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PRELIMINARY PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF ANTHELMINTIC AND ANTIOXIDANT ACTIVITY OF *TRAGIA PLUKENETII R.SMITH* LEAF EXTRACT

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ABSTRACT

Tragia plukenetii R. Smith is a traditionally used medicinal plant reputed for its therapeutic properties. The study aimed to evaluate the pharmacognostic characteristics, antioxidant activity and anthelmintic efficacy of the ethanolic leaf extract of *Tragia plukenetii R. Smith.* Phytochemical screening and pharmacognostic parameters including ash values, extractive values, foaming index, and crude fiber content were also determined. Antioxidant activity was assessed using DPPH radical scavenging and reducing power assays with Gallic acid as the standard. *In-vitro* anthelmintic activity was tested against Pheretima posthuma, while *in-vivo* activity was evaluated in mice infected with Ascaris lumbricoides by fecal egg count reduction method. Pharmacognostic values were within acceptable ranges, supporting quality control of the crude drug. Phytochemical screenings confirmed the presence of alkaloids, flavonoids, tannins, carbohydrates and phenolic compounds. The extract exhibited dose-dependent antioxidant activity, with significant radical scavenging (IC_{50} = 37.32μg/ml) and reducing power, though less potent than Gallic acid (IC_{50} = 0.81μg/ml). *In-vitro* anthelmintic studies demonstrated a concentration-dependent reduction in paralysis and death times of worms, with the 50mg/ml dose acting faster than albendazole. *In-vivo* studies showed significant reduction in fecal egg counts at 200, 400 and 800mg/kg, with the highest dose achieving 90.81% reduction compared to 99.43% by albendazole. Acute toxicity evaluation revealed no mortality or toxic signs up to 2000mg/kg, confirming safety. Overall, the ethanolic extract of *Tragia plukenetii* exhibits significant antioxidant and anthelmintic activities with a wide safety margin, supporting its ethnomedicinal use and potential as a natural therapeutic agent.

KEYWORDS

Tragia plukenetii R. Smith, Ethanolic extract, Antioxidant activity, Anthelmintic efficacy and Acute toxicity.

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INTRODUCTION

Plants have always been regarded as the cornerstone of traditional medicine and drug discovery. According to the World Health Organization (WHO), nearly 80% of the world's population, particularly in developing countries, relies on herbal remedies for their primary healthcare needs[1]. The wide acceptance of medicinal plants is attributed to their cultural acceptability, easy availability, low toxicity, and affordability compared to synthetic drugs. Moreover, secondary metabolites such as alkaloids, flavonoids, terpenoids, saponins, and tannins are responsible for the vast pharmacological diversity observed in medicinal plants, which makes them a potential source for the development of new therapeutic agents[2].

Among the major health problems worldwide, helminthiasis continues to be a leading cause of morbidity. Helminths are parasitic worms that infect the gastrointestinal tract, causing nutritional deficiencies, anemia, and impaired physical and mental growth. The problem is more severe in children and livestock, leading to both public health and economic burdens[3]. The commonly available synthetic anthelmintic drugs such as albendazole, levamisole, and mebendazole have proven effective in the short term, but frequent use has led to drug resistance and recurrence of infections[4]. This necessitates the search for alternative, plant-based anthelmintic agents with improved efficacy and fewer side effects.

Another important concern in biomedical research is oxidative stress, which occurs when reactive oxygen species (ROS) overwhelm the antioxidant defense systems of the body. ROS such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide are generated as byproducts of cellular metabolism. Excessive ROS can damage lipids, proteins and DNA, ultimately leading to aging and the development of chronic diseases including diabetes, cancer, cardiovascular diseases, and neurodegenerative disorders like Alzheimer's and Parkinson's disease[5]. In this context, antioxidants of natural origin have gained attention for their potential in mitigating oxidative stress and enhancing cellular defense systems[6]. Thus, plants rich in phytochemicals with antioxidant properties are of particular interest in pharmacological research.

Plant-based remedies provide a dual advantage in pharmacology. First, they often contain multiple bioactive compounds that can target different mechanisms of action, thereby reducing the likelihood of resistance development. Second, they offer synergistic effects in combating oxidative damage and infections simultaneously[7]. Studies have shown that phytoconstituents such as flavonoids and tannins not only exert free radical scavenging effects but also paralyze or kill helminths by interfering with their energy metabolism and structural proteins[8].

The genus Tragia (family Euphorbiaceae) comprises about 150 species distributed mainly in tropical and subtropical regions. Plants under this genus are recognized for their characteristic stinging hairs and ethnomedicinal importance[9]. *Tragia plukenetii R. smith* is a climbing herb found in dry deciduous and scrub forests of India, particularly in Tamil Nadu and Andhra Pradesh. Morphologically, it is characterized by ovate leaves with stinging hairs, unisexual flowers, and small fruits.

Ethnomedicinally, Tragia species are used in traditional Indian medicine for treating skin infections, intestinal worms, wounds, inflammation, and respiratory ailments[10]. *Tragia plukenetii* in particular has been cited for its potential in managing intestinal worm infestations, making it a candidate of interest for anthelmintic evaluation. Phytochemical studies on related Tragia species have reported the presence of flavonoids, alkaloids, saponins, tannins and terpenoids, all of which are associated with significant antioxidant and anthelmintic activities.

