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Nootropic and Antibacterial Activity of Methanolic *Piper trioicum* Roxb Extracts

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ABSTRACT

The study aims to evaluate the nootropic and antibacterial capacity of methanolic of *Piper trioicum* Roxb. The antibacterial capacity of the extracts of *Piper trioicum* Roxb plant was estimated on gram positive and negative bacteria along withthe determination of the nootropic activity in these extracts using Wistar albino rats. The animals exposed to Scopolamine and treated with *Piper trioicum* Roxb extract (200 and 400 mg/kg) showed significantly ($P<0.05$ and $P<0.01$) decreasing in transfer latency as compared to the negative control. Antibacterial activity of methanolic extract of *Piper trioicum* Roxb was more effective in *Escherichia coli* than other bacteria, but there was no effect on *Proteus mirabilis*.

INTRODUCTION

Piper trioicum Roxb (PT) distributed in South Asian countries is a herb, shrub, or a climber, rarely tree, usually aromatic and belongs to the Piperaceae family[1,2]. The whole plant which is used inthe diabetes, muscular pains, headache, toothache and as the internal remedy for cholera in folk medicine, also possesses rubefacient, diuretic and hepatoprotective activity; while the root is used as diuretic[2]. Sathiskumar et al., revealed that extracts of PT had shown the presence of alkaloids, steroids, flavonoids, phenolic compounds, carbohydrates, tannins and Glycosides[3]. Other reports have demonstrated that the *Piper trioicum* Roxb ethanolic extract is rich in bioactive phytochemicals and it has inhibitory activity on the amylase and lipase thus suggesting that the extract might be useful to limit dietary fat, glucose absorption and the accumulation of fat in adipose tissue[4]. PT ethanolic extract possesses potent alpha glucosidase inhibitory activity[5]. Another studies has reported the antioxidant activity of ethylacetate and methanol extract which correlated with the phenolic content present in the respective extracts[6].Nootropics are agents that enhance the cognitive skills, and "amnstics" are agent that disrupts the learning and memory processes. Learning and memory can be conceived as both a psychological process, as well as a change in synaptic neural connectivity[7].There is, however, no report concerning thenootropic and antibacterial property of the PT. In the present investigation, methanolic extract of PT were used to evaluate their nootropic and antibacterial capacities.

MATERIALS AND METHODS

Collection and Authentication

Plant material of PT was collected from the local areas of Talakona, Andhra Pradesh and authenticatedby Mr. Madhavachetty, Botanist, S.V.University, Tirupati, Andhra Pradesh. The collected material was shade dried and ground into uniform powder using milling machine.

Chemicals

Methanol was purchased from SD Fine chemicals Ltd., (India). *Staphylococcus aureus* (ATCCBAA 1026), *Bacillus subtilis* (ATCC 11774), *Acineto bacterbaumannii* (ATCC 17978) *Escherichia coli* (ATCC 10536), *Proteus mirabilis* (ATCC14153), *Salmonella typhi*

(ATCC13311), and *Pseudomonas aeruginosa* (ATCC 10662) were procured from Microbes Speciality Lab, India. The reference standard Ampicillin was procured from Pradeep Organics and chemicals Pvt. Ltd, Hyderabad. Wistar albino rats purchased from Mahaveer Enterprises (India) were housed in stainless steel wire-bottomed cages under a 12-h light/12-h dark cycle in a temperature- and humidity-controlled room. They were allowed food and water ad libitum.The animal studies were performed in compliance with protocols and policies approved by the Institutional Animal Ethical Committee.Chemicals used for preparation of medium were obtained from SD fine chemicals and Qualigens fine chemicals.

Preparation of Extracts

50 g of shade dried whole plant powder was suspended and extracted with 10 volumes of methanol by shaking at room temperature for 15 hours. The extracts were filtered through filter paper, and the supernatants were pooled. The residue was re-extracted under the same conditions. Pooled extracts were condensed (and methanol was removed) with a rotary evaporator at 50°C. The physical characteristics and percentage yield of methanol extracts were reported. The dried extracts of all solvent were kept in desiccators prior to analysis.

