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Anthelmintic activity of *abutilon indicum* leaf extract on sheep tapeworm *Moniezia expansa* *In vitro*

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Abstract

Abutilon indicum methanol leaf extract was tested against sheep tapeworm in *in vitro* condition with different doses of 25, 50 and 100 mg/ml concentration. Dose dependant cestodicidal activity of the extract was observed in the study with 100 mg/ml concentration and the time taken for the paralysis of the organism amounts to 66.3 ± 0.03 and death was recorded after 93.2 ± 0.09 minutes. No death was recorded even after 8 hours in the negative control (1%DMSO). The positive control was maintained with 10 mg/ml of Albendazole where the paralytic time and death time was recorded as 72.1 ± 0.13 and 93.5 ± 0.11 minutes. Biochemical analysis reveal the decreasing trend in the level of protein in different regions of the tapeworm, However, the changes observed in the level of carbohydrate and lipids were not significant. Light microscopic observation reveals the presence of sub-tegumental layer with circular and longitudinal muscles, presence of paranchymal cell along with glandular cells is also evident. Disintegration of sub-tegumental layer along with necrosis of paranchymal cells and glandular cells was observed in treated groups. GC-MS analysis of the leaf extract shows the presence of potential anthelmintic compounds eugenol and asarone. Separation and purification of potential anti-cestodal compounds will be a better natural alternative to control helminthic infections.

Keywords: anthelmintic, *Abutilon indicum*, eugenol, Asarone, GC-MS, *Moniezia expansa*, light microscopy

1. Introduction

Gastrointestinal helminthiasis causes productive loss to stock breeders around the world, in addition to huge economic loss to the farmers especially in developing countries it also causes a reduction in the normal growth rate, lowers the quality of meat, milk production and wool productivity [1-2]. The sheep tapeworm *Moniezia expansa* is an extremely common infectious parasite in ruminants. The damage caused by *Monieziasis* is much lesser when compared to nematode infections, but it is a major concern among livestock industries on the basis of economic loss incurred due to the infection [3]. The pathogenicity caused by the infection of *M. expansa* shows contrasts variations between young and adult animals due to which the pathological effect of the host has not been established [4].

In temperate regions, synthetic anthelmintics play a vital role in suppressing the helminth infection. Niclosamide, Praziquantel [5], Albendazole and Oxfendazole are the common anthelmintic drugs used for eliminating *M. expansa* in livestock [6]. The routine use of the synthetic anthelmintic drugs may cause side effects on host animal and it is also toxic to the environment [7]. Resistance to synthetic anthelmintics treated against the parasite was also reported [8-9]. The resistance developed in the parasite may be due to the detoxification as well as the excretion of the synthetic anthelmintic drugs by host animal and parasite [10-12]. The plant mediated drugs may be a potential alternative to synthetic anthelmintic drugs. The resistance against the synthetic anthelmintic results in the search of alternative natural resources to overcome the drug resistance.

Abutilon indicum belonging to the family Malvaceae is commonly known as "Thuthi" in Tamil [13] and is a native shrub of the tropical and subtropical region. The plant has been used by Siddha system of medicine to cure jaundice, piles, ulcer and leprosy [14]. The bioactive compounds is promising in the treatment of analgesic [15] hepatoprotective [16] anthelmintic [17] anti-inflammatory [18] and hypoglycemic activity [19]. Alantolactone and isoalantolactone sesquiterpene lactone were identified in *A. indicum*. The objective of the present investigation is to test the efficacy of methanol leaf extract of *Abutilon indicum* for its anthelmintic potential with reference to biochemical and light microscopic studies.

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2. Materials and Methods

2.1 Plant collection and extraction

Fresh disease free leaves of *Abutilon indicum* were collected from the Presidency college campus and identified with the help of the PG Research Department of Plant Biology and Biotechnology, Presidency College, Chennai, India. The leaves were washed with deionized water and cut into smaller pieces and air dried in a shady place. The dried leaves were grounded into fine powder using the blender mixture. The powdered material was stored in air tight containers at room temperature. 25 grams of fine powder were loaded on the column of soxhlet apparatus and the extract was collected using methanol as solvent. The collected extracts were allowed to dry by keeping them in a flask in a water bath at 50°C. The condensed extracts were finally vacuum evaporated and refrigerated until further use.

2.2 Phytochemical Analysis

The extract was analyzed for the presence or absence of secondary metabolites such as alkaloid, flavonoid, tannin, phenol, diterpenoids, steroids, terpenoid, cardiac glycoside, protein and carbohydrates [20].

