

ANTIDIARRHEAL ACTIVITY OF STEM BARK EXTRACTS OF  
*SPATHODEA COMPANULATA* IN RODENTS.

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Summary

The objective of the study is to investigate the aqueous (AQSP) and alcoholic extracts (ALSP) of stem barks of *Spathodea companulata* (Bignoniaceae) for their antidiarrheal activity in rodents. Stem bark was extracted with alcohol and water successively. Preliminary phytochemical investigation was carried out to identify various phytochemical constituents present in the extracts. It was found that the ALSP contains alkaloids, carbohydrates, glycosides, saponins, steroids, flavonoids, tannins and phenolic compounds; AQSP contained carbohydrates, glycosides, saponins, flavonoids, tannins and phenolic compounds. Acute oral toxicity of ALSP and AQSP were conducted as per OECD guidelines 425. Acute toxicity studies revealed that both the extracts are safe upto 2000mg/kg. The antidiarrheal activity was observed in three experimentally induced diarrhea models i.e. Castor oil induced diarrhea; Prostaglandin E2 (PG-E2) induced enteropooling in rats and charcoal meal test in mice. In castor oil induced model ALSP and AQSP showed significant dose dependent reduction of cumulative wet faecal mass. In PG-E2 induced enteropooling model, ALSP (50,100 and 200mg/kg, p.o.) and AQSP (50,100 and 200mg/kg, p.o) inhibit PG-E2 induced secretions. Similarly in charcoal meal test ALSP and AQSP decreased the movement of charcoal indicating its antimotility activity. It was observed that AQSP is having more potent anti-diarrheal activity than ALSP in these models.

**Keywords:** Antidiarrheal activity, *Spathodea companulata*, castor oil, Prostaglandin E2, Charcoal meal test.

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### **Introduction**

Diarrhea, an important health problem worldwide, while not life-threatening, have caused much discomfort affecting the quality of life. especially in developing countries, accounts for more than 5-8 million deaths in infants and children under 5 years, each year [1,2]. In recent years there has been a great interest in herbal remedies for the treatment of a number of ailments .Medicinal plants are promising source of antidiarrheal drugs [3]. Indigenous plants such as *Andrographalis paniculata*, *Asparagus racemosus*, *Butea monosperma*, *Cassia auriculata*, *Ficus hispida*, *Hemidesmus indicus*, *Guiera senegalensis*, *Thespesia populnea* etc are widely used for treatment of diarrhea [4,5]. *Spathodea campanulata* P. Beauv. is a species belonging to the Bignoniaceae family, native from equatorial Africa. [6].This species has many uses in folk medicine the flowers and stem bark extracts were shown molluscicidal activity and also are employed as diuretic and anti-inflammatory [6], while the leaves are used against kidney diseases, urethra inflammations and as an antidote against animal poisons [6]; Hypoglycemic, anti-HIV and antimalarial activities were also observed in stem bark extracts [7,8]; the stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomachaches and diarrhea and scientifically proved for antimicrobial activity [6,9,10]. Hence the present study has been undertaken to investigate the antidiarrheal activity of bark extracts of *Spathodea companulata* in experimentally induced diarrhea in rodents.

### **Materials And Methods**

#### **Drugs and chemicals**

All the solvents used for the extraction process are of Laboratory grade. Castor oil (Medinova Chemicals, Bangalore), Deactivated charcoal (New India chemical enterprises.Kochi), Prostaglandin E2 (Zidus Alidac. Ahmedabad.), Atropine (S.D.Fine chemicals. Mumbai.) and Loperamide (Torrent Pharmaceuticals. Ahmedabad, India) were used for the study.

#### **Plant material**

The bark of the plant was collected in the month of May – June 2007 and authenticated by Dr.K.P.Sreenath, Reader and Taxonomist, Botany Department from Bangalore University. A sample specimen was deposited, bearing voucher number SPC-Coll.no.I.