Nootropic Activity [8,9]

All the animals have been divided into eight groups and placed in separate cages, each consisting of 6 animals. The animals were acclimatized to the laboratory condition for one week before the onset of experiment.The doses of 200mg/kg and 400mg/kg of PT selected as per literature review were dissolved in 1% CMC solution individually and administered through oral route. Positive and negative control groups were received 1% CMC at 10mL/kg orally. Standard group received Piracetam at 200 mg/kg orally. Negative control groups were made by administration of Scopolamine (0.4mg/kg) which induced amnesia to the groups. Group I treated with 1% CMC vehicle (10 ml/kg); Group II treated with 200mg/kg of extract; Group III treated with 400 mg/kg of extract; Group IV treated with Piracetam (200 mg/kg); Group V Amnesic Control (0.4mg/kg) groups received only vehicle against scopolamine-induced amnesia; Group VI methonlic extract (200mg/kg) treated rats against scopolamine induced amnesia; Group VII methonlic extract (400mg/kg) treated rats against scopolamineinduced amnesia; and Group VIII Standard drug Piracetam (200mg/kg)treated rats against Scopolamine-induced amnesia.

The animals were trained for maze task performance by conducting one daily training trial. It took 15 days to get the animal completely trained. During which they did not received any drug. The completely trained animals were chosen for the study. These animals were dosed once in a day with the respective drugs for ten days along with daily training trial. Group I wasreceived vehicle for 15 days. Group II, III and IV received the 200mg/kg of extract, 400mg/kg of extract and 200mg/kgPiracetam for 15 days respectively. Group V was received vehicle + scopolaminefor 15 day. Group VI, VII and VIII received the 200mg/kg of extract, 400mg/kg of extract and 200mg/kg Piracetam for ten days respectively along with scopolamine. After one hour all animals were tested on Morris water maze task performance.

The Morris water maze consists of large circular tank made of black opaque PVC or hard board coated with fiberglass and resin and then surface painted white (1.8-2.0m in diameter and 0.4-0.6m height). The pool is filled with water (20-22^{0}C) to a depth of 0.3-0.4m, and rendered opaque by the addition of small quantityof milk or non-toxic white colour. The pool is fixedwith filling and draining facilities and mounted on a frame so that the water is at waist level. The floor of circular tank is marked off in to four equal quadrants arbitrarily designed north, south, east or west. And escape platform is made of plexiglass with a13 cm square platform attached to a 34 cm long clear plexiglass cylindrical pedestal (3cmDiameter) mounted on a 1sq m (5mm thick) plexiglass base. The top of the platform is covered with a coarse material that provides a good grip for the rat when climbing on a platform. For the hidden platform task, water is added to circular tank to a level 2cm above the top of the platform. Water maze represents a versatile tool in which a number of distinct tasks can be measured. The simplest measure of performance is the Latency to escape from the water on to the hidden platform. The procedure and end point applied in present study for testing learning and memory have been described below. The animals for the experiments were preselected by conducting at least one daily training trail. At the beginning of trail animals are place in the Morris water maze and allowed to swim freely and to seat on hidden platform. The trial was considered to be successful when rat set on the hidden platform within three minutes. Time spent more than three minutes to find hidden platform recorded as error. On the initial day, 7th day and 15th day 60 minutes after the dose, transfer latency was determined for all groups. After 30 minutes each rats was subjects to Morris water maze task performance.

Antibacterial Activity

Methanolic extracts of PT were screened for antibacterial activity done by cup plate method. The activity was compared with standard (Ampicillin) and control sterile water. Various organisms used in the study are Gram +ve bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Acineto bacterbaumannii*) and Gram -ve bacteria (*Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Pseudomonas*

aeruginosa). Different concentrations of extracts equivalent to 100, 200 and 400mcg/ml were prepared by using sterile water. 10 mcg/ml concentration of ampicillin was prepared and used as standards to be studied along with test solutions for their zone of inhibition. Nutrient agar was used to study the antibacterial of the extracts [10, 11]. The zone of inhibition around the cup indicates the antibacterial activity. The control was run simultaneously to assess the activity of sterile water which was used as vehicle for extracts. The study was performed in duplicate. The diameter of the zone of inhibition was measured and recorded.

