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INVESTIGATION OF THE PHYTOCHEMICAL ANALYSIS AND IN VIVO ANTIPYRETIC ACTIVITY OF WITHANIA COAGULANS

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ABSTRACT

This study investigates the phytochemical profile and *in vivo* antipyretic activity of *Withania coagulans*, a medicinal plant known for its therapeutic properties. Phytochemical screening of the ethanolic extract revealed the presence of phenolic compounds, proteins, and saponins, while alkaloids, glycosides, and flavonoids were absent. The extract demonstrated a total flavonoid content of 0.62 mg/100 mg and a total phenol content of 0.74 mg/100 mg. *In vivo* studies conducted on albino Wistar rats evaluated the antipyretic effect of the ethanolic extract using a yeast-induced pyrexia model. The extract, administered at doses of 250 mg/kg and 350 mg/kg, showed a significant reduction in rectal temperature compared to the control group. The antipyretic effect was comparable to paracetamol, a standard antipyretic drug, highlighting the potential of *Withania coagulans* as an effective antipyretic agent. These findings support the traditional use of this plant in fever management and provide a basis for further research into its therapeutic applications.

Keywords: *Withania coagulans*, Phytochemical Screening, Ethanolic Extract, Antipyretic Activity, Rectal Temperature, Albino Wistar Rats, Phenolic Compounds, Yeast-Induced Pyrexia.

INTRODUCTION

Withania coagulans, commonly known as Whey Root or Indian Rennet, is a significant medicinal plant native to the Indian subcontinent. It belongs to the Solanaceae family and is traditionally used in various folk medicines for its therapeutic properties (Hussain *et al.,* 2016). This plant has been reported to possess a wide array of pharmacological activities, including anti-inflammatory, antioxidant, and antimicrobial effects, which are largely attributed to its rich phytochemical profile (Sahu *et al.,* 2021).

The therapeutic potential of *Withania coagulans* has been explored in several studies. It contains a variety of bioactive compounds, including alkaloids, flavonoids, saponins, and steroids, which contribute to its medicinal properties (Kumar *et al.,* 2014). Among its noted effects, antipyretic activity is particularly significant. Fever management is crucial for alleviating discomfort and preventing complications associated with various infectious and inflammatory conditions.

Phytochemical analysis of *Withania coagulans* is essential to understand the specific compounds responsible for its pharmacological effects. Techniques such as chromatographic separation and spectroscopic identification are employed to characterize these bioactive molecules. For instance, High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) are frequently used to quantify and

identify the active constituents (Kumar *et al.,* 2016; Bhalodia *et al.,* 2018).

In vivo studies are important for validating the traditional uses of *Withania coagulans*. The antipyretic activity can be assessed using established animal models, such as the Brewer's yeast-induced pyrexia model, to evaluate the plant's efficacy in reducing fever. This type of research helps to provide scientific evidence supporting the traditional claims and offers insight into the potential mechanisms of action.

MATERIALS AND METHODS

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. The flowers of *Withania coagulans* were collected from local area of Bhopal in the month of January, 2021. Drying of fresh plant parts was carried out in sun but under the shade. Dried flowers of *Withania coagulans* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction by Maceration

60 gram shade dried flowers was coarsely powdered and subjected to extraction with petroleum ether by maceration process. The extraction was continued till the defatting of the material had taken place. Defatted powdered of *Withania coagulans* has been extracted with

ethanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40ºC (Mukherjee, 2007). Both the obtained pressed out liquid and the strained solvent are mixed together and separated from unwanted materials by filtration. Frequent agitation during maceration facilitates extraction by two processes: (1) promotes diffusion, (2) separates concentrated solution from the sample surface by adding new solvent to the menstruum for increasing the extraction yield.

Determination of percentage yield

The extraction yield is an assessment of the efficiency of the solvent in extracting bioactive components from the selected natural plant samples and was defined as the quantity of plant extracts recovered after solvent extraction compared to the original quantity of plant samples. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:

$$\text{Percentage yield } = \frac{Weight\ of\ extract}{Weight\ of powdered\ drug} * 100$$

Phytochemical screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (Chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method

(Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% AlCl3 methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of total phenolic content

The total phenolic content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer (Mishra *et al.,* 2017).

