



Phytochemical Evaluation and Pharmacological Screening of *Saraca celebica* W.J Wilde and *Asparagus fallax* Plant Extract for Central and Peripheral Analgesic Activity

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Received: March 24, 2025

Published: April 09, 2025

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Abstract

Nutraceuticals such as *Saraca celebica* W.J Wilde and *Asparagus fallax* were extracted by ethanolic extract. These extracts were subjected to examine pharmacological action by centrally acting analgesic activity using tail immersion method and peripheral acting analgesic activity using acetic acid induced writhing test in rats. The crude extract of the plant was found to have significant ($p < 0.01$) analgesic activity at the oral dose in the test models of tail immersion, *Saraca celebica* W.J Wilde showed increased time latency than *Asparagus fallax*, where as in acetic acid induced writhing test *Asparagus fallax* showed reduced number of writhes than *Saraca celebica* W.J Wilde at dose levels which have significant ($p < 0.001$) compared to control. The result obtained support the use of the plants *Saraca celebica* W.J Wilde and *Asparagus fallax* in painful condition acting both centrally and peripherally.

Keywords: *Saraca celebica* W.J Wilde Analgesic Activity; *Asparagus fallax*

Introduction

Medicinal plants play a crucial bit in the evolution of potent therapeutic agent [1]. WHO estimated that 80% of the people in the growing countries depend on herbal plants for primary fitness needs [2]. Pain is the most usual trouble for which human seek medical awareness. Analgesics are mostly prescribed drug in clinical observation [3]. Pain is an experience which is tough to explain exactly. Characteristically, it is a response to an unexpected occasion related with tissue damage, such as bruise, swelling or cancer, but chronic pain can develop independent of any liable cause, or continue long after the injury has healed [4]. The international association of pain defines "pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such disease" [5]. Pain might be called a stimulus modality and defender in nature but mostly causes inconvenience simply [6].

Materials

Drugs and chemicals

Ethanol and Acetic acid from S.D FINE chemical private limited, Aspirin was obtained from Cipla.

Plant material

The collected plant part was dried for one week and ground coarse powder with the help of pulveriser.

Animals

Albino male Sprague dawley rats weighing between 120-150 grams were purchased from Sainath enterprises, uppal, after the AIEC clearance. They were kept in polypropylene cages in standard environment conditions at $27.0 \pm 2^\circ$ C temperature and 55-65% relative humidity and 12 hours light/12 hours dark cycle [7,8]. Allowed to adapt the habitat for 2 weeks prior experiment. They were given pellet diet and water ad libitum.

Methodology

Preparation of extract

100 grams of powdered plant material was put in a beaker with suitable solvent so that the entire material is uniformly moistened by the solvent. This mixture is subjected to soxhlet apparatus. The extraction process is continued till whole solvent become colourless and a semisolid extract is obtained. The extract was transferred to an air tight container for protection and further use [9].

Phytochemical screening

Phytochemical screening of ethanolic extract was carried out to detect the presence of Alkaloids (Mayer’s, Wagner’s and Dragendorff’s test), Flavonoids (Lead acetate test), Steroids (Salkowski test, Liebermann burchad reaction), Glycosides (saponin glycosides- foam test), and Phenols (Acetic acid test) [10,11].

Acute toxicity

Safety study was done by fixed dose study (OECD 420) guidelines. 5 animals are tested with same dose of sighting studies (200mg/kg) and are observed for 15 days. All observations are recorded for each animal such as change in skin and fur, eyes and mucous membrane, respiratory system, circulatory system, autonomic and central nervous system [12].

Statistical analysis

Data will be analysed by applying ANOVA (Analysis of variance) followed by Tukey Kramer multiple comparison test using Graphpad Prism software. P-Value (Probability) is calculated for significance and will be expressed as mean ± SEM.

Analgesic activity

Tail immersion

Acute nociception was assessed using the tail immersion test described by Vogel and Vogel (1997) [13]. This method entails immersing the extreme 3 cm of the rats tail in a water bath. Rats weighing 200-230grams are selected and grouped, each having 6 animals. The animals are subjected to tail immersion and the time latency is recorded. Group-I of 6 rats are used as control. Group-II receives standard drug aspirin. Group-III receives *Saraca celebica* W.J WILDE. Group- IV receives *Asparagus fallax*. The temperature is maintained at 55° C. The time latency is noted before and after 0, 5, 10, 15, 20, 25, 30, 45, 60, 90 and 120 minutes following oral administration of standard (aspirin 300mg/kg) or the test drug [14-17].