Statistical Analysis

The data were statically analyzed by student's t-test all the value was expressed as mean ± SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test and values $p < 0.05$ were considered significant.

RESULTS

Nootropic Activity

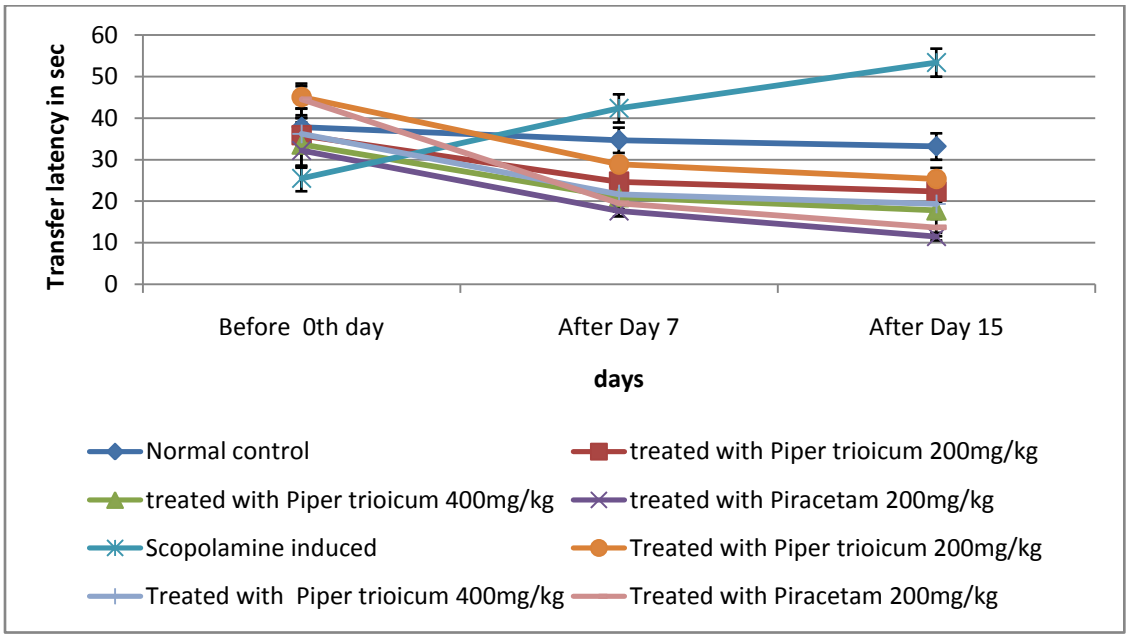
The animals were subjected to transfer latency (TL) to evaluate the retrieval of memory in behavioral paradigm after a period of 7th& 15th days of acquisition trial, to know the effect of extracts on the long term memory. TL of day 0 reflects learning behavior of the animals whereas; TL of day 7th& 15th reflects the retention of the information or memory. The normal control animals have showed non-significant retrieval of memory in this behavioral paradigm. In the positive control groups, the animals treated with Piracetam showed highly significantly (P<0.01) decreasing in the TL. PT (200 and 400 mg/kg) treated animals produced significantly (P<0.05) decreasing in TL. In the negative control group, the animals exposed to Scopolamine, produced significant (P<0.01) loss of memory in behavioral paradigm, which resulted in increase in TL on days 7th& 15th as compared to day 1. In the treatment group, the animals exposed to Scopolamine and treated with Piracetam, reduced the time taken to perform the task in water morris maze resulting in significant (P<0.01) retrieval of memory in behavioral paradigm. The animals exposed to Scopolamine and treated with PT (200 and 400 mg/kg) showed significantly (P<0.01) decreasing in TL as compared to the negative control. A significant increase in cognitive function was observed in rats receiving various doses of methanolic extract of PT. No significant increase in cognitive function observed in normal rats receiving 1% sodium CMC (vehicle) even after 15 days. There is a significant improvement in cognitive function observed in rats with doses of 200mg/kg & 400mg/kg. The degree of percent reduction was more in positive control rats receiving 400mg/kg of extract. Table 1 and figure 1 explains effect of methanolic extract of *Piper trioicum* on transfer latency against scopolamine induced amnesia by Morris water maze model.