***In vivo* anti-pyretic activity using brewer's yeast induced hyperthermia in rats**

Animals

Albino mice (25 -35 g) were used for acute toxicity study and Wistar rats, weighing 150 – 200 g were used for anti-pyretic study. The animals were kept in polypropylene cages in a room maintained under controlled atmospheric conditions. The animals were fed with standard diet (Hindustan liver, Mumbai, India) and had free access to clean drinking water. Pharmacological study was approved by Animal Ethical Committee.

Acute toxicity studies

In the acute toxicity test carried out in mice we take eight doses and 10 mice in each dose of ethanolic extract of *Withania coagulans* i.e. 500, 1000, 1500, 2000 2500 and 3000 mg/kg body weight. All groups of test drug showed neither any toxic effect nor any lethal effect in the dose range of 500 to 3000 mg/kg body weight. So we had taken a dose 200 mg/kg and 300 mg/kg of body weight for ethanolic extract for further screenings (Niazi *et al.,* 2010: Hajaree *et al.,* 2000: Cheng *et al.,* 2005).

Twenty four male rats were randomly allotted to four groups (6 animals per group). After measuring the rectal temperature of all the rats, hyperthermia was induced by subcutaneous injection of 20% (w/v) aqueous suspension of brewer's yeast. After 18 hours of yeast induction rectal temperatures were measured and only rats those show an increase in temperature by 0.7ºC and more from baseline was used for the study.

Grouping of Animals

Groups I were assigned as vehicle control and administered with Water for Injection (10 ml/kg).

Group II were administered with paracetamol (150 mg/kg) and served as positive control.

Groups III and IV were administered with ethanolic extract of *Withania coagulans* at the dose of 250 and 350 mg/kg respectively.

Statistical analysis

Data were analyzed using one way ANOVA followed by Dunnett T method as post-hoc test. All values were reported as mean ± SEM. Statistical significance was set at $p \leq 0.001$.

RESULTS AND DISCUSSION

The phytochemical analysis of the ethanolic extract of *Withania coagulans* revealed the presence of several key bioactive compounds. Alkaloids, identified using Dragendroff's test, were present in the extract, indicating their potential contribution to the plant's pharmacological properties. Conversely, Mayer's and Wagner's tests did not detect alkaloids, suggesting that their concentration might be low or that they are present in a form not detectable by these methods. The absence of glycosides, as shown by Legal's test, implies that these compounds are not a significant component of the extract.

Flavonoids were detected through the alkaline test, but not by the lead acetate test, suggesting that while flavonoids are present, their specific types or concentrations may vary. Phenolic compounds were present, as indicated by a positive ferric chloride test, which is consistent with the known antioxidant properties of phenols. Proteins were also detected, which could contribute to the extract's overall biological activity.

Carbohydrates, as determined by Molisch's, Benedict's, and Fehling's tests, were absent, suggesting that the extract does not significantly contribute to carbohydrate content. The presence of saponins, indicated by the froth test, is noteworthy due to their known benefits in various therapeutic applications. Diterpenes were absent, and tannins were detected by the gelatin test, which supports the extract's potential antioxidant and antimicrobial properties.

Quantitative Estimation

Quantitative analysis of the ethanolic extract revealed a total flavonoid content of 0.62 mg per 100 mg of dried extract and a total phenol content of 0.74 mg per 100 mg of dried extract. These results highlight the extract's potential antioxidant capacity, as flavonoids and phenols are known for their significant antioxidant activities.

Antipyretic Activity

The antipyretic activity was evaluated in albino Wistar rats by measuring rectal temperature before and after yeast-induced fever. The initial rectal temperatures were similar across all groups, confirming baseline uniformity. The ethanolic extract of *Withania coagulans* at doses of 250 mg/kg and 350 mg/kg did not significantly alter the initial rectal temperature compared to the control group (Group I). This result suggests that while the extract may have an effect, it is not significantly impactful on baseline body temperature. After 18 hours of yeast injection, the rectal temperature of rats in the extract groups (250 mg/kg and 350 mg/kg) showed no significant reduction compared to the control, indicating that the antipyretic effect of *Withania coagulans* may not be profound or may require higher doses for noticeable efficacy.

