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IN-VITRO ANTHELMINTIC EVALUATION OF *HIBISCUS ROSA-SINESIS* (L) AND *AZADIRACHTA INDICA* (L) LEAVES AGAINST HELMINTH PARASITE OF THE GOAT: A COMPARATIVE STUDY

K. Jaiswal, S. Mishra and J. Pandey *

Department of Zoology, Babasaheb Bhimrao Ambedkar University, Lucknow - 226025, Uttar Pradesh, India.

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Anthelmintic potential, *Hibiscus rosa-sinesis* (L), *Azadirachta indica* (L), *Haemonchus spp* , Albendazole

Correspondence to Author:
Jyoti Pandey

Research Scholar,
Department of Zoology,
Babasaheb Bhimrao Ambedkar University, Lucknow-226025, Uttar Pradesh, India.

E-mail: jyotiacu@gmail.com

ABSTRACT: The comparative study designed to evaluate the anthelmintic potential of the methanolic extracts of *Hibiscus rosa-sinesis* (L) and *Azadirachta indica* (L) leaves against helminth: *Haemonchus spp*., an abomasal parasite For this study various concentrations (1, 2.5, 5.0, 10.0 mg/ml) of both the extracts were screened. 1 mg/ml concentration of Albendazole (ABZ) was used as a positive control (reference) and Phosphate Buffer Saline (PBS) alone was used as a negative control. The results were represented with respect to time (min) taken to get paralyze followed by the death of the treated parasite in all the tested concentrations. The results of the study disclosed the anthelmintic efficacy, of both the plants when compared with the positive control (reference). The results also established that *Hibiscus rosa-sinesis* (L) leaves show more effective anthelmintic potential as compared with *Azadirachta indica* (L) leaves.

INTRODUCTION: Goat population are the most, broadly adopted livestock and progressively used to boost the income and subsequently improve the household livelihood strategies. Besides, these population had been rigorously suffering from endo-parasitism which is liable to reduce animal performance by lowering productivity, decreased food intake, increase in weight loss and reduced milk production [1, 2, 3]. Helminthiasis is an important endo-parasitic worm disease caused by helminths namely nematode, cestode, and trematode parasitic worms. This parasitic disease has a severe economic impact on livestock development as well as the human well-being of many countries [4, 5].

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Generally, synthetic anthelmintic drugs are used to treat helminth infections. The majority of prevailing anthelmintic drugs are causing several types of side effects such as headache, loss of appetite, weight loss, abdominal discomfort, diarrhoea, nausea, lethargic body condition, vomiting, *etc.* [6, 7]

Large-scale use of these anthelmintics has been developing a new problem, *i.e.*, the development of drug resistance which led to developing an interest in the screening of traditional plants for their anthelmintic potential [8]. The traditional plants are a good source of biodegradable secondary metabolites that were recognized for their medicinal value for centuries to treat a variety of diseases. Herbal medicines are manufactured from various parts of the plant materials such as barks, roots, stems, leaves, seeds, flowers and fruits [8]. Thus, the traditional plants offer herbal alternatives to prepared sustainable and environmentally safe drugs [9]. *Hibiscus rosa-sinensis* (L.) commonly, known as gurhal, is a member of the Malvaceae family. This plant showed antibacterial [10], antioxidant [11], anthelmintic [12, 13, 14], antipyretic [15], antidiabetic [16], anti-inflammatory [17] and hair growth [18] properties.

Azadirachta indica (L.) usually, known as Neem, belongs to family Meliaceae. From ancient times, *A. indica* has been used to cure various types of diseases [19]. Many previous works have proven the therapeutic potential of *A. indica viz.*, antioxidant potency [20], antimicrobial property [21], antiviral potency [22], antifungal [23] anthelmintic activity [24, 25, 26, 27].

MATERIALS AND METHODS:

Plant Collection: Plant leaves were either collected from the field or obtained from the local market of Lucknow, India and put into large cotton bags and brought into the Parasitology and Silkworm Pathology Laboratory of the Department of Zoology (formerly the Department of Applied Animal Sciences) Babasaheb Bhimrao Ambedkar University (B. B. A. U.), Lucknow, Uttar Pradesh. The plants were identified and authenticated by the Department of Plants Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh.