#### **Plant extraction**

The shade dried plant material was powdered. The coarse powder was subjected to successive solvent extraction with petroleum ether, alcohol (70%) in soxhlet apparatus and the marc obtained after alcoholic extraction was macerated with distilled water to obtain aqueous extract. The % yield of petroleum ether, alcoholic and aqueous extracts was found to be 1.2%, 5.33% and 2.5% respectively.

#### **Phytochemical investigation**

The alcoholic (ALSP) and aqueous (AQSP) extracts of *S.companulata* were subjected to preliminary qualitative investigations [11,12].

### **Experimental animals**

Swiss albino mice (18-22g) and Wistar albino rats (150-200 g) of either sex were acclimatized for 7 days under standard husbandry conditions. i.e. room temperature  $26 \pm 1^{\circ}\text{C}$ . relative humidity 45-55% and light: dark cycle 12:12 h. the experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy, Bangalore and conducted according to the guidelines of the Committee for the Purpose of the Control and Supervision on Experiments on Animals (CPCSEA).

### **Acute toxicity studies**

The acute toxicity of AQSP and ALSP was determined in female albino mice (18-22g). After administration with different doses of these extracts, the mortality with each dose was noted at 48 hours (acute) and 14 days (chronic). LD50 was calculated as per OECD guidelines 425 using AOT 425 software [13].

### **Antidiarrheal activity**

#### **A. Castor oil induced Diarrhea**

The method described by Awouters *et al* [14] as followed. Albino rats of either sex weighing 150-200 g were used. They were divided into 8 groups each group containing six animals. Rats were fasted 24 hrs before the test with free access to water. Rats were treated orally with vehicle or aqueous extract or ethanolic extract or standard. One hour after drug treatment, each rat received castor oil (1ml/100g, p.o). Each rat was then housed separately in cage over clean filter paper. Then diarrhea episodes were observed for a period of 4 hours. During this period, first defecation time, frequency of defecation and cumulative wet faecal mass were recorded. Antidiarrheal activity was determined in terms of percentage reduction in cumulative faecal mass with respect to vehicle treated group [5,15].

#### **B. Prostaglandin-E2 induced Diarrhea**

Six groups of rats (150-200 g) consisting of 6 animals in each group were deprived of food and water for 18 hours prior to the experiment. Rats were treated orally with vehicle or ethanolic extract or aqueous extract or loperamide one hour prior to prostaglandin-E2 administration. All the rats were administered with prostaglandins-E2 (100  $\mu\text{g}/\text{kg}$  in 2% v/v Tween 80 orally) except normal control group. Thirty minutes after prostaglandin-E2 all the rats were sacrificed. The whole length of the intestine from the pylorus to the caecum is dissected out and its contents were collected and measured [5,15]. Percentage reduction of intestinal secretion (volume) was calculated.

#### **C. Charcoal meal test**

Albino mice of either sex weighing 20-25 g were used. Mice were fasted for 4 hours before commencing the experiment with free access to water.

After 1 hour of extracts treatment, 1ml of charcoal meal [3% deactivated charcoal in 2% aqueous tween 80 orally] was administered by oral route to all the animals in each group.

After fifty minutes of charcoal treatment each mouse was sacrificed and distance moved by the charcoal meal from the pylorus to caecum was measured to express as a percentage of distance travelled by the charcoal meal in ratio to the intestinal length. Percentage inhibition produced by extracts was calculated [5,15].

#### **Statistical analysis**

Values are expressed as mean  $\pm$  SEM from 6 animals. Statistical difference in mean were analyzed using one way ANOVA (analysis of variance) followed by Dunnett's test.  $p < 0.05$  was considered significant.

### **Results**

#### **Phytochemical investigation**

It was found that the ALSP contains alkaloids, carbohydrates, glycosides, saponins, steroids, flavonoids, tannins and phenolic compounds; AQSP contained carbohydrates, glycosides, saponins, flavonoids, tannins and phenolic compounds .

#### **Acute toxicity studies**

In acute toxicity there was no mortality recorded in all the groups, i.e. ALSP and AQSP up to maximum dose of 2000mg/kg. Hence the extracts were found to be safe till 2000 mg/kg.