2.3 GC-MS Analysis

Methanol leaf extract of *Abutilon indicum* was subjected to GC-MS analysis using Agilent 7890A Gas Chromatograph, 240 Mass Spectrum Detector (MSD). Chromatography was performed on a HP-5 MS capillary column (30m×0.32mm, 0.25 µm film thickness; 5% diphenyl cross-linked 95% dimethylpolysiloxane).

2.4 Collection and identification of worms

Live parasite of *Moniezia expansa* (Cestode) was collected from freshly slaughtered sheep intestine in the Corporation slaughter house, Perambur, Chennai, India. Collected worms were maintained in 0.9 % phosphate buffered saline (PBS, pH 7.3) and identified with help of Department of Veterinary Parasitology, Madras Veterinary College, Chennai.

2.5 Anthelmintic assay

In vitro anthelmintic studies was carried out with *Moniezia expansa* collected from naturally infected sheep. The tapeworm were introduced into the testing medium containing 25, 50 and 100 mg/ml crude methanolic leaf extract to analyze the anthelmintic activity following method of Tandon *et al.*, [21]. The adult mortality assay was followed the worms were sampled into 6 worms in each plate. The following methodology was carried out into the present investigation

1. Albendazole at 10 mg/ml (Positive control)
2. Crude methanol extract (25, 50 and 100 mg/ml)
3. 1% DMSO Negative control

The animals were monitored continuously to observe the change in their motility. The inhibition of worm motility was considered as rational for the anthelmintic activity. The time taken for the paralytic as well as complete inactivity or mortality of the parasite was observed. The experiment was carried out in triplicates to confirm the anthelmintic activity.

2.6 Estimation of metabolites

To determine the changes in the biochemical parameters. The animal was divided into groups containing young, mature and gravid Carbohydrate [22], Protein [23] and Lipid [24] were estimated as per the standard procedure.

2.7 Light microscopic studies

Parts of the mature regions of the worm were fixed at 10% neutral buffered formalin and then embedded in paraffin. Sections of 4 µm thickness were taken and stained with Haematoxylin and Eosin [25].

2.8 Statistical analysis

The obtained results were statistically analyzed using mean, standard deviation, standard error of the mean (SEM)

3. Results

In vitro anthelmintic activity of *Abutilon indicum* was observed in the present investigation. The presence of bioactive compounds such as Alkaloids, Flavonoids, Tannins, Phenol, Terpenoids, Diterpenoids, Steroids and Cardiac glycosides in the methanolic leaf extract are given in (table 1). GC-MS analysis of methanol leaf extract of *A. indicum* (table 2) shows the presence of 34 bioactive compounds with various biological significance, of which the main compounds such as Benzene, 1-2 Dimethoxy-4-(2-Propenyl) (2.32%), Benzene-1, methyl-4-1,2,2 trimethylcyclo (0.873%), Asarone (2.262), 4-(1E)-3-Hydroxy-1-propenyl (1.91%), hexadeconic acid (0.163), Campesterol (11.195%), Alpha-Tocopheroquinone (1.6%), Astaxanthin (0.25%).

The methanolic extract was tested against *Moniezia expansa* sheep tapeworm. The time taken for the paralysis of the parasites at various concentrations of the leaf extract is represented in table III. There is dose dependent activity in both paralytic as well as in the time taken for the complete inactivation. The parasites at 100 mg/ml concentration of methanolic extract reveals the paralysis of the organism after 66.3 ± 0.03 minutes and the death was recorded after 93.2 ± 0.09 minutes. No death was recorded even after 8 hours introduction of parasites into the media consisting of 1% DMSO. The positive control was maintained with 10 mg/ml concentration of Albendazole where the paralytic time recorded was 72.1 ± 0.13 and 93.5 ± 0.11 minutes for the death of the organism. In comparing the results obtained with 100 mg/ml concentration of crude extract it was found to be similar to that of the positive control.

Moreover, in order to evaluate the biochemical changes in the tested animals, carbohydrate, protein and lipids were estimated with different concentrations. The results (table 4) revealed that the amount of carbohydrate decreased in all the tested regions namely, young, mature and gravid proglottids of tapeworm. A decrease in the total protein content was observed in the present study. In control *M. expansa* total protein amounts to 11.43 mg/100 mg of wet tissue, 9.8 mg/100 mg of wet tissue and 14.78 mg/100 mg of wet tissue with respect to young, mature and gravid proglottids respectively, while in experimental group shows a slight decrease in the amount of total protein. However, the changes were not significant and the same trends were observed with lipid.

