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PHARMACOLOGICAL EVALUATION, ANTIOXIDANT AND ANTI-PYRETIC ACTIVITY OF *ARCTIUM LAPPA* ROOTS EXTRACT IN EXPERIMENTAL RATS

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ABSTRACT

The present study was conducted to investigate the phytochemical constituents, antioxidant, and antipyretic activities of *Arctium lappa* root extracts in experimental animal models. The plant roots were extracted sequentially using petroleum ether and methanol via Soxhlet extraction. Preliminary phytochemical screening revealed the presence of alkaloids and saponins in both extracts, while glycosides and carbohydrates were found only in the methanolic extract. Quantitative estimation showed that the methanolic extract contained significant levels of total phenolic content (61.66 mg GAE/g) and flavonoid content (17 mg RE/g). Antioxidant activity assessed by DPPH assay showed a dose-dependent radical scavenging effect, with an IC_{50} of 48.74 µg/mL, indicating moderate activity compared to ascorbic acid. The antipyretic effect was evaluated using Brewer's yeast-induced pyrexia in Wistar rats. The methanolic extract at 200 mg/kg exhibited a significant reduction in rectal temperature, comparable to the standard drug paracetamol. The findings suggest that *Arctium lappa* root extract possesses promising antioxidant and antipyretic properties, potentially due to the presence of phenolic compounds and alkaloids.

Keywords: *Arctium lappa*, phytochemical screening, antioxidant activity, DPPH assay, antipyretic activity, Brewer's yeast-induced pyrexia, total phenolic content, flavonoids, herbal medicine, Soxhlet extraction.

INTRODUCTION

Herbal medicines derived from medicinal plants have been extensively used in traditional systems of medicine for the treatment of various diseases, owing to their safety, efficacy, and minimal side effects. *Arctium lappa*, commonly known as burdock, is a biennial plant native to Europe and Asia and has been widely utilized in traditional Chinese and Ayurvedic medicine for its diverse therapeutic properties (Chan *et al.*, 2011). The roots of *Arctium lappa* are particularly rich in bioactive constituents such as lignans, flavonoids, polyphenols, tannins, and inulin, which have been attributed to its wide range of pharmacological effects including antimicrobial, hepatoprotective, antidiabetic, and anti-inflammatory activities (Predes *et al.*, 2011; Lin *et al.*, 2014). Its antioxidant potential is mainly linked to the scavenging of free radicals and the prevention of oxidative stress-induced cellular damage, which is a important mechanism involved in many chronic conditions including inflammation and fever (Kong *et al.*, 2008).

Fever is a common symptom associated with many pathological conditions and is often a result of the release of pyrogenic cytokines

and prostaglandins. Plant-derived natural compounds have shown promising results in modulating these pathways and reducing elevated body temperatures (Olajide *et al.*, 2000). Given its traditional use and phytochemical profile, *Arctium lappa* root extract could serve as a potential natural remedy with antipyretic properties.

The present study aims to scientifically validate the traditional claims by evaluating the antioxidant and antipyretic activities of *Arctium lappa* root extract in experimental rat models. By assessing its pharmacological profile, the study seeks to explore the therapeutic potential of this medicinal plant in managing oxidative stress and pyrexia-related conditions.

MATERIALS AND METHODS

Materials

The study utilized a variety of analytical-grade reagents and solvents from reputed suppliers. Glacial acetic acid, nitroprusside, sodium hydroxide, and ammonia were procured from Merck. Petroleum ether was obtained from Researchlab, while concentrated sulfuric acid (H_2SO_4) was sourced from Fizmerck. Ethanol was purchased from Molychem, and both 95% alcohol and concentrated hydrochloric acid (HCl) were provided by Clorofiltind. Magnesium was supplied by Himedia, and chloroform came from Clorofiltind. Additionally, a 1% copper sulfate solution was obtained from Rankem. These reagents were used in various phytochemical and pharmacological evaluations throughout the study.

Plant collection and authentication

A total of 300 grams of the medicinal plant *Arctium lappa* (commonly known as burdock) roots were collected for the study. The roots were thoroughly cleaned to remove dirt and debris. Initially, they were shade-dried at room temperature for three days, followed by oven drying at 45°C until complete dehydration was achieved. The dried roots were then stored in airtight glass containers in a cool, dry environment to prevent contamination and degradation. The plant material was authenticated by a qualified plant taxonomist to confirm its botanical identity and ensure the purity of the selected species.