#### **Antidiarrheal activity**

##### **Castor oil induced diarrhea**

The standard drug Loperamide(1mg/kg), ALSP (50,100 & 200mg/kg) and AQSP (50,100 & 200mg/kg) of *Spathodea companulata* bark significantly reduced the mean weight of the faeces when compared to untreated control rats. The aqueous extract has shown more significant activity than alcoholic extract, the results are shown in **Table No. 1**.

##### **Prostaglandin E2 induced diarrhea**

The standard drug loperamide (1mg/kg), ALSP (50, 100 & 200mg/kg) and AQSP (50,100 & 200mg/kg) of *Spathodea companulata* bark significantly inhibited PGE2 induced enteropooling in rats compared with PGE2 control animals. PGE2 induced a significant increase in fluid volume of the rat intestine when compared with the vehicle control animals. Results are shown in **Table No. 2**.

##### **Charcoal meal test**

The standard drug atropine (2 mg/kg), ALSP (50, 100 & 200mg/kg) and AQSP (50,100 & 200mg/kg) of *Spathodea companulata* bark significantly decreased the propulsion of charcoal meal through the gastrointestinal tract, as compared with the control group. Results are shown in **Table No. 3**.

Table.1.Effect of *Spathodea companulata* bark extracts on Castor oil induced diarrhea

Group	Treatment	Dose mg/kg	Number of defaecations (counts/4 h)	Mean weight of faeces $\pm$ S.E.M. after 4 hrs. (gm)	Percentage of inhibition
<b>I</b>	Control	10ml/kg	6.22 $\pm$ 0.31	7.29 $\pm$ 0.305	----
<b>II</b>	Lopermide	1	1.54 $\pm$ 0.178	1.51 $\pm$ 0.187	79.29
<b>III</b>	ALSC	50	4.52 $\pm$ 0.281	4.074 $\pm$ 0.217**	44.12
<b>IV</b>	ALSC	100	2.38 $\pm$ 0.42	3.538 $\pm$ 0.426***	51.47
<b>V</b>	ALSC	200	2.49 $\pm$ 0.198	2.848 $\pm$ 0.325***	60.94
<b>VI</b>	AQSC	50	3.19 $\pm$ 0.173	3.27 $\pm$ 0.360***	55.15
<b>VII</b>	AQSC	100	2.13 $\pm$ 0.43	2.404 $\pm$ 0.125***	67.03
<b>VIII</b>	AQSC	200	1.31 $\pm$ 0.182	1.26 $\pm$ 0.094***	82.72

Values are expressed as mean  $\pm$  S.E.M. n = 6: \*\*  $P < 0.05$ , \*\*\*  $P < 0.001$  as compared to control group using one-way ANOVA followed by Dunnett's test.

Table.2.Effect of *Spathodea companulata* bark extracts on PG-E2 induced diarrhea

Group	Treatment	Dose mg/kg	Mean Volume of intestinal fluid $\pm$ S.E.M. (ml)	Percentage of inhibition
<b>I</b>	Normal rats (2% Tween 80)	10ml/kg	0.21 $\pm$ 0.11	-----
<b>II</b>	Control (2% Tween 80)	10ml/kg	2.61 $\pm$ 0.118	-----
<b>III</b>	Lopermide	1	0.78 $\pm$ 0.110***	70.11
<b>IV</b>	ALSC	50	2.16 $\pm$ 0.118***	17.24
<b>V</b>	ALSC	100	1.66 $\pm$ 0.068***	36.40
<b>VI</b>	ALSC	200	1.16 $\pm$ 0.188***	55.56
<b>VII</b>	AQSC	50	1.84 $\pm$ 0.146***	29.50
<b>VII</b>	AQSC	100	1.06 $\pm$ 0.110***	59.39
<b>IX</b>	AQSC	200	0.68 $\pm$ 0.089***	73.95

