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EXPLORING THE ANTHELMINTIC POTENTIAL OF *AZADIRACHTA INDICA* AND *ANDROGRAPHIS PANICULATA* EXTRACTS AND THEIR SYNERGISTIC EFFECTS

Ramesh Kumari Dasgupta[1*], Aniruddha Das[2], Rahul Maiti[2], Sajid Mondal[2]

[1] Professor, Department of Pharmaceutical Technology, Brainware University, Kolkata, West Bengal, India.

[2]Students, Department of Pharmaceutical Technology, Brainware University, Kolkata, West Bengal, India.

Address for Correspondence: **Dr. Ramesh Kumari Dasgupta**

Email: rkd.pt@brainwareuniversity.ac.in

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ABSTRACT

Helminth infections represent a significant global health concern, particularly in developing nations, contributing to malnutrition and other ailments. The rise of resistance to synthetic anthelmintics underscores the urgent need for alternative treatment strategies. This study investigated the phytochemical composition and anthelmintic efficacy of crude extracts from *Azadirachta indica* (AI) and *Andrographis paniculata* (AP), both individually and in combination, against helminths, with Albendazole serving as the standard reference. Anthelmintic activity, assessed by the time taken for paralysis and death of helminths, demonstrated a dose-dependent effect for all extracts (50, 75, and 100 mg/ml).Albendazole exhibited the most rapid action, inducing paralysis and death at 13±0.5 min and 22±0.4 min respectively, at 100 mg/ml. *A. indica* extract showed anthelmintic properties, with paralysis at 37±0.6 min and death at 50±0.3 min at 100 mg/ml. *A. paniculata* extract was more potent than AI, causing paralysis at 24±0.1 min and death at 27±0.1 min at the same concentration. Notably, the combined extract of AI and AP displayed a synergistic or additive effect, significantly enhancing anthelmintic activity. At 100 mg/ml, the combination induced paralysis in 19±0.4 min and death in 21±0.8 min, comparable to Albendazole's efficacy (paralysis at 75 mg/ml: 17±0.9 min; death at 100 mg/ml: 22±0.4 min). These findings suggest that the polyherbal combination of A. indica and A. paniculata is a promising candidate for a viable alternative or complementary therapy for helminth infections, potentially mitigating drug resistance.

INTRODUCTION

Anthelmintic activity refers to the ability of a substance to kill or expel parasitic worms (helminths) from the body. These worms can cause a variety of health problems in humans and animals, ranging from mild discomfort to serious illness. Helminth infections represent a significant global health concern, particularly in developing nations, contributing to malnutrition and other ailments. The World Health Organization estimates that 2 billion people harbour parasitic worm infections [1] According to the World Health Organization, more than 1.5 billion people, or 24% of the world's population, are infected with soil- transmitted helminths (STHs) [2].

In recent decades, continuous and intensive use of synthetic anthelmintics has been the only method to control gastrointestinal nematodes. However, resistance to all available anthelmintic drug classes has been reported in livestock species. Resistance to an anthelmintic drug is often observed within a few years of introduction of the drug, indicating a remarkably high rate of resistance development, which likely results from a combination of large, genetically diverse parasite populations, and strong selection pressure for resistance.[3]. Plants are an ideal source of naturally occurring compounds that can be used as alternative dewormers in livestock [4]. Plants have been an essential source of human medicine for millennia. The use of herbal medicinal products and supplements has increased tremendously over the past three decades, with not less than 80% of people worldwide relying on them for some part of primary healthcare. Although therapies involving these agents have shown promising potential with the efficacy of a good number of herbal products clearly established. Number of herbal medicines are generally considered to be safe and effective; it is said that these have fewer side effects. [5]. In our study we investigated the phytochemical composition and anthelmintic efficacy of crude extracts from *Azadirachta indica* (AI) and *Andrographis paniculata* (AP), both individually and in combination, against helminths.

Azadirachta *indica (AI)* and *Andrographis paniculata (AP)* both the plants belong to *Meliaceae and Acanthacea* family respectively.

The extracts of these plants are bitter in taste even though the *Andrographis paniculate* is known as king of bitters. These plants are grown in the tropical and sun tropical countries. In India these are found in all the states and dry leaves of these plants are used in storage of food grains [6]. In India, *Azadirachta indica* is called "Divine Tree", "Life giving tree", "Nature's Drugstore", "Village Pharmacy" and "Panacea for all diseases" [7].