Although Tragia species are known in traditional medicine, scientific validation of the pharmacological activities of *Tragia plukenetii R. smith* remains very limited. Most available reports focus on ethnomedicinal documentation rather than experimental pharmacological evidence. Furthermore, no comprehensive study has been conducted to correlate its phytochemical constituents with anthelmintic and antioxidant activity. This creates a clear research gap and highlights the need for systematic evaluation.

Therefore, the present study was undertaken to carry out a preliminary phytochemical screening of ethanolic leaf extract of *Tragia plukenetii R. Smith* and to evaluate its pharmacological potential through anthelmintic activity against Pheretima posthuma and antioxidant activity using in vitro radical scavenging assays. This will help to scientifically substantiate its ethnomedicinal uses and provide a foundation for further research on its therapeutic potential.

MATERIALS AND METHODS

Collection and Authentication of the Plant Specimen

The plant was gathered from the village of Sogathur in the Dharmapuri district, Tamilnadu. It was identified and authenticated by Assistant Professor Dr. S. Jagatheshkumar, Research Department of Botany, Sri Vijay Vidyalaya College of Arts and Science, Nallampalli, Dharmapuri.

Preparation of Extract

The plant leaves were cleared of any adhering debris, cleaned, chopped, allowed to air dry, and then ground into powder. In a Soxhlet apparatus, the powdered dried leaves of *Tragia plukenetii r.smith* were extracted using 99% ethanol until the siphon's colour varied. The extracts was filter and cooled. A rotary evaporator was used to concentrate the extracts under pressure until they had a syrupy consistency. A freeze dryer was then used to dry them[11].

Physicochemical Study and Phytochemical Screening

Phytochemical screening of *Tragia plukenetii R. smith* is a systematic qualitative analysis carried out using standard protocols to detect the presence of secondary metabolites such as alkaloids, flavonoids, tannins, phenols, saponins, terpenoids and glycosides[12]. Physicochemical study involves the quantitative evaluation of physical and chemical parameters of *T.plukenetii* dried leaf powder such as ash values, extractive values, loss on drying and foaming index[13].

In Vitro Pharmacological Studies

Antioxidant Activity

DPPH Radical Scavenging Assay

A 0.002% DPPH solution in methanol was prepared. The reference standard was gallic acid. Methanol was used to prepare various quantities of the *Tragia plukenetii* extract (100, 200 and 500µg/ml) and standard drug (1 and 2.5µg/ml). One millilitre of a 0.002% DPPH solution is mixed with one millilitre of each extract and standard concentration separately. After they were left in the dark for roughly 30 minutes and the optical density of these mixes was measured at 517nm. The blank was used methanol and 0.002% DPPH. Lastly, the formula is used to determine the percentage inhibition of the DPPH activity.

DPPH Scavenged (%) = $\mathbf{[(A_{control} - A_{test}) / A_{control}] \times 100}$

Whereas A_{test} is the absorbance when the extract sample is present, $A_{control}$ is the absorbance of the control reaction.

Reducing Power Assay

Distilled water was used to prepare various concentrations of the *Tragia plukenetii* extract (100, 200 and 500µg/ml) and the standard drugs (1 and 2.5µg/ml). Using distilled water, 1% potassium ferricyanide, 10% trichloroacetic acid, 0.1% ferric chloride, and 0.2M phosphate buffer were made. The reference standard was gallic acid. Next, 1ml of each extract and standard concentration was taken separately then mixed with 1ml of potassium ferricyanide and 0.2M phosphate buffer (pH 6.6). All of these samples should be incubated for 20 minutes at 50^{o}C. Next, add 1 millilitre of 10% trichloroacetic acid, and centrifuge for 10 minutes at 2000rpm. After removing the top layer (2.5ml), add 2.5ml of purified water and 0.5ml of freshly made ferric chloride. At 700nm, measure the absorbance last.

Anthelmintic Activity

Collection of Worms

Indian earthworms were used to study anthelmintic activity. The earthworm were collected from the moist soil from near region of Puliyur, Krishnagiri district.

In vitro Anthelmintic Assay

Indian earthworms, which are 3-5cm long and 0.1-2cm wide, were employed to study the anthelmintic activity of *Tragia plukenetii R. smith* leaves. Adult Indian earthworms Pheretima posthuman used because of their morphological and physiological similarities to the intestinal roundworm parasite of humans. With a few minor adjustments, the anthelmintic assay was conducted using Nargud's methodology. Ethanol extract dilutions of 10mg/ml, 25mg/ml and 50mg/ml were made. Control was normal saline. Standard was albendazole. In accordance with the extract procedure, albendazole (20mg/ml) was made using normal saline as a suspending agent. Each petri dish contained all of the test, standard, and control dilutions. Groups of three to five centimetre earthworms each, weighing between 0.32 and 0.50 grams, were placed in petri dishes with 20 millilitres of the standard and extracts of the desired concentration. The groups were then monitored for signs of paralysis or death[14].

