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Anthelmintic and Analgesic Activities of *Trachyspermum Khasianum* H. Wolff

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Objectives: *Trachyspermum khasianum* H. Wolff is a rare medicinal plant characteristically used by the traditional healers in traditional medicine for the treatment of throat-pain, toothache, and stomach ache. The study was designed to determine the anthelmintic and analgesic properties of the aerial parts of *Trachyspermum khasianum* H. Wolff (Family: Apiaceae). The aqueous and ethanol extract of *T. khasianum* H. Wolff was prepared and subjected for evaluation to determine the possible therapeutic effects.
Methods: Anthelmintic activities of the extracts were determined by observing the time taken to paralyze and the time taken for the death of earthworms (*Eisenia foetida*) as compared to the standard drug–Albendazole (20 mg/ml) and control. Analgesic potential of the extracts was evaluated using Eddy's hot plate method to understand the analgesic activity in rats (Wistar rats) at 100 mg/kg and 200 mg/kg body weight doses and compared with the standard reference (Diclofenac sodium: 10 mg/kg of animals).
Results: The extracts showed a significant dose-dependent anthelmintic effect at the different concentrations (10, 20, and 40) mg/ml, compared to that of the standard drug (20 mg/ml). Also, the results suggested that the plant extracts possess significantly analgesic activity in rats.
Conclusion: The studies indicate that *Trachyspermum khasianum* shows anthelmintic and potent analgesic activities. Further research should be carried out to identify the specific phytoconstituents responsible for both analgesic and anthelmintic activities and its possible mechanism of action.

Keywords: albendazole, analgesic, anthelmintic, diclofenac sodium, trachyspermum khasianum

INTRODUCTION

Traditional systems of medicine are one of the widely practiced systems of medicine in the north-eastern part of India. Since ancient times plants are believed to have miraculous healing properties and playing a vital role in the management of different disease conditions. Treatment strategies exploring the medicinal properties of plants are most popular in developing countries like India, due to some of the unique features include abundant availability, low cost, effective acceptability, better compatibility, and fewer side effects. The traditional healers of the North-eastern states of India have successfully treated several aliments using different ethnomedicinal plants. *Trachyspermum khasianum* H. Wolff is a plant of Northeast, India is considered for this study. *Trachyspermum khasianum* H. Wolff, locally known as "Jatira Skei" is a rare medicinal plant of Meghalaya, belonging to the family Apiaceae. As per C.B. Clarke et al., the Trachyspermum comes from Greek words as *Trachy* mean rough and *spermum* mean seeded. *T. khasianum* H. Wolff is grown in the terrestrial region with sandy soil. It is a biennial or perhaps perennial herb 30-60 cm tall. This plant is believed to have miraculous medicinal properties. The traditional healers of Meghalaya use the aerial parts along with *Zingiber officianalis* (Zingiberaceae) for the treatment in the traditional medicine of toothache, throat pain, and stomachache. Taking into consideration the above-mentioned facts, the

present research has been planned to determine or validate the medicinal values of the plant as well as to investigate the anthelmintic effect and analgesics properties of *Trachyspermum khasianum* H. Wolff by using a standard laboratory procedure [1, 2].

The medicinal plants or medicinal herbs have been discovered and used in traditional medicine practices since prehistoric times which play a crucial role in improving and maintaining human health [3]. According to the World Health Organisation, traditional medicines are used by approximately 80% of the world population. Traditional medicines have been used in medicinal preparations for the treatment of various human and animal diseases [4]. Thus, human beings have been using herbs, organic materials as well as materials from the marines for their benefits in term of food and medicines. Among the substances having medicinal value, herbs have been extensively used for the therapy due to their easily available [5]. Therefore, the medicinal properties of plants are due to some chemical substances or constituents that produce a specific physiological action on the human body which is called phytochemicals. Therefore, it is assumed that these phytoconstituents may be responsible for the activities [6]. Most of the fundamental concepts of their medicinal systems are still unexplainable by using the modern technique [7].