Histological studies were carried out to analyze the normal architecture of the tapeworm. A thick tegument was observed within the body wall covered by microtrichies. The subtegument layer contains circular and longitudinal muscles. Presence of paranchymal cell along the glandular cells were also evident. Damages are witnessed in the outer tegumental layer along with necrosis of sub-tegument layer. Vacuulations were observed in paranchymal cells. The glandular and secretory cells showed morphological disintegrations. (Figure 2)

4. Discussion

Moniezia expansa is the tapeworm infecting sheep and ruminants. *Moniezia expansa* biotransforms various xenobiotics such as benzimidazole in *in vitro* conditions [26]. The major reason for the development of resistance against the anthelmintic drugs is due to the extraction of the drug by the host and parasites [27]. In spite of the resistance to drugs developed mainly in the helminth parasites synthetic chemotherapy was commonly used till date. Application of ethano-veterinary medication to control the livestock disease is gaining importance [28]. The present study has been designed to evaluate the anthelmintic potential of the methanol leaf extract of *A. indicum* on *Moniezia expansa*. The observation of results suggests the dose dependent anthelmintic potential of *Abutilon indicum*. The helminthocidal activity of plant extracts was due to the presence of active principal bioactive compounds, namely alkaloids, phenolic acid, tannins and saponins etc. The present study is in line with the above findings due to the presence of alkaloid, flavonoids, and phenolic compounds, present in the methanolic extract which are responsible for the anthelmintic activity of *Abutilon indicum*.

In view to study the metabolic changes in the parasites *in vitro* condition the amount of carbohydrate, protein and lipid were estimated in different regions of the tapeworm. The biochemical assay was performed on homogenates. A decreasing trend of total carbohydrate, protein levels was observed in the extract treated group of parasites. However, no significant alteration was observed in the total lipid content of the organism. By active mediated transport the cestodes were capable of taking glucose from the carbohydrates present in the host diet nutrition [29]. Moreover, it is evident from the present study the metabolic activity of the parasite was influenced by the extract and the organism utilizes endogenous carbohydrate resulted in the decrease. Structural protein are the key component of the cell, *in vitro* treatment with the methanol extract reveals no marked changes in the level of proteins.

The tegument of the helminth parasites plays a major role in the uptake of amino acid from the surroundings [30]. The total protein content of the homogenates of the young, mature and gravid proglottids shows very meagre alteration in the amount of protein. However, the light microscopic observation reveals the disintegration of tegumental layer along with the damages in sub-tegumental and muscle fiber of the parasites. Asarone, a compound with antiparasitic nature interfere with the absorption of protein and lipid in the parasite and lead to the damage of the intracellular membrane system and cell lysis [31]. The bioactive compound present in the methanol extract might have interacted with the same mechanism by causing damage in the cellular architecture of the parasite. Similar results were observed on *Moniezia expansa* with different extract of various plant [32, 33, 34]. The phenolic compounds break energy metabolism and interfere with the cell surface glycoproteins resulting in the death of the parasite [35]. It is evident from the present study the phenolic compounds such as tannins and flavonoids are present.

The alkaloids are responsible for the cause of paralysis in the parasites [36]. Hence, the phenolic compound and alkaloids present in the methanol leaf extract of the *Abutilon indicum* which is responsible for the paralysis and death of the parasites in *in vitro* condition. The plant extracts were proven to possess anthelmintic compounds [37, 38, 39]. It is evident from the present study that the *Abutilon indicum* methanolic leaf extract possesses a potential anthelmintic bioactive

compounds such as flavonoid, alkaloid, tannins and phenols. Further analysis and purification to identify the anthelmintic compounds present in the plant extract GC-MS analysis was performed and the results suggest the presence of the anthelmintic compounds Benzene, 1-2, dimethoxy-4-(2-Propenyl) (eugenol) and Asarone. Hence the purification and separation of Eugenol and Asarone may throw light on the identification of navel anthelmintic compounds.