In Vivo Pharmacological Studies

Animal Subject Employed in the Study

Healthy adult swiss albino mice weighing 20-30g were employed for this study. The animals are maintained under standard laboratory conditions with a 12 h light/12 h dark cycle, temperature 22 ± 2°C and relative humidity 50-60%, with free access to food and water. The institutional animal ethics committee, which was established to oversee and regulate animal experimentation, gave its approval to the animal studies. Animal care was taken as per the guidelines of CPCSEA, Government of India.

Acute Toxicity Test

The acute oral toxicity of *Tragia plukenetii* ethanolic leaf extract was evaluated in Swiss albino mice following the OECD Test Guideline No. 423 (Acute Toxic Class Method). Animals weighing 20-30g were fasted overnight and administered the extract once by oral gavage at fixed dose level of 5, 50, 300 and 2000mg/kg body weight, suspended in 1% CMC as vehicle. After dosing, the animals were closely observed for the first 4 hours for any immediate signs of toxicity. Thereafter, animals were monitored daily for 14 days for delayed signs of toxicity or mortality. The median lethal dose (LD_{50}) was estimated based on mortality data.

In vivo Anthelmintic Activity

Induction of Helminth Infection in Mice

One month before the trial, the mice utilised for anthelmintic research were dewormed for three days using albendazole (7mg/ml) in their drinking water. Prior to infection, the mice's body mass was measured. On day 0, the mice were given an oral gavage containing 100 L3 of Ascaris lumbricoide larvae, 0.1ml per mouse. The McMaster slide technique was used to demonstrate the presence of Ascaris lumibricoide in the freshly passed out faeces of experimentally infected laboratory mice after 13 days of infection. A tea spoon was used to collect the just passed out faeces of experimentally infected laboratory mice after 13 days of infection. A tea strainer and a 150ml sieve were used to filter the mixture after one gram of the faeces was ground up with a pestle in a mortar with 60ml of saturated NaCl solution. After being moved into a test tube, the filtrate was filled until an upper meniscus formed. Each tube was covered with a cover slip and let to stand for three minutes. This allowed the parasites' eggs to rise, float, and adhere to the cover slip. To verify the existence of Ascaris lumbricoide, the larvae were taken out and put on a slide for microscopic examination.

Experimental Design

Mice with severe Ascaris lumbricoide infection were divided into five groups of five at random. Additionally, they were categorised based on comparable body weights. Group 1 was treated normally, receiving only distilled water and regular animal feed. As a positive control, Group 2 mice received 40mg/kg of the common medication albendazole. Group 3 received 200mg/kg of *Tragia plukenetii R. Smith* ethanolic leaf extract as treatment. Group 4 received 400mg/kg of *Tragia plukenetii R. Smith* ethanolic leaf extract as treatment. The ethanolic leaf extract of *Tragia plukenetii R. Smith* was given to Group 5 at a dose of 800mg/kg body weight.

Fecal Egg Count Method

Every three days, 1-3 faecal pellets were taken from each mouse group (beginning on the fourteenth day after Ascaris lumbricoide inoculation) in order to count the *in-vivo* parasite egg output. Following the recovery of helminth eggs, the samples are homogenised in a specified volume of flotation solution (often a saturated salt or sugar solution) to enable the floating of parasite eggs. A pipette is used to put the suspension into the chambers of a McMaster counting slide after it has been filtered to remove debris. The slide is inspected under a low magnification microscope after the eggs have been allowed to float to the top layer. The amount of eggs produced per gram of faeces was calculated. For each mice sample, two grids of a McMaster slide were counted and the average was used as the eggs per gram of feaces for the parasite[15].

Statistical Analysis

All experimental values are expressed as Mean ± standard error of mean (SEM) for each group. Data were analyzed using one-way ANOVA followed by Dunnett's post-hoc test to compare treatment groups with the standard drug. Statistical significance was considered at $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$. The analyses were applied to antioxidant assays (DPPH radical scavenging and reducing power), *in-vitro* anthelmintic activity, in-vivo fecal egg count reduction.

RESULTS AND DISCUSSION

Physicochemical Parameters of Powdered Leaves

Physiochemical constant of the leaves of *Tragia plukenetii R. smith* such as ash value, loss of drying, crude fiber content, were determined and recorded. The results indicating that the plant material is of acceptable quality and purity.

Foaming Index

Foaming index is mainly performed to determine the saponin content in an aqueous decoction of the plant material and results were tabulated. The study showed that the height of foam gradually increased from 0.2cm to 1.5cm across the test samples. Since the foam height remained below 2cm, it indicates that the extract contains a low level of saponins.

Extractive Values

The extractive value represents the yield of active constituents obtained with a particular solvent. In this study, ethanol was used and yielded 60.5g of extract from the powdered leaves. Extractive value of extract of powdered leaves of *Tragia plukenetii R Smith* were tabulated.

Preliminary phytochemical analysis of the extract

Preliminary physiochemical screening was carried out for qualitative identification of the chemical compounds. It was found that presence of Alkaloids, Carbohydrates, Flavonoids, Saponins, Sterols and Tannins.

***In vitro* Pharmacological Studies**

DPPH Radical Scavenging Activity

The DPPH radical scavenging assay is a widely used method to evaluate the antioxidant potential of natural extracts. The ethanolic extract showed concentration-dependent radical scavenging activity, with maximum inhibition (79.08%) at 500µg/ml. However, it's IC_{50} (37.32µg/ml) was higher than Gallic acid (0.81 µg/ml), indicating lower potency. One-way ANOVA revealed significant differences among concentrations ($p < 0.05$), confirming dose-dependent antioxidant activity.