Acetic acid induced writhing

In this method acetic acid is administered intraperitoneally to the experimental animal to create pain sensation. Rats weighing 200-230grams are selected and grouped, each having 6 rats. Acetic acid is injected intraperitoneally. Group-I of 6 rats is used as control given acetic acid. Group- II receives standard drug aspirin. Group-III receives *Saraca celebica* W.J WILDE. Group-IV receives *Asparagus fallax*. Animals are given the test and the standard drug (aspirin 300mg/kg) at various pre-treatment times prior to acetic acid. The test samples and vehicle are administered orally 30 minutes prior to acetic acid injection. Then the animals are placed on the observation table. The rats are observed for 10 min and the writhes are noted [18-20].

Result and Discussion

Phytochemical evaluation

Saraca celebica W.J Wilde

S. No	Tests	Ethanol
1.	Carbohydrates:	
	i. Benedict’s Test	++
	ii. Fehling’s Test	+
	iii. Molisch Test	+++
2.	Test For Gums	+
3.	Test For Proteins:	+
	i. Biuret Test	+
	ii. Millon’s Test	+
4.	Test For Amino Acids:Ninhydrin Test	+
5.	Test For Alkaloids:	
	i. Dragondroff’s Test	+
	ii. Mayer’s Test	+
6.	Test For Phenols: Acetic Acid Test	+
7.	Test For Oils: Solubility For Benzene	+
8.	Test For Saponin Glycosides: Foam Test:	+
9.	Test For Steroids: Salkowski Test:	+++
10.	Test For Flavonoids:	+++

Table 1: Phytochemical evaluation of *Saraca celebica* W.J Wilde.

Asparagus fallax

S. No	Tests	Ethanol
1.	Carbohydrates:	
	i. Benedict's Test	+++
	ii. Fehling's Test	+++
	iii. Molisch Test	+++
2.	Test For Gums	+
3.	Test For Proteins:	+
	i. Biuret Test	+
	ii. Millon's Test	+
4.	Test For Amino Acids:Ninhydrin Test	+
5.	Test For Alkaloids:	+
	i. Dragondroff's Test	+
	ii. Mayer's Test	+
6.	Test For Phenols: Acetic Acid Test	+
7.	Test For Oils: Solubility For Benzene	+
8.	Test For Saponin Glycosides: Foam Test:	+
9.	Test For Steroids:Salkowski Test:	+++
10.	Test For Flavonoids:	+++

Table 2: Phytochemical evaluation of *Asparagus fallax*.

The phytochemical evaluation of various extracts of *Saraca celebica* W.J Wilde showed presences of all the main chemical constituents like, alkaloids, phenols, glycosides, steroids and flavonoids. While other extracts of 1- butanol, n- heptane, acetone indicates the non-appearance of the vital constituents. Various extracts of *Asparagus fallax* showed the absences of the phytochemical evaluation of various extracts of *Saraca celebica* W.J Wilde showed presences of all the main chemical constituents like, alkaloids, phenols, glycosides, steroids and flavonoids. While other extracts of 1- butanol, n- heptane, acetone indicates the non-appearance of the vital constituents. Various extracts of *Asparagus fallax* showed the absences of the vital constituents while this ethanolic extracts showed the presences of the important constituents such as alkaloids, glycosides, steroids, phenols and flavonoids. So ethanolic extracts of both *Saraca celebica* W. J Wilde and *Asparagus fallax* was selected for main trail.

Safety study

The plant under the study were put to safety study by fixed dose method and screened by blind screening method which were found safe which was assessed by the test for somatic and auto-

nomnic activities by the blind screening method by Irwin (1982) which is a comprehensive observational technique whose scoring is observed for awareness, mood, locomotor activity, CNS excitation, posture, motor incoordination, muscle tone, reflexes, autonomic, miscellaneous and death.

Preclinical studies

Peripherally acting (acetic acid induced writhing)

S. No	Group	MEAN ± SEM
1	Untreated	15.5 ± 1.478
2	Standard	4.16 ± 0.6009
3	<i>Saraca celebica</i> w.j wilde	14.66 ± 2.459
4	<i>Asparagus fallax</i>	8.83 ± 1.493

Table 3: Acetic acid induced writhing.
While ***P < 0.001 between groups.