Preparation of Extract: The plant leaves were dried in the oven at ambient temperature (40 ºC- 45 ºC) for 2 to 3 days till the materials were completely dry, and then ground to a fine powder with the help of an electric grinder. 100 g of fine powder of each plant was processed for extraction using 500 ml methanol as a solvent separately, in a Soxhlet apparatus, for 2 days at room temperature. Furthermore, the extracts were concentrated by water bath, and then dried at 20 ºC-25 ºC and stored in airtight bottles at 4 ºC until use [9, 28, 29, 30].

Worm's Collection: Gastrointestinal (GI) tracts of goats were collected randomly from the local slaughterhouses located in different parts of Lucknow, Uttar Pradesh.

The GI tracts were kept in the polythene bags and brought to the Parasitology and Silkworm Pathology Laboratory of Dept. of Zoology (formerly the Department of Applied Animal Sciences) BBAU, Lucknow. Each part of the gastrointestinal tract was examined carefully for the presence of helminth parasites (*Haemonchus spp*.) by following the standard method [31, 32]. Collected parasites were transferred to fresh normal saline (0.9 %).

Phytochemical Analysis: Phytochemical examinations were carried out for both plant extracts as per the standard procedures [33, 34, 35, 36] with slight modification.

Terpenoids:

Salkowski's Test: 5 ml of test solution was dissolved in 1 ml of chloroform, and then 1 ml of sulphuric acid was added carefully. The appearance of reddish-brown colour indicates the presence of terpenoids.

Tannins:

$FeCl_3$ Test: 5 ml of test solution was mixed with an equal volume of $FeCl_3$ solution. A dark green or black colour precipitate indicates the presence of tannins.

Alkaloids:

Mayer's Test: 5 ml of test solution was mixed with a few drops of Mayer's reagent. The formation of cream-coloured precipitate confirms the presence of alkaloids. 5 ml of the extract was mixed with an equal volume of distilled water and heated up to 60 ºC and allowed to cool for 10 min. Thereafter, 5 ml of NaOH (Sodium hydroxide) solution was added, and at that time, the colour of the mixture changed to yellow, but after the addition of 5 ml dilute HCl mixture, the colour changes from yellow to colourless, which indicates the presence of alkaloids.

Flavonoids:

Alkaline Reagent Test: 5ml of the test solution was taken and treated with 2-3 drops of sodium hydroxide solution (NaOH). The development of an intense yellow colour, which disappears with the addition of dilute acid (HCl), confirms the presence of flavonoids.

Saponin:

Foam Test: test solution was vigorously shaken with 2 ml of distilled water. If foam produced persists for 10 min, it confirms the presence of saponins.

Phenol:

FeC_{l3} Test: 5 ml of plant extract mixed with equal volume 10 % aqueous ferric chloride, Bluish - green colour appears, confirms the presence of phenol.

Glycosides: 5 ml of the extract was mixed with 2.5 ml of dilute H_2SO_4 and boiled for 10-15 min, then cooled and counterbalanced with 10% NaOH. After that, Fehling's solution A and B were added. The formation of brick-red colour precipitation indicates the presence of glycosides.

***In-vitro* Anthelmintic Efficacy Analysis:** The anthelmintic assay of both plants was performed by following the standard protocol37 3839 with lesser modifications. Both plant extract concentrations and drug solution were prepared freshly before starting the assay.

Same-sized ten actively motile adult worms were chosen and placed in Petri dishes containing various concentrations (1 mg/ml, 2.5 mg/ml, 5 mg/ml, and 10 mg/ml) of both methanolic plant extracts (ME) in Phosphate Buffer Saline (PBS). Anthelmintic allopathic drug Albendazole (ABZ: 1 mg/ml) was used as a reference, while PBS was taken as the control. Three replicates of each concentration were taken for the experiment.