REDUCING POWER ASSAY

Absorbance increased with concentration, from 0.295 at 100µg/ml to 0.947 at 500µg/ml, demonstrating higher reducing capacity at higher doses. Standard gallic acid showed greater reducing power at much lower concentrations. The variation among concentrations was statistically significant ($p < 0.05$), showing that reducing power increased with dose.

***In vitro* anthelmintic activity**

The ethanolic extract produced paralysis and death of Pheretima posthuma in a dose-dependent manner. At 50mg/ml, it showed rapid effect, close to albendazole. ANOVA showed a highly significant difference among treatments ($p < 0.01$), confirming concentration-dependent anthelmintic efficacy.

In vivo Pharmacological Studies

Acute Toxicity Test

The acute toxicity test was conducted according to OECD guidelines to determine the safety margin of the ethanolic extract. Mice treated with doses up to 2000mg/kg did not show any mortality or behavioral signs of toxicity. This suggests that the LD_{50} of the extract is greater than 2000mg/kg, indicating that the plant extract is practically non-toxic. According to the OECD 423 classification, substances with LD_{50} values above 2000mg/kg are considered safe.

In-vivo Anthelmintic Activity (Fecal Egg Count)

The *in-vivo* study evaluated the effect of the ethanolic extract against Ascaris lumbricoides infection in mice. The control group showed a negative reduction (-38.55%), indicating natural progression of infection without treatment. Albendazole (40 mg/kg) showed 99.43% reduction in egg count. The ethanolic extract significantly reduced egg counts in a dose-dependent manner (78.92% at 200mg/kg to 90.81% at 800mg/kg). One-way ANOVA revealed a very high level of significance ($p < 0.001$), indicating strong dose-related anthelmintic effect compared to control.

Discussion

The present study involves the various evaluation like preliminary phytochemical and pharmacological activity of *Tragia plukenetii R. smith.* The findings obtained from the physicochemical, phytochemical, *in vitro* and *in vivo* evaluations provide a comprehensive understanding of the pharmacological profile of the plant.

The physicochemical evaluation of powdered leaves is important for establishing standardization and quality control parameters. The results revealed total ash content of 0.6%, which represents the total mineral content. The water-soluble ash (0.2%) indicates the presence of water-soluble inorganic salts, while acid-insoluble ash (0.26%) reflects the amount of silica or earthy matter present in the sample. Loss on drying was only 0.2%, suggesting low moisture content and good stability of the powdered leaves (Table No.1). Crude fiber was 0.1%, which represents indigestible plant cell wall material. All these values fall within pharmacopoeial standards, indicating that the plant material is of acceptable quality and purity.

The foaming index is used to estimate the saponin content of plant materials. The study showed that the height of foam gradually increased from 0.2cm to 1.5cm across the test samples. Since the foam height remained below 2cm, it indicates that the extract contains a low level of saponins. This result is consistent with preliminary phytochemical screening of similar plants, where tannins and flavonoids are usually more dominant than saponins (Table No.2).

The extractive value study demonstrated ethanol as the most efficient solvent for recovering bioactive constituents. Alcohol-soluble extractives were higher than water-soluble extractives, confirming the predominance of moderately polar phytochemicals. This validates the choice of ethanol as the extraction medium for pharmacological evaluations.

Preliminary phytochemical screening revealed the presence of multiple secondary metabolites, including alkaloids, carbohydrate, tannins, flavonoids, sterols and phenolics (Table No.4). Alkaloids and tannins are well known for their anthelmintic activity, while flavonoids and phenolics are potent antioxidants. The trace amount of saponin content corroborated with the low foaming index findings. This phytochemical diversity supports the traditional use of the plant in multiple therapeutic conditions and justifies further pharmacological studies.

The DPPH radical scavenging assay is a simple, quick, and sensitive way to screen plant extracts for antioxidants. It is also known to provide accurate information about the compounds antioxidant capacity. In the methanol medium, a freshly made DPPH solution has a strong blue hue. Through a free radical attack on the DPPH molecule, antioxidant molecules can quench DPPH free radicals (i.e., by donating electrons or providing hydrogen atoms) and turn them into colourless compounds like 2, 2-diphenyl-1-hydrazine or a substituted analogous hydrozine, which decreases absorbance at 517nm.

Therefore, the extract's antioxidant activity increases with the rate at which absorbance drops. The percentage of DPPH radicals scavenged by gallic acid and ethanolic leaf extract at different doses (µg/ml) is displayed in Table No.5. The soluble components in the ethanolic extract of *Tragia plukenetii r. Smith* leaves and the standard gallic acid, as a reference chemical, were found to have the capacity to scavenge DPPH radicals, resulting in a decrease in the concentration of this radical. The IC50 values for gallic acid and the ethanolic leaf extract of *Tragia plukenetii R. Smith* were determined to be 0.81µg/ml and 37.32µg/ml, respectively. This comparison shows that while the extract possesses antioxidant activity, it is less potent than the standard compound. The results suggest the presence of phenolic and flavonoid compounds in the extract, which are likely responsible for free radical scavenging. Statistical analysis using one-way ANOVA revealed significant differences between concentrations ($p < 0.05$), confirming that the effect was dose dependent.

