether A.R (60-80°C). The solvents and other chemicals used during experimental protocol were of analytical grade. Normal saline were purchased from authorized pharmaceuticals.

Anthelmintic activity

Albendazole is a broad-spectrum anthelmintic. Albendazole causes degenerative alterations in the tegument and intestinal cells of the worm by binding to the colchicine-sensitive site of tubulin, thus inhibiting its polymerization or assembly into microtubules. Albendazole also has been shown to inhibit the enzyme fumarate reductase, which is helminth-specific. This action may be considered secondary to the effect on the microtubules due to the decreased absorption of glucose.

Determination of Anthelmintic Activity

Six groups containing four earth worms of nearly size (8±1) cm of the same type were selected and released into 15ml normal saline as a vehicle. Albenzadole in same concentration as that of extract was included as standard reference and normal saline water as control.The plant extract and the standard albendazole were prepared in the concentration 20, 40, 60, 80 % w/v by dissolving them

in a minimum quantity of distilled water and taken in different petridishes. Flaccid paralysis of the worms followed by death occurs.[14] The time taken to complete paralysis and death were recorded. The mean paralysis and lethal time for each concentration was recorded. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors.

RESULTS AND DISCUSSION

Phytochemical analysis of the crude extracts revealed presence of tannins as one of the chemical constituents. Tannins are chemically polyphenolic compounds and were shown to produce anthelmentic activities. Tannins may be formed by condensations of flavan derivatives which have been transported to woody tissues of plants. Alternatively, tannins may be formed by polymerization of quinone units[15]. This group of compounds has received a great deal of attention in recent years, since it was suggested that the consumption of tannin-containing beverages, especially green teas and red wines, can cure or prevent a variety of ills[16]. Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation[17]. It is possible that tannins contained in the extracts of *M.jalapa* Linn produced similar effect[18].Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death. The qualitative chemical evaluation of the extracts of the plant *Mirabilis jalapa* Linn in series of solvents was carried out and revealed the presence of alkaloids, glycosides, tannins, flavonoids, carbohydrates and proteins in detectable amounts. The methanolic extracts of roots displayed significant anthelmentic properties at higher concentration.

Table 1: Results from the anthelmintic activity of methanolic extracts (by soxhlet method) of *Mirabilis jalapa* Linn

Crude extract/ Standard	Concentration (% w/v)	Time taken (min)	
		Paralysis	Death
Methanol extract	20	26.2±0.43^{h}	31.8±1.53^{h}
	40	21.3± 0.57^{g}	24.7±0.28^{g}
	60	14.4± 0.40^{f}	16.8±0.92^{f}
	80	12.6±0.63^{e}	13.5±0.42^{e}
Albendazole	20	6.1±0.42^{d}	7.3±0.75^{d}
	40	4.2±0.37^{c}	5.53±0.34^{c}
	60	3.28±0.22^{b}	4.21±0.27^{b}
	80	2.3±0.38^{a}	3.24±0.52^{a}

Values are expressed as mean ± S.D for 6 worms in each group. Values not sharing a common superscript a, b, c, d, e, f, g, h differ significantly at P < 0.05. Unit= mins. Control worms were alive up to 24 hrs of observation.

The extract showed anthelmentic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 80% w/v concentrations. The extract caused paralysis in 12.6 min and death in 13.5 min, the reference drug albendazole showed the same at 2.3 min and 3.24 mins. The methanolic extracts of roots of *M.jalapa* Linn displayed significant anthelmintic properties at higher concentrations. The extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 80%w/v concentration. The methanolic extract of *M.jalapa* Linn caused paralysis in 12.6 min and death in 13.5 min. The reference drug albendazole showed the same at 2.3 min and 3.24 min.

CONCLUSION

The result of anthelmintic activity on earthworm phertima prosthuma was given in table 1 and figure 3, reveal that, the different concentration used for methanolic extracts shown has paralysis and death of earthworms and it was compared in the same concentration with albendazole as reference drug. The data presented here indicates that the extracts of *Mirabilis jalapa* Linn showed significant anti bacterial effects and the results support the traditional use of the plant. The traditional claim of *Mirabilis jalapa* Linn as an anthelmentic have been confirmed as the methanolic extract displayed activity against the worms used in the study. Further studies to isolate and reveal the active compound contained in the crude extracts of *M. jalapa* Linn and to establish the mechanism of action are required. The anthelmintic activity was determined for the methanolic extract which displayed significant anthelmentic properties at higher concentration. The extract showed anthelmentic activity in dose dependent manner which is effective against parasitic infections of humans.

Figure 3: Showing the anthelmintic activity of methanolic extract of *Mirabilis jalapa*

It can be concluded that active constituents responsible for anthelmintic activity are present in the methanolic extract of *M.jalapa*. The plant can be further explored for its phytochemical profile to identify the active constituents responsible for the anthelmintic activity.

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