II PHYTOCONSTITUENTS

Azadirachta indica and *Andrographis paniculate* are the rich source of phenols, flavonoids, alkaloids, tannins, saponins, glycosides, amino acids and terpenoids. The bitter tase of neem is due limoids, which is tetranortriterpeoid. *Azadirachta indica* has azadirachtin, nimbolinin, nimbin, nimbidinin, nimbidol, gedunin, salannin, quercetin, sitosterol, 6-desacetylnimbinene, n-Hexacosanol, nimbandiol, nimbolide, ascorbic acid, 7- desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoyl- gedunin, 17- hydroxy azadiradione [8]. The bitter taste of kelmegh is due to the presence of the lactone andrographolide named kalmeghin. Andrographolides paniculata has Andrographolides, 14-Deoxyandrographolide, Neoandrographolide ,14-Deoxy 11,12-didehydro andrographolide, Andrographiside, Isoandrographolide, Andrograpanin, 14-Deoxy-11-oxo-andrographolide, 14-Deoxy-11- hydroxy andrographolide, 3-O- β-D-glucosyl-14-deoxyandrographolide, 14-Deoxy-12-hydroxyandrographolide,6'-Acetyl-neo-andrographolide,3,14-Dideoxyandrographolide, 3-Oxo-14-deoxyandrographolide,3-O-β -D-glucopyranosyl 14,19-dideoxy Andrographolide,3-O- β -D-glucopyranosyl Andrographolide, Andrographolactone, 8,17-Epoxy-14- deoxy andrographolide, 14-Deoxy-17-betahydroxyandrographol, 12- Hydroxyandrographo lide,3-Oxo-14-deoxy-11,12-Didehydroandrographolide,7-Hydroxy-14-deoxy andrographolide, Apigenin, 7-O-methylwogonin, 7,8-Dimethoxy-2-D-glucopyranosyl oxyflavone, 7-O-methyl-dihydrowogonin Skullcapavone-1,2'methoxylether [9]

III PHARMACOLOGICAL ACTIVES

Azadirachta indica (Neem) has a lot of pharmacological actives, It is used traditionally as amedicine in Ayurveda. It has antioxidant activity [10], anticancer activity [11], anti-Inflammatory activity [12,13,14], antidiabetic activity [15,16,17], antibacterial activity [18], antiviral activity [19], antifungal activity [20, 21,22]. *Andrographis paniculate* (kalmegh), herbaceous plant extract contains a lot of active compounds with multiple biological activities against various diseases. It has antibacterial activity [23], anti-diabetic [24,25] anti-Inflammatory activity [26,27] antiviral effects [28]., antipyretic and analgesic activity [2] and effects on cardiovascular disease [30].

IV ANTHELMINTIC ASSAY

The plant materials whole) are collected from the surrounding village area of Nakibasan, Tamluk, East Medinipur, Barasat, West Bengal. Each plant material was extracted for 24h with (1:1) menthol and water using Soxhelt's apparatus [31]. After extraction the solvents were evaporated by using rotary evaporator and solid mass was stored at 4 oC for evaluating the anthelmintic activity. Indian earthworm (*Pheretima posthuma*) was collected from the water-logged area of soil, Habra. (North 24 Parganas Dist.), West Bengal. Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. They were washed with normal saline to remove all fecal matter. The earthworms of 5-8 cm in length and 0.1-0.1.2 cm in width were used for all experimental protocol.

Extracts from the *Azadirachta indica* and *Andrographis paniculate* were investigated for their anthelmintic activity against *Pheretima posthuma*. Various concentrations (50, 75 and 100 mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was included as standard reference and saline water as control [32]. with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings[33,34,35]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro [36,37,38]. In the first set of experiment, four groups of six earthworms were released in to 25 ml of solutions of Albenzadole, and extracts of Azadirachta indica and Andrographis paniculate in distilled water. The remaining groups were treated for different concentrations. All drug and extract solutions were freshly prepared before starting the experiment. Albenzadole was used as reference standard while saline water as control. Observations were made for the time taken to paralysis and death of individual worms. 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.

V RESULT AND DISCUSSION

Helminth infections are among the commonest infections in man, affecting a large proportion of the world's population. In developing countries, they pose a major threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia, and pneumonia many helminths. Some helminth also live-in tissues, or their larvae migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. But now a days they are becoming resistant to synthetic anthelmintics. The challenges posed by multi-drug resistance in synthetic anthelmintics, the potential of plant extracts as an alternative helminth control option offers a ray of hope [39]. The anthelmintic activity of *Azadirachta indica* (AI) and *Andrographis paniculata* (AP) extracts, both individually and in combination, against helminth was investigated using Albendazole as a standard reference drug. The results, presented as the mean time taken for paralysis and death of the organisms ± standard deviation, demonstrate a dose-dependent effect for all extracts. The control group, treated with normal saline, showed no signs of paralysis or death throughout the observation period, indicating the viability of the helminth and the inertness of the solvent (Table 1).