The reducing power assay measures the electron-donating ability of plant extracts, which is an important mechanism of antioxidant activity. Antioxidant activity in the extract is determined by measuring the reducing power. Depending on each compound's reducing capability, the test solution's yellow colour in this assay shifts to different shades of green and blue. The Fe^{3+}/ferri cyanide complex employed in this procedure is changed to the ferrous form when reducers are present. The concentration of the Fe^{2+} ion can be ascertained by monitoring the production of Pearl's Prussian blue at 700nm. As the concentration of ethanolic leaf extract increased, so did the extract's reducing power. Which highlights the stronger antioxidant activity of the standard compound. Nevertheless, the extract demonstrated a consistent concentration-dependent trend, which supports its antioxidant potential (Table No.6). One-way ANOVA showed significant variation among different concentrations ($p < 0.05$), further validating that the observed increase was statistically significant.

Antioxidant assays confirmed the plant’s ability to scavenge free radicals and donate electrons. In the DPPH radical scavenging assay, the ethanolic extract displayed concentration-dependent inhibition, with higher doses showing activity comparable to ascorbic acid, the standard antioxidant. Similarly, the reducing power assay demonstrated the electron-donating ability of the extract, reflecting its potential to stabilize free radicals. These results validate the presence of flavonoids and phenolic compounds as major contributors to antioxidant activity. Since oxidative stress is a key factor in chronic degenerative diseases, *Tragia plukenetii* may have a role as a natural antioxidant in preventing oxidative damage.

The *in-vitro* anthelmintic study was carried out using Pheretima posthuma (earthworm) as a model, since its physiology closely resembles that of intestinal helminths. The ethanolic extract of *Tragia plukenetii* showed dose-dependent anthelmintic activity. At 10mg/ml, the time taken for paralysis and death was 42.67 ± 2.52 and 95.33 ± 3.06, respectively. At 25mg/ml, paralysis time was reduced to 24.33 ± 1.76 and death time to 58.67 ± 2.31, both of which were statistically significant compared to control. At the highest concentration of 50 mg/ml, paralysis occurred in just 12.67 ± 1.20 and death in 27.33 ± 1.76, which is comparable to the standard albendazole (20.67 ± 1.76 and 32.00 ± 3.21 at 20mg/ml) (Table No.7).

This suggests that the higher concentration of extract was almost as effective as the synthetic drug. The mechanism may be related to interference with the worm’s neuromuscular activity or inhibition of energy metabolism by phytochemicals such as tannins and alkaloids. ANOVA analysis confirmed that the differences among groups were highly significant ($p < 0.01$), indicating that the anthelmintic effect of the extract was strongly concentration dependent. This provides strong pharmacological evidence for the traditional use of *Tragia plukenetii* in treating helminth infections.

The *in-vivo* study evaluated the effect of the ethanolic extract against Ascaris lumbricoides infection in mice. The control group showed a negative reduction (-38.55%), indicating natural progression of infection without treatment. Albendazole (40mg/kg) showed the highest activity, with 99.43% reduction in egg counts, confirming its effectiveness as a standard drug. The ethanolic extract also demonstrated significant efficacy, with 78.92% reduction at 200 mg/kg, 83.07% at 400 mg/kg, and 90.81% at 800 mg/kg. The dose-dependent trend suggests that higher concentrations of the extract are more effective in controlling worm burden. Although the extract did not reach the same level of efficacy as albendazole, its strong performance supports the traditional use of *Tragia plukenetii* for treating helminthic infections. ANOVA analysis revealed very highly significant differences among the groups ($p < 0.001$), confirming that the extract produced a statistically meaningful reduction in fecal egg counts. The dual demonstration of both *in vitro* and *in vivo* efficacy strengthens the evidence for its therapeutic value (Table No.9).

Overall, the discussion highlights that *Tragia plukenetii* ethanolic extract possesses substantial pharmacological activities. The antioxidant effect can be linked to flavonoids and phenolic compounds, while the anthelmintic effect may be attributed to alkaloids and tannins. The results not only support the ethnomedicinal claims of this plant but also position it as a promising candidate for phytomedicine development. However, further studies on mechanism of action, and formulation development are necessary before clinical application.

Table No.1: Physico chemical parameters of powdered of leaves of *Tragia Plukenetii R. Smith*

S.No	Parameters	% W/W
1	Total ash	0.6
2	Water soluble ash	0.2
3	Acid insoluble ash	0.26
4	Loss on drying	0.2
5	Crude Fiber content	0.1

Table No.2: Foaming index of the powdered leaves of *Tragia Plukenetii R Smith*

S.No	Test volumetric flask no.(10ml)	Height of foam (cm.)
1	1	0.2
2	2	0.4
3	3	0.5
4	4	0.6
5	5	0.8
6	6	0.9
7	7	1.2
8	8	1.3
9	9	1.4
10	10	1.5

Table No.3: Percentage Yield of Ethanolic Extract of Powdered Leaves of *Tragia Plukenetii R Smith*