Extraction Procedure

The extraction of *Arctium lappa* root was carried out using the continuous hot percolation method in a Soxhlet apparatus. The dried and powdered root material was placed in the thimble of the Soxhlet unit. Initially, extraction was performed using petroleum ether as a non-polar solvent at a temperature of 60°C. After exhaustive extraction, indicated by the absence of color change in the siphon tube, the plant residue (marc) was air-dried and re-extracted using methanol as a polar solvent.

Each solvent extraction was continued until no further solvent color change was observed, indicating complete extraction. The obtained extracts were then concentrated using a rotary vacuum evaporator (Buchi-type) at 40°C to remove the solvents. The dried extracts were weighed, and the percentage yield for each was calculated using the following formula:

$$\% \ Yield = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} x100$$

Phytochemical investigation

Experiment was performed to identify presence or absence of different

phytoconstituents by detailed qualitative phytochemical analysis. The colour intensity or the precipitate formation was used as medical responses to tests. Following standard procedures were used (Sharma *et al.,* 2020).

Quantitative Phytochemical Estimation

Total Phenolic Content (TPC)

The total phenolic content (TPC) of *Arctium lappa* extract was estimated using the Folin–Ciocalteu method. Briefly, 0.2 mL of the plant extract (from stock solution) was mixed with 2.5 mL of folin–ciocalteu reagent and 2.0 mL of 7.5% sodium carbonate solution. The mixture was then diluted with distilled water to a final volume of 7 mL. The reaction mixture was incubated at room temperature for 2 hours. After incubation, the absorbance was measured at 760 nm using a UV-visible spectrophotometer. A calibration curve was prepared using standard solutions of Gallic acid (20–100 µg/mL). The phenolic content was expressed as milligrams of Gallic Acid Equivalent (GAE) per gram of dry extract (Dwivedi *et al.,* 2019). The Folin–Ciocalteu reagent reacts with reducing compounds, including phenolics, to form a blue chromogen that can be measured spectrophotometrically.

Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method. In this assay, 0.5 mL of *Arctium lappa* extract was combined with 2 mL of distilled water, followed by the addition of 0.15 mL of 5% sodium nitrite solution. After 6 minutes, 0.15 mL of 10% aluminum chloride solution was added, and the mixture was allowed to stand for another 6 minutes. Subsequently, 2 mL of 4% sodium hydroxide was added, and the solution was thoroughly mixed. The absorbance was measured at 510 nm using a UV-visible spectrophotometer. A standard calibration curve was constructed using Rutin (20–100 µg/mL), and results were expressed as milligrams of Rutin Equivalent (RE) per gram of dry extract (Sharma *et al.,* 2020).

Antioxidant Activity – DPPH Assay

The antioxidant activity of *Arctium lappa* root extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A stock solution of the extract (1 mg/mL in methanol) was prepared. From this, various concentrations (20–100 µg/mL) were tested. Each test solution (1 mL) was mixed with 2 mL of 0.1 mM DPPH methanolic solution. The reaction mixtures were vortexed and incubated in the dark at room temperature for 30 minutes. The absorbance was then recorded at 517 nm using a UV-visible spectrophotometer (Shimadzu 1700). A control sample containing only DPPH solution was also incubated and measured under the same conditions. Methanol served as the blank (Jain *et al.,* 2018). The antioxidant capacity was calculated using the following formula:

$$\%\ \text{Inhibition} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} X100$$

Acute Toxicity Study

The acute toxicity study was performed in accordance with the OECD guideline (1996) using the acute toxic class method. This method follows a stepwise procedure involving the use of three animals of the same sex per step. The test compound was administered orally, and the dose was selected from predefined levels: 5, 50, 300, and 2000

mg/kg body weight. Based on the observed mortality and/or moribund condition of the animals in each step, decisions were made to either terminate the test or proceed to the next dose level using three additional animals. On average, two to four steps were required to assess the acute toxicity of the test substance. The study helps determine the dose at which the substance exhibits no observable adverse effects or induces mortality.