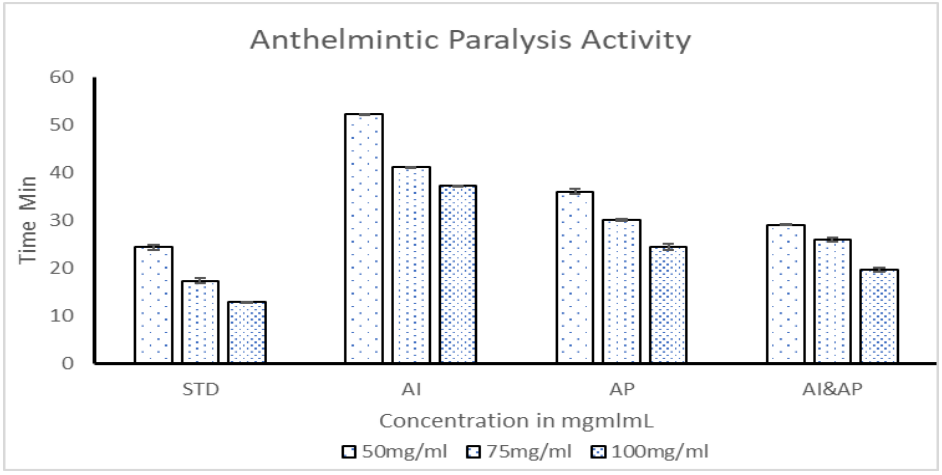
Table 1: Anthelmintic activity extracts of Azadirachta indica and Andrographis paniculate

Treatment	Concentration mg/ml	Time taken for paralysis (min)	Time taken for death (min)
Control (Normal Saline)			
Albendazole (STD)	50	24±0.4	28±0.8
	75	17±0.9	24±0.4
	100	13±0.5	22±0.4
Extract of *Azadirachta indica* (AI)	50	52±0.2	66±0.5
	75	41±0.6	59±0.3
	100	37±0.6	50±0.3
Extract of *Andrographis paniculate* (AP)	50	36±0.6	40±0.4
	75	30±0.3	31±0.8
	100	24±0.1	27±0.1

Extract of Azadirachta indica and Andrographis paniculate (AI &AP)	50	29±0.4	30±0.5
	75	26±0.8	28±0.8
	100	19±0.4	21±0.8

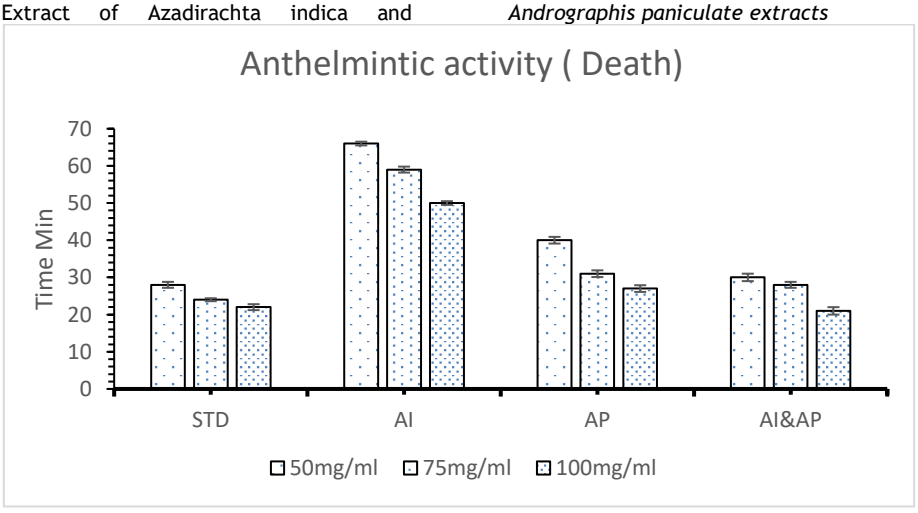
Albendazole, the standard drug, exhibited the most rapid anthelmintic action. At concentrations of 50, 75, and 100 mg/ml, it induced paralysis at 24±0.4 min, 17±0.9 min, and 13±0.5 min, respectively, and death at 28±0.8 min, 24±0.4 min, and 22±0.4 min, respectively. This establishes a baseline for potent anthelmintic efficacy (Figure 1 and Figure 2).

The extract of *Azadirachta indica* (AI) also demonstrated anthelmintic properties, albeit with a slower onset of action compared to Albendazole. Paralysis was observed at 52±0.2 min, 41±0.6 min, and 37±0.6 min, while death occurred at 66±0.5 min, 59±0.3 min, and 50±0.3 min for concentrations of 50, 75, and 100 mg/ml, respectively. This indicates that A. indica possesses anthelmintic compounds, but they are less potent or slower acting than Albendazole at the tested concentrations.