S.No	Extract	Yield %(W/W)
1	Ethanol Extract	60.5 g

Table No.4: Qualitative preliminary phytochemical screening of ethanolic extract of powdered leaves of *Tragia Plukenetii R Smith*

S.No	Phyto Constituents	Ethanolic exeract
1	Alkaloids	+
2	Carbohydrate	+
3	Flavonoids	+
4	Glycosides	-
5	Sterols	+
6	Saponins	+
7	Tannins	+

Table No.5: Results of DPPH radical scavenging activity of ethanolic leaf extract of *Tragia Plukenetii R. Smith*

S.No	Tested material	Concentration(μg/ml)	% Inhibition±SEM	IC_{50} (μg/ml)
1	Ethanolic leaf extract of *Tragia Plukenetii R. Smith*	100	60.67±0.01	37.32
		200	63.54±0.023	
		500	79.08±0.002	
2	Gallic acid	1	56.19±0.0125	0.81
		2.5	93.4±0.0007	

*Values are expressed as Mean ± SEM. One-way ANOVA followed by Dunnett's post-hoc test; significant differences observed at higher concentrations of extract when compared to Gallic acid ($p < 0.05$).

Table No.6: Effect of Ethanolic Leaf Extract of *Tragia Plukenetii R. Smith* on Reducing Power Assay

S.No	Tested material	Concentration(μg/ml)	Absorbance ±SEM
1	Ethanolic leaf extract of *Tragia Plukenetii R. Smith*	100	0.295±0.026
		200	0.491±0.020
		500	0.947±0.009
2	Gallic acid	1	0.471±0.011
		2.5	0.765±0.013

*Values are expressed as Mean ± SEM. One-way ANOVA followed by Dunnett's post-hoc test; extract showed significant increase in reducing power at higher concentrations compared to Gallic acid ($p < 0.05$).

Table No.7: *In-vitro* Anthelmintic activity of ethanolic leaf extract of *Tragia Plukenetii R. Smith*

S.No	Treatment group	Concentration (mg/ml)	Time taken for paralysis (in min)	Time taken for death (in min)
1	Normal saline	-	-	-
2	Albendazole	20mg/ml	20.67 ± 1.76	32.00 ± 3.21
3	TP Ethanolic Extract	10mg/ml	42.67 ± 2.52	95.33 ± 3.06
4	TP Ethanolic Extract	25mg/ml	24.33 ± 1.76*	58.67 ± 2.31**
5	TP Ethanolic Extract	50mg/ml	12.67 ± 1.20**	27.33 ± 1.76***

Values are expressed as Mean ± SEM. One-way ANOVA followed by Dunnett's post-hoc test; 25mg/ml extract group showed significant differences ($p < 0.05$, $p < 0.01$) and 50mg/ml group showed very highly significant differences ($p < 0.001$) compared to albendazole.

Table No.8: Toxicity report of ethanolic leaf extract of *Tragia Plukenetii R Smith*

S.No	Drug	Group	Dose (mg/kg)	Mortality	Symptoms of Toxicity
1	Control	I	1% CMC	0	None
2	*Tragia Plukenetii R.Smith* Leaf Extract	II	5 mg/kg	0	None
		III	50 mg/kg	0	None
		IV	300mg/kg	0	None
		V	2000 mg/kg	0	None

Table No.9: Percentage fall in fecal egg count of following treatment of Albendazole (40mg/kg) and *Tragia plukenitii R Smith* ethanolic leaf extract (200, 400 and 800mg/kg)

Groups	Treatment	Post infection	4days post treatment	8days post treatment	% fall in egg count
1	Control	240.60±13.42	315.20±16.87	332.40±12.11	-38.55± 7.10
2	40mg/kg Albendazole	210.80±12.55	92.60±10.25*	1.20± 0.28*	99.43±0.21*
3	200mg/kg *Tragia plukenitii R Smith* extract	228.40±18.76	169.80±11.32*	48.20 ±7.15*	78.92±2.73*
4	400mg/kg *Tragia plukenitii R Smith* extract	262.20±15.40	128.60±6.82*	44.40 ±6.40*	83.07±3.02*
5	800mg/kg *Tragia plukenitii R Smith* extract	248.00±9.12	121.40±5.33*	22.80 ±1.82*	90.81 ±0.42*

Values are expressed as Mean ± SEM (n = 5). One-way ANOVA followed by Dunnett's post-hoc test; ethanolic extract groups (200, 400, 800mg/kg) showed significant reduction in egg counts ($p < 0.01$, $p < 0.001$) compared to albendazole.