Gastrointestinal nematodes in ruminants are the main causes of animal diseases in temperate and tropical areas [8]. Worm or parasitic infections are among the most widespread infections in humans, affecting a large proportion of the world's population. In some developing countries and less developed countries, they pose a major challenge to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia, and pneumonia [9]. Helminthiasis is a disease in which a part of the body is infested with different species of these parasites, which are broadly classified into tapeworms, flukes, and roundworms [10]. Helminths are worm-like organisms that live and absorb nourishment from the living host and disturb the hosts' nutrient absorption mechanism [11]. Albendazole, Mebendazole, etc. are among the group of anthelmintic drugs that expel parasitic worms (helminths) and other internal parasites from the body by either stunning or killing them without causing significant damage to the host [12]. Anthelmintic from the plant sources may play a crucial role in the treatment of these parasite infections [13].

Pain is an unpleasant sensation but a protective mechanism occurs in the human body [14]. Analgesics are the drugs that selectively relieve pain sensation by acting in the CNS or on peripheral pain mechanisms, by increasing the pain threshold to external stimuli without altering consciousness. Analgesics are divided into two groups such as opioid analgesic and non-opioid analgesic [15]. Most of the currently available analgesics drugs such as NSAIDs and opioid analgesics are probably having their side effects such as gastric erosions, leg swelling, constipation, and so on, as well as tolerance and dependence, so the use of these drugs have not been a successful one. Therefore, analgesic drugs lacking those side effects, researches have been searched for alternatives NSAIDs and opiates analgesics drugs worldwide [16]. Thus, re-evaluate analgesics drugs having lower side effects possibly to those of herbal sources is an important need [17]. Hence, the current study was to validate the folklore claims for the analgesic activities of this plant scientifically.

MATERIALS AND METHODS

1. Materials

All Chemicals and reagents were obtained from the Hi-Media laboratory, Mumbai, and solvents were procured from SD Fine Chemical, Mumbai. Diclofenac Sodium was procured from IPCA laboratories, Mumbai, and Albendazole suspension was purchase from a medical shop. Adult earthworms (*Eisenia foetida*) were purchased from Bio-Resources Development Centre, Shillong, Meghalaya vide bill no. BRD6/2015/Pt.III/20 dated 04/04/2019. Male albino rats were collected from the Institutional Animal Ethical Committee (IAEC) approved animal house of our institute.

2. Methods

1) Collection and identification of plant materials

Fresh plants (aerial parts) of *Trachyspermum khasianum* H. Wolff were collected from West Khasi Hills District, Meghalaya, India in September 2018. The herbarium sheet was prepared and authenticated by Dr. N. Devi., Professor and Head, Department of Botany, Guwahati University (Reference No. Herb./GUBH/2018/97 dated on 11/10/2018). After the collection of plants, the fresh plants were washed and dried at room temperature for 2-3 weeks [18].

2) Experimental animals

All the animals were acclimatized at ambient temperature for 7 days along with food and water *ad libitum*. The experimental

study protocol was approved by the Institutional Animal Ethical Committee (IAEC) (AdtU/IAEC/2018/009 dated 01/12/2018).

3) Extraction of plant

A fresh plant (aerial parts) was collected, shade dried, and powdered in an electrical grinder. The coarsely powdered plants were sieved through sieve no. 30. The powdered were extracted with 96% ethanol and water as a solvent at room temperature using the cold maceration process. 100 grams of the plant materials were soaked in 250 ml of solvent separately for 72 hours in a conical flask. Filtrations were done using Whatmann No.1 filter paper and the filtrate was collected in a conical flask and then covered using aluminium foil. Both the extracts obtained were concentrated using a rotary vacuum evaporator at low temperature (40-50℃) and then, transferred into a closed container for further use [19].