Experimental Protocol for Animal Studies

All animal experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC), ensuring ethical compliance and proper handling. The study was conducted on Wistar albino rats, weighing 200 ± 50 grams, of either sex. Animals were housed in groups of six in standard polypropylene cages under controlled environmental conditions (22 ± 2°C temperature), with free access to standard pellet diet (Golden Feed, New Delhi) and clean drinking water.

Brewer's Yeast-Induced Pyrexia Model

To evaluate the antipyretic activity of *Arctium lappa* root extract, the Brewer's yeast-induced pyrexia model in rats was employed. The animals were divided into five groups (n = 6). Pyrexia was induced by a subcutaneous injection of 15% w/v Brewer's yeast suspension below the nape of the neck. The rectal temperature was recorded using a telethermometer just before and 18 hours after yeast administration to confirm the development of fever.

Following the induction of pyrexia, the treatment was administered orally to the respective groups:

- **Group I**: Normal control (received no yeast or treatment)
- **Group II**: Yeast control (received yeast but no treatment)
- **Group III**: *Arctium lappa* extract at 100 mg/kg body weight
- **Group IV**: *Arctium lappa* extract at 200 mg/kg body weight
- **Group V**: Standard treatment with Paracetamol at 150 mg/kg body weight

Post-treatment, the rectal temperature of all groups was monitored at 1, 2, 3, and 4 hours to assess the antipyretic effect (Sengar *et al.,* 2015).

RESULTS AND DISCUSSION

The current study investigated the phytochemical profile, antioxidant activity, and antipyretic potential of *Arctium lappa* root extracts in experimental models. The sequential extraction using petroleum ether and methanol by Soxhlet apparatus yielded 0.55% and 2.06%, respectively, as shown in Table 1. The higher yield with methanol indicates its greater solubilizing ability for polar phytochemicals present in *A. lappa* roots.

Phytochemical screening (Table 2) revealed that both extracts contain alkaloids and saponins, while glycosides and carbohydrates were detected only in the methanolic extract. The absence of flavonoids, tannins, phenolic compounds, and steroids in both extracts suggests a selective extraction profile, potentially due to the plant's phytochemical distribution or the nature of solvents used. The presence of alkaloids and glycosides might contribute to the pharmacological activities observed.

The antioxidant activity was assessed using the DPPH free radical scavenging assay. Methanolic extract of *A. lappa* demonstrated

dose-dependent radical scavenging activity, with a maximum inhibition of 60.43% at 100 µg/mL (Table 4). However, this was notably lower than the standard ascorbic acid, which showed 85.55% inhibition at the same concentration (Table 3). The IC_{50} value of the methanolic extract (48.74 µg/mL) was significantly higher than that of ascorbic acid (21.77 µg/mL), suggesting a moderate antioxidant potential.

Quantitative phytochemical analysis showed that the methanolic extract of *Arctium lappa* contains a total phenolic content of 61.66 mg GAE/g and total flavonoid content of 17 mg RE/g (Tables 7 and 10). These bioactive compounds are known for their free radical scavenging ability and may underlie the moderate antioxidant effect observed in the DPPH assay. The antipyretic activity was evaluated using Brewer's yeast-induced pyrexia in Wistar rats. As shown in Table 11**,** yeast administration significantly elevated rectal temperature in rats. Treatment with *A. lappa* extract at 100 mg/kg showed a mild reduction in temperature over 4 hours, whereas the 200 mg/kg dose exhibited a more pronounced and sustained antipyretic effect, reducing the temperature from $40.01 \pm 0.1°C$ to $38.10 \pm 0.2°C$. The standard drug paracetamol produced the most significant temperature reduction, affirming the validity of the model.

The antipyretic action of *Arctium lappa* may be attributed to the presence of alkaloids, glycosides, and phenolic compounds, which could inhibit prostaglandin synthesis or modulate cytokine levels. The extract's performance, particularly at 200 mg/kg, demonstrates a promising but moderate effect compared to standard therapy.