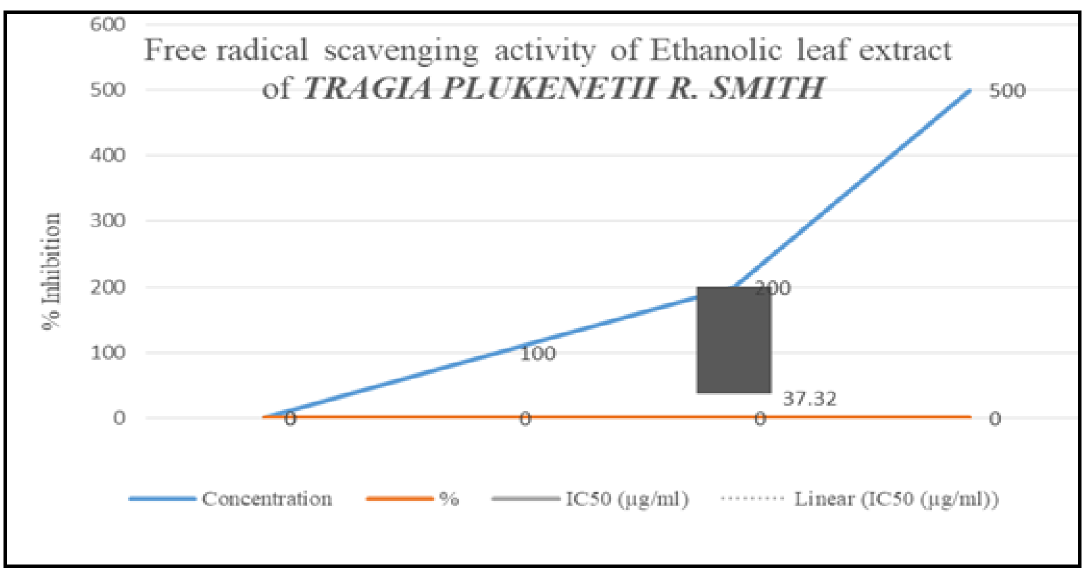

Figure No.1: Free radical scavenging activity of ethanolic leaf extract of *Tragia Plukenetii R. Smith*

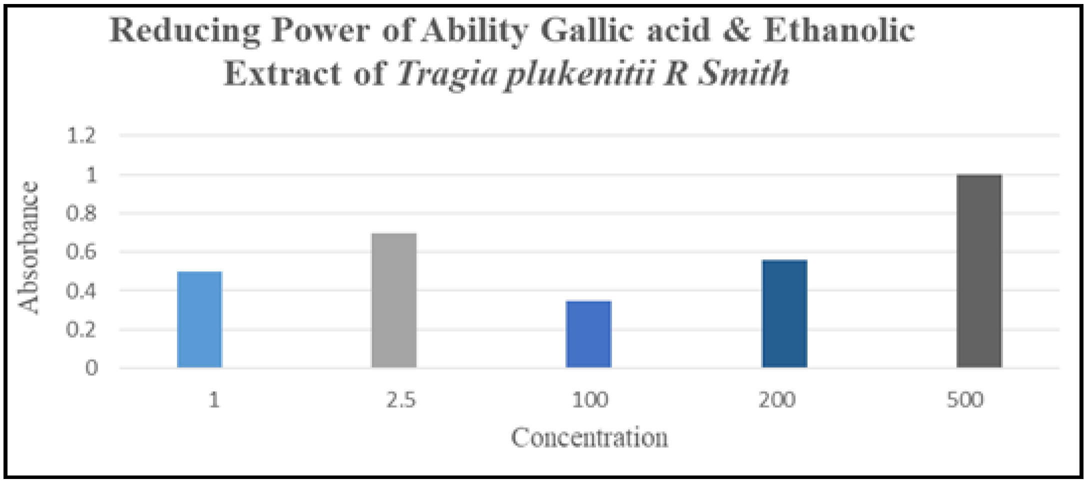

Figure No.2: Reducing power of ability gallic acid and ethanolic extract of *Tragia plukenitii R. smith*

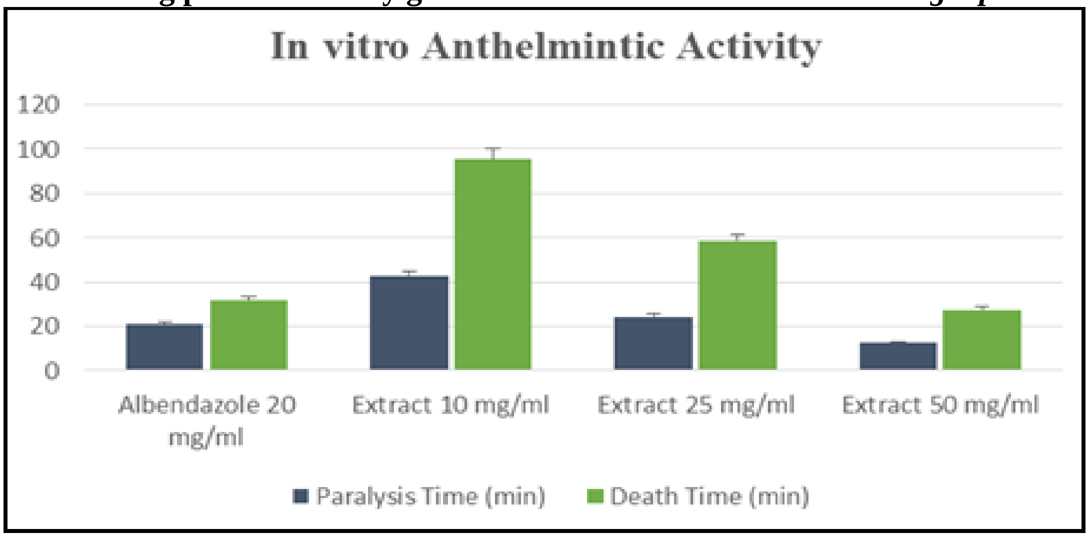

Figure No.3: Anthelmintic activity of *Tragia plukenetii R. smith* ethanolic leaf extract