STD- Albendazole- Extract of Albendazole, AI Extract of Azadirachta indica, AP Extract of Andrographis paniculate, AI&AP- Extract of Azadirachta indica and Andrographis paniculate)

Fig. 1. Anthelmintic paralysis activity of *Azadirachta indica* and *Andrographis paniculate extracts*



STD- Extract of Albendazole, AI Extract of Azadirachta indica, AP Extract of Andrographis paniculate, AI&AP- Extract of Azadirachta indica and Andrographis paniculate

Fig. 2. Anthelmintic activity of Azadirachta indica and Andrographis paniculat extracts

Similarly, the extract of *Andrographis paniculata* (AP) showed notable anthelmintic activity, proving to be more effective than Azadirachta indica extract. At 50, 75, and 100 mg/ml, paralysis was recorded at 36±0.6 min, 30±0.3 min, and 24±0.1 min, respectively. The corresponding times for death were 40±0.4 min, 31±0.8 min, and 27±0.1 min. While more effective than AI, AP extract still did not match the rapid action of Albendazole (Figure2) Interestingly, the combined extract of *Azadirachta indica* and *Andrographis paniculata* (AI & AP) exhibited a synergistic or additive effect, resulting in significantly enhanced anthelmintic activity compared to the individual extracts, and approaching the efficacy of Albendazole at higher concentrations. At 50 mg/ml, the combination induced paralysis at 29±0.4 min and death at 30±0.5 min. These times were further reduced at 75 mg/ml (paralysis: 26±0.8 min, death: 28±0.8 min) and 100 mg/ml (paralysis: 19±0.4 min, death: 21±0.8 min). Notably, at the 100 mg/ml concentration, the combination therapy's time to induce paralysis (19±0.4 min) was comparable to Albendazole at 75 mg/ml (17±0.9 min), and its time to cause death (21±0.8 min) was very close to Albendazole at 100 mg/ml (22±0.4 min).

We can say that all tested extracts demonstrated dose-dependent anthelmintic activity. While Albendazole was the most potent agent, the combination of *Azadirachta indica* and *Andrographis paniculata* extracts showed promising results, exhibiting a synergistic effect that significantly enhanced their individual activities. This suggests that a polyherbal formulation could be a viable alternative or

complementary therapy in the management of helminth infections, potentially offering a broader spectrum of action or reducing the risk of resistance development associated with single-drug therapies. Further research to identify the active phytochemical constituents and elucidate the precise mechanism of their synergistic interaction is warranted.

CONCLUSION

This study successfully demonstrated the anthelmintic potential of *Azadirachta indica* (AI) and *Andrographis paniculata* (AP) extracts. All tested extracts exhibited dose-dependent anthelmintic activity against the target helminths. *A. paniculata* extract was more potent than *A. indica* extract when used individually. Crucially, the combination of AI and AP extracts resulted in a significant synergistic or additive effect, markedly enhancing their anthelmintic properties. At the highest concentration (100 mg/ml), the combined formulation induced paralysis and death in times closely approaching those of standard drug (Albendazole). This potentiation suggests that a polyherbal approach using *A. indica* and *A. paniculata* could be a highly promising and viable alternative or complementary strategy for managing helminth infections. Such a formulation may offer benefits in terms of efficacy and potentially in combating the growing issue of anthelmintic resistance. Further investigation into the specific active compounds and their mechanisms of synergistic interaction is warranted to fully explore and develop this promising herbal combination for therapeutic use.

FUTURE ASPECTS

The future of neem (*Azadirachta indica*) and kalmegh (*Andrographis paniculata*) as anthelmintics appears bright, driven by the growing preference for natural treatments and the try for novel alternatives to conventional drugs hampered by resistance and adverse effects. Future research should prioritize the isolation and characterization of specific active compounds. These plants are used individually or in combination with other herbs. Mechanistic studies are crucial to fully understand their anthelmintic actions, paving the way for optimized formulations, potentially including nanoformulations to enhance bioavailability. Rigorous clinical trials in humans are essential to validate their safety and efficacy, ideally through comparisons with existing treatments. Sustainable cultivation practices must be implemented to ensure consistent availability. Integrating modern scientific findings with traditional medicinal uses can further solidify their role, alongside increasing awareness and education about their benefits. Given the rising challenge of anthelmintic resistance, neem and kalmegh warrant investigation as potential solutions for managing resistant helminth strains. Ultimately, a collaborative, interdisciplinary approach will be key to fully realizing the potential of these plants in global helminth management.\

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest whatsoever.

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