Table 1: Percentage yield of crude extracts of *Arctium lappa* extract

S. No.	Plant name	Solvent	Theoretical weight	Yield (gm)	% yield
1	*Arctium lappa*	Pet ether	288	1.60	0.55%
2		Methanol	298	6.15	2.06%

Table 2: Phytochemical testing of extract

Phytochemical test	Presence or absence of phytochemical test	
	Pet. Ether extract	**Methanolic extract**
Alkaloids		
Dragendroff's test	Present	**Present**
Mayer's reagent test	Present	**Present**
Wagner's reagent test	Present	**Present**
Hager's reagent test	Present	**Present**
Glycoside		
Borntrager test	Absent	**Present**

Killer-Killiani test	Absent	**Present**
Carbohydrates		
Molish's test	Absent	**Present**
Fehling's test	Absent	**Present**
Benedict's test	Absent	**Present**
Barfoed's test	Absent	**Present**
Flavonoids		
Shinoda's Test	Absent	**Absent**
Tannin and Phenolic Compounds		
Ferric Chloride test	Absent	**Absent**
Gelatin Test	Absent	**Absent**
Lead Acetate Test	Absent	**Absent**
Saponin		
Froth Test	Present	**Present**
Test for Triterpenoids and Steroids		
Salkowski's test	Absent	**Absent**
Libbermann-Burchard's test	Absent	**Absent**

Table 3: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	**Absorbance**	**% Inhibition**
20	0.482	51.313
40	0.433	56.262
60	0.342	65.454
80	0.283	71.414
100	0.143	85.555
Control	0.990	
IC50	**21.77**	

Table 4: DPPH radical scavenging activity of methanol extract of *Arctium lappa*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.518	44.108
40	0.464	49.837
60	0.453	51.027
80	0.412	55.459
100	0.366	60.432
Control	0.925	
IC50	**48.74**	

Table 5: Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.143
2.	40	0.178
3.	60	0.193
4.	80	0.230
5.	100	0.278

Table 6: Total phenolic content

S. No.	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1.	0.136	61.66 mg/gm

Table 7: Total phenolic content of extract *Arctium lappa*

Extract	Total phenolic content (mg/gm equivalent of Gallic acid)
Methanol	61.66

Table 8: Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.175
2.	40	0.200
3.	60	0.277
4.	80	0.318
5.	100	0.329

Table 9: Total flavonoid content

S. No.	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.147	17 mg/gm
2	0.160	
3	0.191	

Table 10: Total flavonoid content of extract *Arctium lappa*

Extracts	Total Flavonoid content (mg/gm equivalent of rutin)
Methanol	17

Table 11: Brewer's yeast induced pyrexia in rats

Rectal temperature (°C)					
Groups	**18 h after Yeast administration**	**Temperature after treatment**			
		1h	**2h**	**3h**	**4h**
Normal control	37.33±0.1	37.55±0.2	37.74±0.1	37.64±0.2	37.59±0.1
Yeast induced pyrexia group	40.10±0.1	40.06±0.3	40.02±0.2	39.99±0.1	39.81±0.3
***Arctium lappa* extract(100mg/kg)**	40.09±0.1	40.01±0.3	39.98±0.1	39.90±0.2	39.64±0.3
***Arctium lappa* extract(200mg/kg)**	40.01±0.1	39.90±0.1	39.73±0.2	38.99±0.1	38.10±0.2
Paracetamol (150mg/kg bw)	39.91±0.2	39.38±0.2	38.96±0.2	38.32.±0.1	37.65±0.2

CONCLUSION

The present study provides valuable insight into the pharmacological potential of *Arctium lappa* root extracts. The methanolic extract demonstrated a significant presence of phytochemicals such as alkaloids, glycosides, carbohydrates, and saponins, which are known for their therapeutic activities. Quantitative analysis confirmed that the extract is rich in phenolic and flavonoid compounds, which likely contributed to its observed antioxidant activity as demonstrated by the DPPH radical scavenging assay. Moreover, the methanolic extract exhibited a significant antipyretic effect in Brewer's yeast-induced pyrexia model in rats, particularly at a dose of 200 mg/kg, showing temperature-lowering effects comparable to

the standard drug, paracetamol. These findings validate the traditional use of *Arctium lappa* in treating fever and oxidative stress-related disorders. In conclusion, *Arctium lappa* root extract, particularly the methanolic fraction, possesses notable antioxidant and antipyretic properties. Further studies are warranted to isolate the active constituents, explore the mechanisms of action, and evaluate its efficacy through clinical trials for potential development into natural therapeutic agents.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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