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Original article

Anthelmintic potentials of *Andrographis paniculata*: A preliminary study

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ABSTRACT

Andrographis paniculata (Acanthaceae), a potential medicinal plant, distributed in waste lands, open forests in India. *A. paniculata* is used in the treatment of typhoid, snake bite, scorpion sting, malaria and cancer. The acetone, chloroform and aqueous extracts were evaluated for its anthelmintic activity against adult earth worms (*Phertima prosthuma*). Three concentrations (10, 50 and 100 mg/ml) of each extract were which involved the determination of time of paralysis and time of death of the test worms. It was found that the acetone and aqueous fraction of *Andrographis paniculata* of was proved to be one of the best alternative cum effective herbal remedy to control helminthic infections.

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1. Introduction

Helminthes infections, entitled as helminthiasis are the most pervasive infection and a foremost degenerative disease distressing a large proportion of world's population and they pose a large threat to public health (Bundy, 1994). It is also a major problem to livestock production throughout the tropics. The World Health Organization estimates that a staggering two billion people harbor parasitic worm infections. Parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact (Ashok Kumar, 2010). The helminth parasites mainly subsist in human body in intestinal tract, when they increase in number they migrate towards other parts of the body (Tripathi, 2003). Majority of helminthes infections are generally restricted to tropical regions (Thompson et al., 1995). Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in

nature. Human with parasitic worms in their bodies can experience a variety of symptoms, including intestinal discomfort, weight loss abdominal bloating, and signs of malnutrition such as hair loss, anemia, eosinophilia and pneumonia. According to the WHO, only a few drugs are used in treatment of helminthes in humans. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. Anthelmintic drugs which are chemical compounds that 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 (Coles, 1997; Geert and Dorny, 1995) and drastic side effects caused to the host (Tagbota and Townson, 2001; Sondhi et al., 1994). Hence, alternative, cost effective, anthelmintics which should not cause any or less side effects and should not develop resistance in helminthes must be identified from natural sources to treat these parasite infections. 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. 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. A traditional medicinal plant *Andrographis paniculata* commonly known as "king of bitter" belonging to family Acanthacea. It is an important annual medicinal herb widely distributed in Madhya Pradesh, India. It is one of the most important herb in Pharmacopoeia and widely used plant in Ayurvedic formulations. It is used to overcome sannipata type of fever, difficulty in breathing, hemopathy burning sensation, cough, skin diseases, fever, ulcer and worms. It is also useful in acidity and liver complaints (Aiyer and Kolammal, 1962). The whole plant has variety of therapeutic values. It has immunosuppressive properties and is useful in treatment of wounds, ulcers leprosy, skin infections, diarrhea, sore throat, common cold and hypertension Panchang (stem, leaves, flowers, root and seeds) of the plant is being used in various formulation of Indian system of medicine for the treatment off ever, malaria and sore throat. It has also been used traditionally for sluggish liver as antidote in case of colic dysentery and dyspepsia. Having all these key points in mind, in the current study an attempt has been made to study the anthelmintic activity of traditional medicinal plant *Andrographis paniculata*.

2. Materials and methods

2.1. Collection and authentication of plant materials

The leaves of *Andrographis paniculata* were collected from the local field of Lalgudi, Tiruchirappalli, 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.

2.2. Preparation of extracts

The collected leaves of *Andrographis paniculata* were surface sterilized with sterile distilled water to remove the dirt and soil particles adhered on them. Seeds were shade dried at room temperature (32°C) for two weeks. The shade dried leaves were pulverized into a coarse powder and stored at room temperature for further use.

2.3. Extraction of plant material

Coarse plant powder was first defatted with acetone and then extracted with different solvents alcohol and aqueous extracts of *Andrographis paniculata* leaves were obtained by standard hot and cold extraction methods. (Harborne et al., 1999).

2.4. Aqueous extraction

50 g of coarsely powdered *Andrographis paniculata* leaves 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 420 µm 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°C and utilized throughout the studies.

2.5. Acetone extraction

100 g of *Andrographis paniculata* leaves were mixed with 300 ml of acetone, 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 acetone under reduced pressure and controlled temperature (40-50°C). The fraction was dried into powder form and placed in air tight containers, stored at 4°C and utilized throughout the studies (Harborne et al., 1999).

2.6. Chloroform extraction

100 g of *Andrographis paniculata* leaves were mixed with 300 ml of chloroform, 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 chloroform fraction was dried into powder form and placed in air tight containers, stored at 4°C and utilized throughout the studies (Harborne et al., 1999).

2.7. Assay of anthelmintic activity

2.7.1. Experimental animals

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. Moreover, they belong to same group of Annelida (Mueller, 1774). 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.

2.7.2. Drugs

The solvents and other chemicals of analytical grade were used during experimental protocol. All the test and standard drug solutions were prepared freshly before starting the experiments.