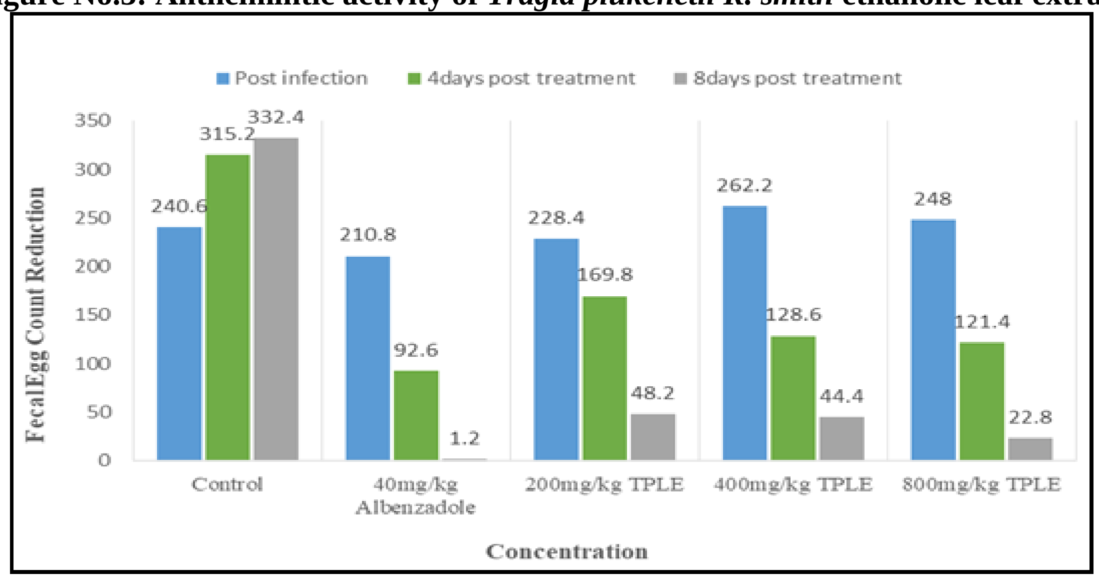

Figure No.4: Fecal egg count reduction

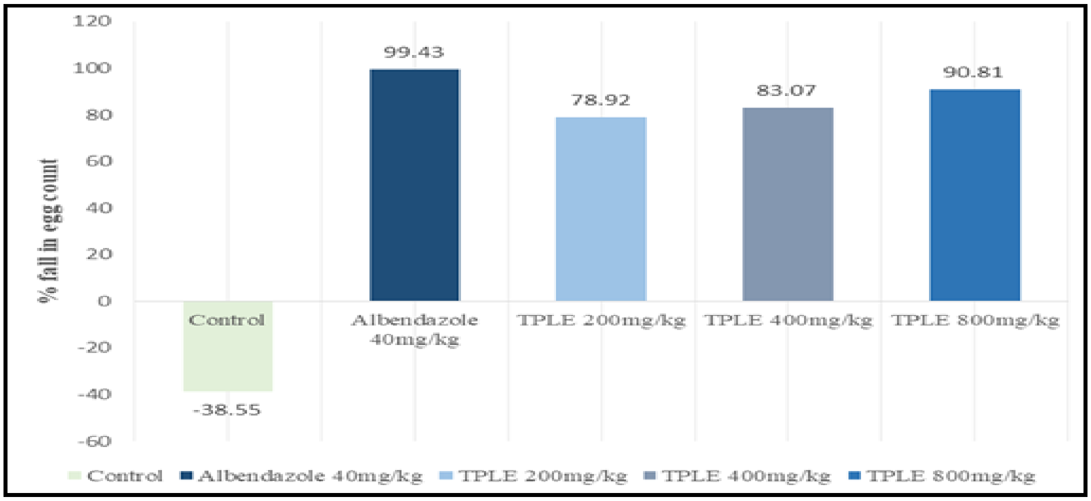

Figure No.5: Percentage fall in fecal egg count of Ascaris Lumbricoide

CONCLUSION

The plant *Tragia plukenetii R. smith* was collected and authenticated successfully. The dried plant leaves was extracted using ethanol by Soxhlet extraction technique. The Physicochemical Parameters revealed the Ash values and moisture content were within standard limits, indicating purity and low adulteration in the plant material. The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrate, flavonoids, tannins, sterol and phenolic compounds, which are responsible for its pharmacological effects. *In vitro* antioxidant assays (DPPH and reducing power) confirmed strong free radical scavenging activity, indicating potential in reducing oxidative stress related damage. In addition, strong antioxidant potential suggests additional health benefits. *In vitro* anthelmintic assays showed that the TPLE produced dose-dependent paralysis and death of worms, with results comparable to the standard drug albendazole. *In vivo* fecal egg count reduction in mice confirmed the extract's anthelmintic efficacy with statistical significance. In conclusion, the *Tragia plukenetii R. Smith leaf extract* exhibits promising antioxidant and anthelmintic properties. The antioxidant effect can be linked to flavonoids and phenolic compounds, while the anthelmintic effect may be attributed to alkaloids and tannins.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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