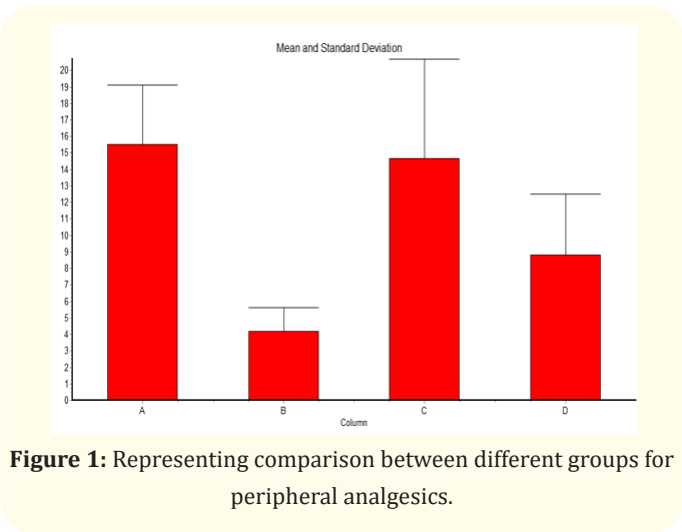


Figure 1: Representing comparison between different groups for peripheral analgesics.

Centrally acting (tail immersion test)

Ethanolic extract of *Saraca celebica* W.J Wilde and *Asparagus fallax* were treated with peripheral (acetic acid induced writhing) and central analgesic activity (tail immersion) methods. Statistical analysis found that.

Ethanolic extract of *Asparagus fallax* were subjected to peripheral analgesic activity by acetic acid induced writhing method statistical analysis found that

S. No	Group	MEAN ± SEM
1	Untreated	3.06 ± 0.282
2	Standard	3.628 ± 0.42
3	<i>Saraca celebica</i> w.j wilde	2.696 ± 0.194
4	<i>Asparagud fakkax</i>	3.27 ± 0.465

Table 4: Tail immersion.

P-value is 0.32. While *p > 0.1 between each group.

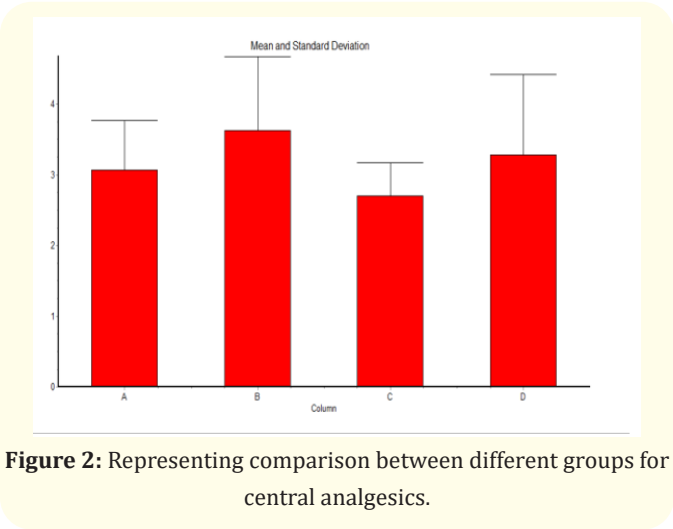


Figure 2: Representing comparison between different groups for central analgesics.

- Standard vs Control P < 0.001***
- Control vs Test 1 P < 0.05*
- Control vs Test 2 P < 0.05*
- Standard vs Test 1 P < 0.01**
- Standard vs Test 2 P < 0.05*
- Test 1 vs Test 2 P < 0.05*

Peripheral analgesic activity between groups, under study of *Asparagus fallax* was originated to be more significance in showing peripheral analgesic in comparison with other group.

Ethanollic extracts of *Saraca celebica* W.J Wilde were also subjected to central analgesic activity by tail immersion method. Statistical analysis found that

- Standard vs Control P < 0.01**
- Control vs Test 1 P < 0.01**
- Control vs Test 2 P < 0.01**
- Standard vs Test 1 P < 0.01**
- Standard vs Test 2 P < 0.01**
- Test 1 vs Test 2 P < 0.05*

In central analgesic activity between groups under study *Saraca celebica* W. J Wilde was originated to be more significant in showing central analgesic activity in comparison with other groups. The main trail results were alike to the previous studies [21].

Conclusion

Saraca celebica W.J Wilde and *Asparagus fallax*, the plant under the study was found to be safe which were studied using fixed dose method by blind screening method pf scoring. Somatic and autonomic effects of drugs were studied which was found to be safe.

Both the plants under the trails were found to possess the analgesic activity with verifying degree of activities in comparison with one another when studied for central and peripheral analgesic effects and found that both the plant effects by different mechanism of action i.e., *Asparagus fallax* has significant peripheral analgesic activity and *Saraca celebica* W.J Wilde has central analgesic activity.

The analgesic property of the plant under the study may be because of the important chemical constituents such as alkaloids, glycosides, flavonoids, phenols and steroids. These plants can be studied further by fractionation to pin point the extract constituents for analgesic activity.

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