2.7.3. Screening of anthelmintic activity

The anthelmintic assay of the selected plant extracts were carried out as per the method of Ajaiyeoba et al. (2001). 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, 50 and 100 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. 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 triplicates and analyzed statistically.

2.7.4. 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).

2.7.5. Thin layer chromatography

Thin layer chromatography technique was performed based on the method prescribed by 3333333333333333 to separate the different compounds present in the fraction which showed the maximum anthelmintic activity.

2.7.6. Fourier-transform spectroscopy

2.7.6.1. FTIR analysis

FT-IR analysis of selected medicinal plant was carried out for the extracts which showed maximum anthelmintic activity to identify the possible biomolecules responsible for anthelminic activity.

3. Results and discussion

3.1. Anthelmintic activity

Infectious diseases including helminthes infection are the leading threat across the world. As a global concern the antibiotic and anthelmintic resistance by pathogens and helminthes has emerged. Many of the anthelmintics and antibiotics have been out of use as drug resistance has been developed by the pathogens and worms. Natural products, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial and anthelmintic compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases. Therefore, much attention has been given to folk medicine in order to look for new leads to develop better drugs to treat resistant bacteria Rojas et al. (2003).

The anthelmintic activity of aqueous, acetone and chloroform fractions of *Andrographis paniculata* was assayed in *in vitro* condition and the results are noted. Dose dependant activity was observed in all the fractions giving shortest time of paralysis and death at 100 mg/ml concentration. As far as Paralysis Time (PT) was concerned, it was low in the case of both aqueous and acetone (80 min) followed by chloroform (90 min) at 100 mg/ml concentration. Death time (DT) was low in acetone fraction (83 min) followed by aqueous (93 min). Chloroform fraction was showing least activity against the worm (Table 1).

Albendazole by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. All the extracts demonstrated paralysis as well as death of worms at a time comparable to Albendazole especially at higher concentration of 100 mg/ml.

Table 1
Anthelmintic activity of leaves of *Andrographis paniculata*.

Treatment	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Cold water extract	10±0.86	80±0.66	93±0.88
	50±0.22	93±0.54	96±0.92
	100±0.30	97±0.72	96±0.80
Acetone extract	10±0.28	80±0.12	83±0.64
	50±0.46	95±0.86	97±0.38
	100±0.18	97±1.00	99±0.48
Chloroform extract	10±0.86	90±0.56	92±0.22
	50±0.16	115±0.38	116±0.92
	100±0.42	116±0.26	150±0.86

3.2. Phytochemical analysis

Preliminary phytochemical screening of aqueous, acetone and chloroform fractions of leaf extracts of *Andrographis paniculata* revealed the presence of alkaloids, tannins and coumarins. Steroids, volatile acids and quinines were absent in all the three fractions. Alkaloids, terpenoids, flavonoids, steroids and polyphenolic compounds were present only in aqueous and acetone fractions and absent in chloroform fraction (Table 2). The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1952). According to Middleton and McLanghlin (1992), the flavonoids have long been recognized to possess antiallergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities as well as to affect some aspects of mammalian metabolism. Farwuar (1996) included protection against free radicals, platelet aggregation, microbes, ulcers and hepatoxins. The phytochemical analysis revealed the presence of flavonoids in the herbal plants.

Alkaloids and polyphenolic compounds are the major chemical constituents present in the active fractions. Polyphenolic compounds have shown anthelmintic activity (Smith et al., 1964). Some synthetic phenolic anthelmintics e.g. niclosamide, oxiclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin et al., 1997). Another possible antihelmintic

effect of tannins is that they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death (Mali et al., 2008; Athnasiadou et al., 2001).

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). It is possible that, tannins contained in the extract of *Lantana camara* produced similar effects. In another study, polyphenols from bryophytes were shown to have anthelmintic activity against *Nippostrongylus brasiliensis*. Several authors have reported that an increase in the supply of digestible protein does improve the resilience and resistance of sheep to gastrointestinal nematodes (Van Houtert et al., 1996).

Table 2
Preliminary phytochemical screening of *Andrographis paniculata*.

S.No	Test	Water	Acetone	Chloroform
1	Alkaloids	+	+	+
2	Terpenoids	+	+	-
3	Steroids	-	-	-
4	Coumarins	+	+	+
5	Tannins	+	+	+
6	Flavonoids	+	+	-
7	Phenols	+	-	+
8	Volatile oils	-	-	-
9	Quinone	-	-	-
10	Saponin	+	+	-