3. Anthelmintic activity

The study was carried out following the process as referred by Duvey [20]. The extracts of *Trachyspermum khasianum* H. Wolff were evaluated for anthelmintic activity in *Eisenia foetida* (earthworms) [21]. The collected earthworms were first washed with distilled water and acclimatized at an ambient temperature 30 min before the experiment. Test samples of the extracts were prepared at different concentrations to include 10, 20, and 40 mg/ml [22]. Albendazole suspension was used as standard and distilled water was used as a control. The earthworms were divided into nine groups, each group was consisted of 5 earthworms. The earthworms were placed in Petri dishes containing the different concentrations of extract solution as well as the standard drug [23]. The chronological group arrangements are given as follows:

Group-1: Received distilled water which served as the control.
Group-2: Received Albendazole suspension at a dose of 20 mg/ml which served as the standard.
Group-3: Received 10% Ethanol solvent.
Group-4: Received Ethanolic extract at a dose of 10 mg/ml.
Group-5: Received Ethanolic extract at a dose of 20 mg/ml.
Group-6: Received Ethanolic extract at a dose of 40 mg/ml.
Group-7: Received Aqueous extract at a dose of 10 mg/ml.
Group-8: Received Aqueous extract at a dose of 20 mg/ml.
Group-9: Received Aqueous extract at a dose of 40 mg/ml.

Earthworms (*Eisenia foetida*) were kept under close observation, the paralysis time (PT) and death time (DT) for individual worms were recorded. Paralysis was said to occur when no movement of earthworms at any sort could be observed except when the worms were shaken vigorously [24]. Death of worms was ascertained by the absence of motility when dipped in warm water (50℃) followed by the fading of the body colors. The mean paralysis time and mean lethal time for each sample was expressed as a Mean ± Standard error of the mean [25]. All the results were shown in Table 1.

4. Analgesics activity

1) Hot plate method

The study was carried out following the process as referred by Venkatachalam et al. [16]. Analgesic activity of the ethanolic and aqueous extracts of *T. khasianum* H. Wolff was assessed using Eddy's hot plate method [26]. The test samples were administered orally and the standard drug (10 mg/kg) was given intraperitoneally before the experiment. The Rats were placed on heated plates and the time is taken for either paw licking or jumping was considered as the reaction time or latency time. Rats were immediately removed from the hot plates on paw licking or jumping [27]. The animals were taken randomly and divided into 6 groups containing 5 animals in each group designated as group-I, group-II, group-III, group-IV, group-V, and group-VI as mentioned below [28].

Group I: Normal control (Distilled water)
Group II: Positive control (Diclofenac Sodium: 10 mg/kg)
Group III: Ethanolic extract (100 mg/kg)

Table 1. Anthelmintic activity of plants *Trachyspermum khasianum* H. Wolff

Groups	Concentration (mg/ml)	*Einesia foetida* (earthworms)	
		Paralyzing time (min)	Death time (min)
Distilled water (control)	-	-	-
10% Ethanol	-	5 ± 0.5	8 ± 1
Aqueous extract	10	14 ± 0.25	23 ± 1
Aqueous extract	20	12 ± 0.5	20 ± 0.25
Aqueous extract	40	9 ± 1	14 ± 1.25
Ethanolic extract	10	5 ± 0.5	12 ± 0.5
Ethanolic extract	20	4 ± 0.25	10 ± 0.5
Ethanolic extract	40	3 ± 0.5	6 ± 1
Standard (albendazole)	20	6 ± 0.5	11 ± 1

Min is minutes. mg/ml is milligram per milliliters. SEM is the standard error of the mean.

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Group IV: Ethanolic extract (200 mg/kg)
Group V: Aqueous extract (100 mg/kg)
Group VI: Aqueous extract (200 mg/kg)

The animals were individually placed on the hot plate maintained at 55 ± 0.5℃. The reaction time (in seconds) was noted as the time at which animals reacted to the thermal pain stimulus either by paw licking or jump response, whichever appeared first. The cut off time for the reaction was 15-20 seconds to avoid damage to the paw [29]. In all cases, the reaction time was recorded before (0 min) and each 30 minutes interval of time after oral administration of the samples and groups of animals were subjected for trial on a hot plate, and the response was observed in Table 2 [30]. The percentage (%) time of inhibition due to the effect of various sample and standard reference were calculated using the following formula:

% Inhibition of reaction time = (Mean reaction time of the test sample – Mean reaction time of the control group) / Mean reaction time of the control group [31].