The observations were noted the time taken for the paralysis and the death of the individual worms at 1, 2, 3, 4, 5, 6, 7, and 12 h and readings were noted in minutes. The paralyzed worms were placed in Phosphate Buffer Saline (PBS) for 30 min for the possible rescue in the motility of the worm after each interval of time. After the completion of the assay (after 12 h), alive and dead parasites were counted under a dissecting microscope and recorded.

The time taken for paralysis and death was investigated on the basis of the behaviour of the worm, *i.e.,* no recovery in motility even after placing in PBS whereas death was determined on the basis of the complete loss of motility with yellowing in body colour [29, 30, 38, 40, 41].

Statistical Analysis: The data were expressed as Mean ± S.E.M of 10 worms for each concentration. Data analysis was done using One-way ANOVA followed by Tukey- post hoc with the help of SPSS (version 20.00). The difference in the value at $P \leq 0.05$ was set as statistical significance.

RESULTS AND DISCUSSION: Parasitic invasions and concomitant infections are serious health concerns to livestock industries [42]. Among different parasitic diseases, helminth infections have not only a vast range of infections but frequently develop resistance against the used anthelmintic drugs worldwide [43, 44].

The anthelmintic resistance characteristic, along with the drug toxicity and the presence of drug residues in animal products, has led to a revitalization of interest in using plant-based drugs [45]. The existing anthelmintics basically eradicate worms either by paralyzing or making them starved until death has occurred. Helminth worms are parasitic and have no means of the energy-storing process. To combat this situation, they have a voraciously feeding habit of meeting their metabolic needs.

Any interference in the metabolic process results in depletion of energy, and parasites become paralyzed and momentarily lose their ability to sustain their place in the gut [46].

Additionally, these drugs bind to free proteins in the host's intestinal tract and create a hindrance in the energy driving mechanism or adhere to the cuticular glycoprotein of the parasite and generate an interruption in the motility causes the death of the worm [5, 47].

Preliminary phytochemical screening of both plant extracts expressed the presence of biologically active metabolites. This study revealed the presence of alkaloids, flavonoids, terpenoids, tannins, phenols, saponins, glycosides in both extracts of *Hibiscus rosa-sinensis* (L) plant as well as *Azadirachta indica* (L.) **Table 1**.

Almost the same findings were reported by the many scientists in the screening of *H. rosa-sinensis* plant 48 as b well as in *A. indica* [49, 50]. Phenolic group-based synthetic anthelmintic drugs such as oxyclozanide, niclosamide, bithionol, *etc.*, are functioned by interfering with the energy generation kinetics of helminth parasites by uncoupling of oxidative phosphorylation [36].

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF METHANOLIC LEAVES EXTRACT OF *HIBISCUS ROSA-SINESIS* (L) LEAVES AND *AZADIRACHTA INDICA* (L)

Phytochemical Constituents	*Hibiscus rosa-sinesis* (ME)	*Azadirachta indica* (ME)
Alkaloids	++	+
Flavonoids	+	+
Terpenoids	+	+
Tannins	++	+
Phenols	++	+
Saponin	+	+
Glycosides	+	+

Where; +: Positive, ++: Strong positive, -: Negative, ME: Methanolic Extract

Flavonoids, phenols, and tannins are polyphenolic compounds biologically active against micro-organisms, liver contaminants, inflammation, allergic reactions, tumor, and free radicals [51, 52]. So, the anthelmintic efficacy of these compounds might be due to their ability to bind with the free proteins, which are necessary for the nourishment of the parasitic larvae that leads to the nutrients scarcity and, finally, ending with the larval starvation condition. It can also bind to the cuticular glycoprotein and create a hindrance in the parasite's motility or bind to the glycoproteins of the gastrointestinal tract and obstruct its metabolism by impeding the oxidative phosphorylation that indirectly affects the CNS [47, 53, 54].

Alkaloids are a biologically active compound and work as anthelmintic. It might be suppressed the transportation of sucrose from the stomach to the small intestine due to the antioxidant property which, consequently, reduces the formation of nitrate and generates interference in indigenous homeostasis that severely affects the central nervous system and caused paralysis [48, 55]. Several types of research reported the mechanism of action of saponin as anthelmintic. They stated, saponin has membrane permeabilizing efficiency due to the pore-forming property causing vacuolization and disintegration of the membrane, which leads to the breakdown of helminths cuticular surface [52, 56].