3.3. FTIR analysis

FTIR assay was carried out for the acetone fraction of *Andrographis paniculata* to identify the possible biomolecules responsible for anthelmintic activity. This spectrum showed different absorption bands indicated the presence of active functional groups. The intensity peaks are slightly increased for the period of peak like 3924, 3790, 2256, 1730 and 1645 cm^{-1} as well as some intensity peaks decreased like 1242, 1, 437 cm^{-1} . Fig. 1 showed the band at 3924 correspond to alcohols, phenols. The peak at 3790 indicate to C=C stretching vibrations to aromatics. The peak at 3404 represents to N-H stretch primary, secondary amines, amides. The peak at 595 corresponds to C-Cl, C-Br stretching vibrations to alkyl halides. The band at 2080 corresponds to C-N stretching vibration. The weak band at 437 indicates C-Br stretch alkyl halides vibrations and it corresponds to the presence of alcohols, carboxylic acids, ethers, esters and aliphatic amines in the plant extract (Table 3). The compounds responsible for the active functional groups may exhibit anthelmintic activity (Fig. 1). Alkene, alkynes, aromatic amines, alkanes, aliphatic amines, Alkyl halides alcohols, phenols were also found in the fractions which could be responsible for anthelmintic activities.

Table 3
FTIR result for leaves extract of *Andrographis paniculata*.

S.No	Frequency range	Bond	Type and group
1	3924	O-H stretch, free hydroxyl	Alcohols, phenols
2	3790	O-H stretch, H-bonded	Alcohols, phenols
3	3404	N-H stretch	Primary, secondary amines, amides
4	2901	C-H stretch	Alkanes
5	2975	C-H bend	Alkanes
6	2256	H-C=O: C-H stretch	Aldehydes
7	1730	C-Cl stretch	Alkyl halides
8	1945	-C(triple bond)C- stretch	Alkynes
9	2245	-C(triple bond)C- stretch	Alkynes
10	1242	C-N stretch	Aliphatic amines
11	881	C-H "loop"	Aromatics
12	438	C-Br stretch	Alkyl halides

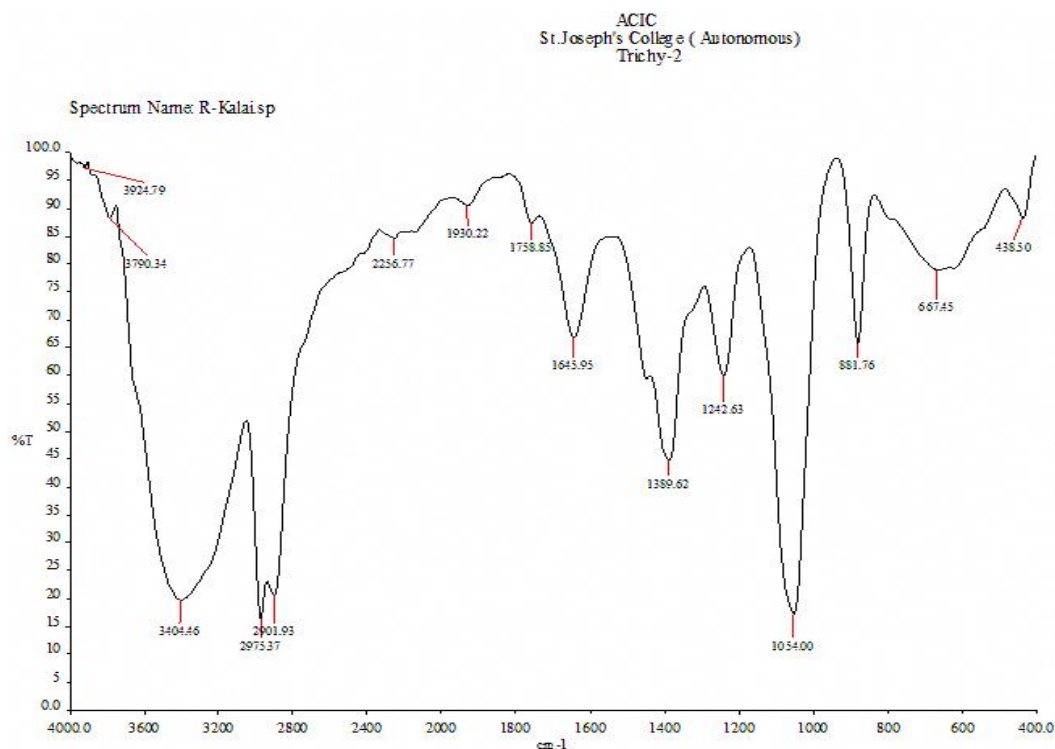


Fig. 1. FTIR result for leaves extract of *Andrographis paniculata*.

4. Conclusion

From the above preliminary study, it may be concluded that, the acetone and aqueous fraction of *Andrographis paniculata* was proved to be one of the best alternative cum effective herbal remedy to control helminthic infections. Preliminary studies must be strengthened by further investigations to isolate and identify the possible active phytoconstituents responsible for the anthelmintic activity. After sufficient scientific validations, *Andrographis paniculata* leaf component could be recommended as alternative safe, economic anthelmintic drug candidate.

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