Values are expressed as mean  $\pm$  S.E.M. n = 6: \*\*\*  $P < 0.001$  as compared to control group using one-way ANOVA followed by Dunnett's test

Table.3.Effect of *Spathodea companulata* bark extracts on Charcoal meal test

Group	Treatment	Dose mg/kg	Mean % movement (cm)	Percentage of inhibition
I	Control (2% Tween 80)	10ml/kg	86.17954	-----
II	Atropine (2 mg/kg)	1	24.075***	72.06296
III	ALSC	50	63.293***	35.05706
IV	ALSC	100	46.953***	45.51614
V	ALSC	200	32.853***	61.87728
VI	AQSC	50	62.416***	27.57314
VII	AQSC	100	46.167***	46.4287
VIII	AQSC	200	30.358***	64.77267

Values are expressed as mean  $\pm$  S.E.M. n = 6: \*\*\* P <0.001 as compared to control group using one-way ANOVA followed by Dunnett's test

### Discussion

Alcoholic and aqueous extracts of stem bark of *Spathodea companulata* that have not been studied so far, were evaluated for their antidiarrheal potential against castor oil induced diarrhea and prostaglandin-E2 induced enteropooling in albino Wistar rats and antimotility effect in charcoal meal test in Swiss albino mice.

ALSP and AQSP exhibited significant antidiarrheal activity against castor oil induced diarrhea in rats; The AQSP (100 & 200mg/kg) and AQSP (100 & 200mg/kg) extracts showed almost similar antidiarrheal activity as that of loperamide (1mg/kg). It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in hyper secretory response and diarrhea [16, 17].

Ricinoleic acid markedly increases the PG-E2 in portal venous and gut lumen and also causes an increase in secretion of water and electrolytes in to the small intestine [18, 19]. Ricinoleic acid also produces irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [20]. Inhibition of prostaglandin biosynthesis delayed castor oil induced diarrhea [14]. Based on these observations, it seems that the antidiarrheal effect of ALSP and AQSP may be due to the inhibition of prostaglandin biosynthesis or by decreasing the peristaltic movement.

To ensure that ALSP and AQSP modify the action of prostaglandin, effect of ALSP and AQSP on PGE2 induced diarrhea was studied in rats. ALSP and AQSP significantly inhibited the PG-E2 induced intestinal fluid accumulation (enteropooling). It has been shown that E type of prostaglandins cause diarrhea in experimental animals as well as human beings [21]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport [22]. These observations tend to suggest that ALSP (50,100 & 200mg/kg) and AQSP (50,100 & 200mg/kg) reduced diarrhea by inhibiting PG-E2 induced intestinal accumulation of fluid.

Studies showed that activated charcoal readily adsorbs drugs and chemical on the surface of the charcoal particles and their by preventing absorption [23]. Hence gastrointestinal motility test with deactivated charcoal was carried out to find out the effect of ALSP and AQSP on peristaltic movement. The extracts appear to act on all parts of intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model; at the doses 50,100 and 200mg/kg of ALSP and 50, 100 and 200mg/kg of AQSP.

The results also showed that ALSP and AQSP suppressed the propulsion of charcoal meal there by increased the absorption of water and electrolytes. The inhibition of peristaltic movement with alcoholic and aqueous extracts of stem bark of *Spathodea companulata* may be due to the anti histaminic and anticholinergic actions. From these models we can suggest that ALSP and AQSP non-specifically inhibit diarrhea either by decreasing intestinal motility or by decreasing the prostaglandin biosynthesis. The result indicates that ALSP and AQSP possess significant antidiarrheal activity due to their inhibitory effect both on gastrointestinal propulsion and fluid secretion.

The data obtained is consistent with literature reports on antidiarrheal activity of *Spathodea companulata* stem bark using gastrointestinal motility test in mice and castor oil induced diarrhea and intraluminal accumulation of fluids in rats.

The inhibitory effect of the plant extracts justified the use of plant as a non-specific antidiarrheal agent in folklore medicine. Further detailed investigations are needed to determine the phytoconstituents which are responsible for antidiarrheal activity.

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