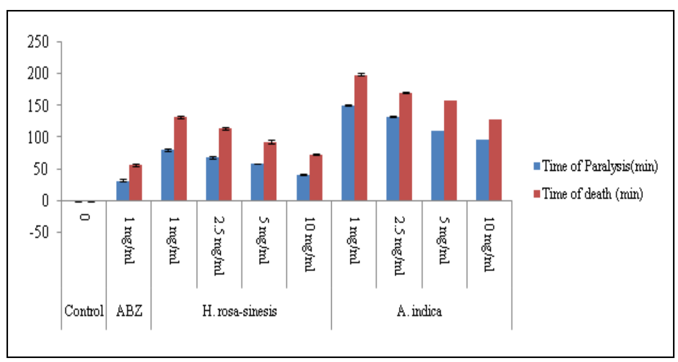
Anthelmintic analysis result disclosed a significant ($p \leq 0.05$) anthelmintic efficacy of methanolic extract of both plants, *i.e., Hibiscus rosa-sinensis* (L) and *Azadirachta indica* (L.) as compared to control at all tested concentration. Extract of *Hibiscus rosa-sinensis* (L) showed significantly more potent anthelmintic activity in both paralysis and death assay than *Azadirachta indica* (L.) at all concentrations except 10.0 mg/ml where the efficacy for the paralysis varies non-significantly ($P \geq 0.05$) as compared to the reference drug albendazole **Table 2** & **Graph 1**. This analysis also revealed the potency of both plants as anthelmintic. Both plant extracts expressed the dose-dependent process of anthelmintic activity, *i.e.*, at higher concentrations (10 mg/ml); both plants took less time to paralyze and die.

The same finding was reported by many researchers using different plants [46, 57]. The comparative study of both plants revealed the *Hibiscus rosa-sinesis* showed high potency for anthelmintic activity than *Azadirachta indica*. The disparity in the result could be due to the quantity of biologically active metabolites in both plant extracts [52, 54].

TABLE 2: *IN–VITRO* ANTHELMINTIC ASSAY OF METHANOLIC LEAVES EXTRACT OF *HIBISCUS ROSA-SINESIS* (L.) AND *AZADIRACHTA INDICA* (L.) AGAINST TEST PARASITE

Treatment	Concentration (mg/ml)	Paralysis time (min) (mean±SEM)	Death time (min) (mean±SEM)
Control		0	0
Albendazole		32±2.3*	57.3±5.5*
H rosa-sinesis(ME)	1	81.3±1.8*[a]	132.3±1.7*[a]
	2.5	68.6±2.0*[a]	114.3±2.3*[a]
	5	58.3±2.0*[a]	93.6±2.6*[a]
	10	41.3±1.2*	73.6±2.4*[a]
A indica (ME)	1	150.6±0.6*[a]	199.3±2.9*[a]
	2.5	133.3±0.8*[a]	170.3±1.4*[a]
	5	110.0±1.1*[a]	158.0±2.0*[a]
	10	97.0±1.0*[a]	129.3±1.2*[a]

Values are mean±SEM, (n=10), All superscripts indicate significance at $P \geq 0.05$, *compared to control, [a]compared to albendazole (one-way ANOVA followed by Tukey *post hoc* test),: ME: Methanolic extract



GRAPH 1: ANTHELMINTIC ASSAY (WORM PARALYSIS AND DEATH) OF METHANOLIC LEAVES EXTRACT OF *HIBISCUS ROSA-SINESIS* (L.) AND *AZADIRACHTA INDICA* (L.) AS COMPARED TO STANDARD DRUG ABZ: Albendazole, min: Minute

CONCLUSION: This study concluded that the phytochemical compounds responsible for anthelmintic potential are present in both plant extracts. However, it would be required to explore both plants for an *in-vivo* study to validate this finding. Further, the work will focus on the isolation and characterization of active principles compounds responsible for the anthelmintic activity of leaves extracts of both plants.

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