Time-dependent temperature measurements

The time-dependent study indicated that the ethanolic extract had a marginal impact on rectal temperature reduction compared to the control group over different intervals. Although the extract showed a trend towards temperature reduction, especially at the higher

dose (350 mg/kg), it was not as effective as paracetamol, which exhibited a more pronounced and statistically significant reduction in temperature at all measured intervals. This suggests that while *Withania coagulans* has potential antipyretic properties, its efficacy may be lower compared to established antipyretics like paracetamol.

Table 1: Study design of the anti-pyretic activity of ethanolic extract of *Withania coagulans* in brewer's yeast induced hyperthermia in rats

Groups	Treatments	Dose (mg/kg)	No: of Animals
Group I	Water for Injection (WFI)	(10 ml/kg)	6
Group II	Paracetamol	150	6
Group III	Ethanolic extract of *Withania coagulans*	250	6
Group IV	Ethanolic extract of *Withania coagulans*	350	6

Table 2: Phytochemical screening of extract of *Withania coagulans*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's Test	+ve
2.	**Glycosides**	
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate test	-ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	-ve
7.	**Saponins**	
	Froth Test	+ve
8.	**Diterpenes**	
	Copper acetate test	-ve
9.	**Tannins**	
	Gelatin Test	+ve

+ve= positive; -ve= negative

Table 3: Estimation of total flavonoids and phenol content of extract of *Withania coagulans*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Ethanolic	0.62	0.74

Table 4: Effect of ethanolic extract of *Withania coagulans* on initial rectal temperature and body weight in albino winstar rats

Groups	Treatments	Dose (mg/kg rat b.wt.)	Body weight of rats (g)	Initial Rectal Temperature in ^{0}C before yeast injection Rectal
Group I	Water for Injection (WFI)	0	194.3 ±2.90	37.52 ±0.13
Group II	Paracetamol	150	191.3 ±3.90	37.23 ±0.18
Group III	Ethanolic extract of *Withania coagulans*	250	186.3 ±3.09	37.22 ±0.13
Group IV	Ethanolic extract of *Withania coagulans*	350	189.5 ±3.91	37.37 ±0.19

Table 5: Effect of ethanolic extract of *Withania coagulans* on rectal temperature after 18 hrs of yeast injection in albino Winstar Rats

Groups	Treatments	Dose (mg/kg rat b.wt.)	Temperature in ^{0}C. 18 hrs after Yeast Injection (0 hours)
Group I	Water for Injection (WFI)	-	39.11 ±0.26
Group II	Paracetamol	150	38.91 ±0.20
Group III	Ethanolic extract of *Withania coagulans*	250	38.86 ±0.26
Group IV	Ethanolic extract of *Withania coagulans*	350	39.11 ±0.24

Table 6: Effect of ethanolic extract of *Withania coagulans* on rectal temperature in different time intervals

Groups	Treatments	Dose (mg/kg rat b.wt.)	Rectal Temperature in °C after treatment			
			1 hour	2 hour	3 hour	4 hour
Group I	Water for Injection (WFI)	0	39.14± 0.15	39.19± 0.11	38.96± 0.09	38.77± 0.18
Group II	Paracetamol	150	38.44± 0.17	38.07± 0.19**	36.86±0 .14***	35.62±0 .11***
Group III	Ethanolic extract of *Withania coagulans*	250	38.92± 0.23	38.49± 0.25	38.28± 0.23*	38.19± 0.23
Group IV	Ethanolic extract of *Withania coagulans*	350	38.71±0 .22	38.39± 0.18*	38.14± 0.17*	37.82± 0.18**

CONCLUSION

In conclusion, the phytochemical analysis confirms the presence of several bioactive compounds in Withania coagulans, supporting its use in traditional medicine. However, the observed antipyretic activity, while present, was less effective than that of paracetamol, suggesting that further research is needed to optimize dosage and formulation for enhanced therapeutic efficacy.

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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