Table 1: Effect of Methanolic Extract of *Piper trioicum* on Transfer Latency against Scopolamine Induced Amnesia by Morris Water Maze Model.

Group	Subgroup	Transfer Latency (in sec)		
		Before 0th day	After Day 7	After Day 15
Norma Control	Normal control	37.83±4.438	34.67±3.040	33.17±3.177
Positive Control	*Piper trioicum* 200mg/kg	35.83±4.269	24.67±3.593*	22.33±2.201*
	Piper trioicum 400mg/kg	33.67±5.302	21.00±3.483	17.83±1.939*
	Piracetam 200mg/kg	32.17±4.094	17.67±1.308**	11.50±0.922**
Negative Control	Scopolamine induced	25.50±3.085	42.33±3.393**	53.33±3.373**
Treatment Group: Scopolamine + Drug	*Piper trioicum* 200mg/kg	45.00±2.671	28.83±1.815**	25.33±2.704**
	Piper trioicum 400mg/kg	36.17±3.807	21.67±1.430**	19.33±2.076**
	Piracetam 200mg/kg	44.50±3.202	19.50±0.738**	13.67±1.606**

**P<0.01; *P<0.05

Figure 1: Effect of Methanolic Extract of *Piper trioicum* on Transfer Latency Against Scopolamine Induced Amnesia by Morris Water Maze Model



Antibacterial Activity

In the present study antibacterial activity of ampicillin and methanolic extract of PT with different concentration were performed and compared. The Zone of inhibition of different concentration of methanolic extract like 100, 200 and 400mcg/ml against Gram +ve bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Acineto bacterbaumannii*) and Gram –ve bacteria (*Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*). Antibacterial potential of methanolic extracts of the plant were present in the table 2. The results show that all the test organisms were inhibited significantly by methanol extracts in a dose dependant manner as compared to the sterile water as a control. The result values were compared with standard ampicillin. Antibacterial activity of methanolic extract of PT was more effective in *Escherichia coli* than other gram positive and gram negative bacteria, but there was no effect on *Proteus mirabilis*. From the results, we concluded that the extract was not active against *Proteus mirabilis*.

Table 2: Antibacterial Activity of Methanolic Extract of *Piper trioicum*

Sl.No	Bacterial strains used	Zone of inhibition in mm			
		Piper trioicum extract (µg/ml)			Standard drug (µg/ml)
		100	200	400	10
		Gram Positive			
1.	*Staphylococcus aureus*	11.67	12.67	14.17	16.17
2.	*Bacillus subtilis*	10.0	11.0	13.5	18.33
3.	*Acineto bacterbaumannii*	8.75	9.25	10.0	12.75
		Gram Negative			
4.	*Escherichia coli*	12.33	12.67	15.0	15.67
5.	*Proteus mirabilis*	----	----	----	21.0
6.	*Salmonella typhi*	9.5	11.5	11.0	14.3
7.	*Pseudomonas aeruginosa*	8.0	9.0	12.0	14.5

DISCUSSION

The previous studies had shown that the phenolic components, flavonoids and condensed tannins would probably play an important role in the antioxidant activity of the PT extracts. Through this investigation it can be said that this extract also had shown

highest antibacterial and nootrotic activity than those extracted by using methanol. The results revealed that PT plants generally regarded as a disposable by-product, has potential for exploitation to promote human and animal health.

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