Table 1: Phytochemical analysis of methanol leaf extract of *Abutilon indicum*

Phytoconstituents	Result
Alkaloids	++
Flavonoids	+++
Tannins	+
Phenol	++
Terpenoids	++
Diterpenoids	+
Steroids	+
Cardiac glycosides	+
Protein	-
Carbohydrate	-

+++ Strong Positive ++ Mild Positive + Positive – Not Present

Table 2: GCMS analysis of methanol leaf extract of *Abutilon indicum*

S. No	Compounds	RT	Area %
1	Benzene, 1-2 Dimethoxy-4-(2-Propenyl)	15.3	2.32
2	Benzene-1, methyl-4-1,2,2 trimethylcyclo	17.77	0.873
3	Asarone	21.61	2.262
4	4-(1E)-3-Hydroxy-1-propenyl	25.05	1.91
5	hexadectonic acid	29.62	0.163
6	Campesterol	55.34	11.95
7	Alpha-Tocopheroquinone	53.43	1.16
8	Astaxanthin	55.74	0.25

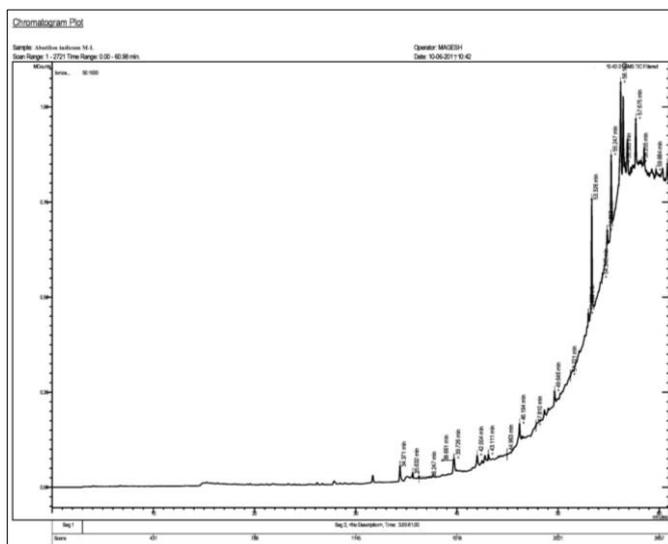


Fig 1: GCMS Chromatogram of *Abutilon indicum* leaf methanol extract

Table 3: Anthelmintic Activity of methanol extract of *Abutilon indicum* against *Moniezia expansa*

Concentration	Paralysis Time	Death Time
100mg	66.3±0.03	93.2±0.09
50mg	75.4±0.08	102.3±0.03
25mg	88.3±0.15	128.3±0.18
Albendazole	72.1±0.13	93.5±0.11
Control	-	No death after 8 hrs

Table 4: Biochemical analysis of control and extract treated *M.expansa*

Metabolites	Control (mg/100mg of wet tissue)			Experimental (mg/100mg of wet tissue)		
	Young proglottid	Mature proglottid	Gravid proglottid	Young proglottid	Mature proglottid	Gravid proglottid
Carbohydrate	11.23±0.34	14.48±0.25	12.33±0.09	9.53±0.03	11.22±0.31	11.22±0.311
Protein	11.43±0.12	9.8±0.09	14.78±0.30	10.3±0.08	9.36±0.03	14.28±0.08
Lipid	6.58±0.80	6.63±0.08	6.54±0.067	6.50±0.15	6.5±0.15	6.5±0.03

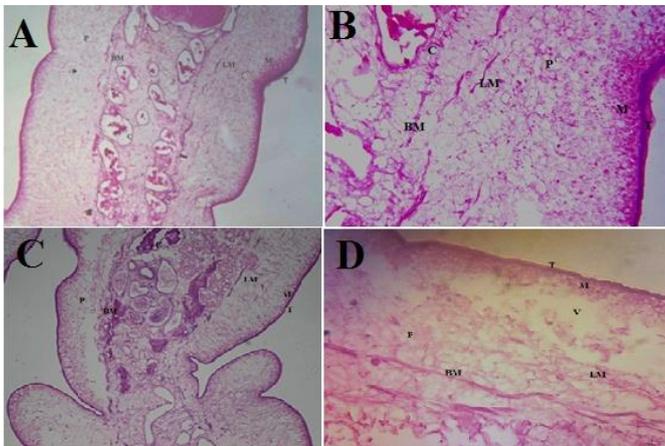


Fig 2: Photomicrograph of *Moniezia expansa* treated with *Abutilon indicum* methanol leaf extract. (A) Control 4X (B) Control 10X (C) Treated 4X (D) Treated 10X P: Parenchyma cells BM: Basal Membrane LM: Longitudinal muscle M: Microtriches T: Tegument V: Vacuolation

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