5. Statistical analysis

The data of analgesics and anthelmintic activities were expressed as Mean ± SEM (Standard error of Mean) as shown in Tables 1 and 2. The statistical significance of the difference between the data obtained from the animal experiments using one-way analysis of Variance (ANOVA) test and followed by Tukey post hoc test for multiple comparisons. At ($p < 0.05$) were considered statistically significant.

RESULTS

In this study, the anthelmintic activity of the entire plant extract of *T. khasianum* H. Wolff was carried out on adult earthworm (*Einesia foetida*) as shown in Fig. 1. Different concentrations (10, 20, and 40) mg/ml of the aqueous and ethanolic extracts were studied and paralysis and death time was found to be reported as 23, 20, 14, and 12, 10, 6 minutes respectively. The results also exhibit more significant activity ($p < 0.05$) at a concentration (40 mg/ml) against *Einesia foetida* (earthworm). The time taken for paralysis and death of earthworms were recorded in Table 1 and shown graphically in Fig. 2.

The analgesics effect of the aqueous and ethanolic extracts of *T. khasianum* H. Wolff was evaluated through a hot plate test method using two different doses of 100 mg/kg and 200 mg/kg of plant extract. The percentage (%) inhibition time was recorded at an interval of 30, 60, and 90 min after administration of test extract in the experimental rats. About 30 minutes, the percentage inhibition of two different doses (100 and 200 mg/kg body weight) were recorded as 128.9%, and 278.1% for aqueous extracts and 186.0% and 313.2% for ethanol extract, while compared with the standard drug showed 142.1%. Then, after 90 minutes, the percentage inhibition of two different doses (100 and 200 mg/kg body weight) was recorded as 339.7% and 481.9% for aqueous extracts and 351.7% and 512.1% for ethanol extract respectively while compared with the standard drug as shown in Table 2 and shown graphically in Fig. 3. The results of the hot plate test revealed a significant therapeutic response at a dose of 200 mg/kg for both water and ethanolic extracts were reported as 13.5 ± 0.202 sec and 14.2 ± 0.336 sec respectively.

DISCUSSION

Both aqueous and ethanolic extract of the plant were evaluated for anthelmintic activity and the effective dose-dependent response was observed on death rate and paralysis tested warms. It was also noticed that the ethanolic extract of *T. kha-*

Table 2. Analgesics activity of plants *Trachyspermum khasianum* H. Wolff

Groups	Dose (mg/kg)	Reaction time in sec (Mean ± SEM) before and after drug administration(s)				% inhibition		
		0 min	30 min	60 min	90 min	30 min	60 min	90 min
Control (distilled water)	-	2.08 ± 0.213	2.28 ± 0.278	2.36 ± 0.248	2.32 ± 0.242	-	-	-
Standard (diclofenac sodium)	10	2.02 ± 0.086	5.52 ± 0.174	7.14 ± 0.103	10.9 ± 0.212	142.1	202.5	369.8
Aqueous extract	100	2.24 ± 0.098	5.22 ± 0.235	7.66 ± 0.163	10.2 ± 0.257	128.9	224.6	339.7
Aqueous extract	200	2.16 ± 0.108	8.62 ± 0.183	12.24 ± 0.232	13.5 ± 0.202	278.1	418.6	481.9
Ethanol extract	100	2.2 ± 0.1	6.52 ± 0.180	8.12 ± 0.107	10.48 ± 0.240	186.0	244.1	351.7
Ethanol extract	200	2.08 ± 0.169	9.42 ± 0.246	13.18 ± 0.260	14.2 ± 0.336	313.2	458.5	512.1

Sec is seconds. mg/kg is milligrams per kilograms. SEM is standard error of mean.

Figure 1. Anthelmintic activity of selected plant with different doses using earthworms.

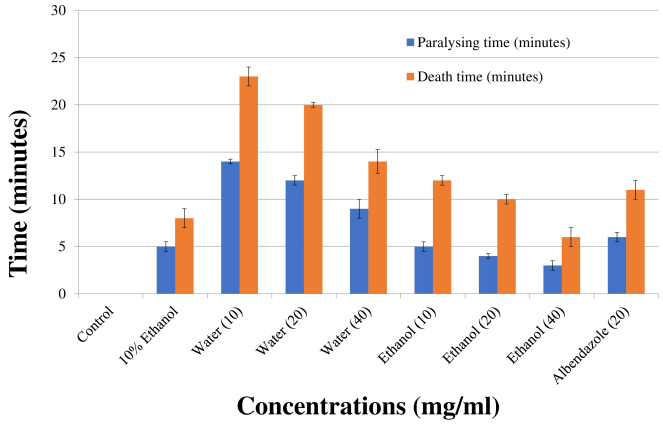

Figure 2. Effect of anthelmintic activity of selected plants in earthworms. Results are given as mean ± SEM. Significance at $p < 0.05$.

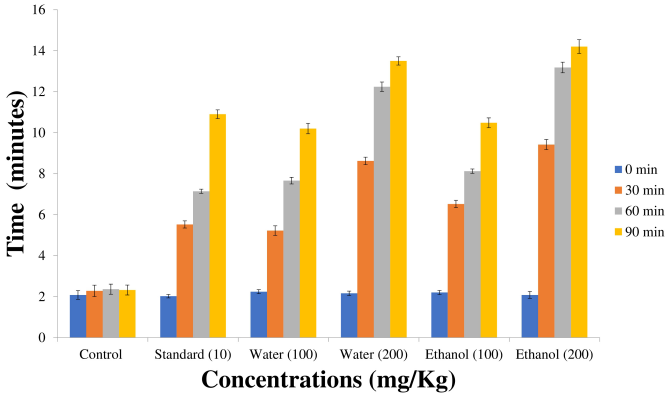

Figure 3. Effect of analgesic activity of selected plants in rats. Results are given as mean ± SEM of five animals in each group. Significance at $p < 0.05$.

sianum H. Wolff shows more potent action than the aqueous extracts. Further, as compared to standard drug albendazole at a concentration of 20 mg/ml, significant anthelmintic activity was observed at a concentration of 40 mg/ml for both ethanolic extract and aqueous extract of *T. khasianum* H. Wolff (Table 1).

The anthelmintic activity of aqueous and ethanol extracts was found to be concentration-dependent. The anthelmintic activity shown by *T. khasianum* H. Wolff extracts may be due to the presence of phytoconstituents available in form of alkaloids, tannins, phenols, and flavonoids, etc [25].

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The hot plate method is the most commonly used thermal nociception model to assess the complex response of narcotic analgesia. The result of the hot plate test revealed a significant therapeutic effect at a dose of 200 mg/kg for water and ethanolic extracts at 13.5 ± 0.202 sec and 14.2 ± 0.336 sec respectively. Therefore, the extracts of this plant showed antinociceptive effects in this test since the reaction time of test groups has increased to a significant level. The plant extracts at a dose of 200 mg/kg show the potent analgesic effect in a dose-dependent manner. However, considering the fact that narcotic analgesics drugs inhibit both peripheral and central mechanisms of pain, while NSAIDs inhibit only peripheral pain. It may be stated that the aqueous and ethanolic extracts of the plant might possess anti-nociceptive activity mediated via a central mechanism. The analgesic effect of this plant suggests that it might have been acting through the central or peripheral mechanism or both central and peripheral mechanisms [32]. Hence, this study validates its use in pain management by the traditional healers.

CONCLUSION

Based on studied, it can be concluded that the aerial parts of plant extracts of *Trachyspermum* H. Wolff possess significant ($p < 0.05$) anthelmintic and analgesic activities. Further research is required to be carried out to identify the responsible phytoconstituents for both analgesic and anthelmintic activities and their mechanism of action.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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