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Evaluation of Analgesic and Antimicrobial potential of Hydroalcoholic extract of leaves of *Coleus aromaticus* in albino mice

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Abstract

The study describes the biological activity of the dried leaves of *Coleus aromaticus* belong to the family Lamiacea. Successive extraction was carried out for identification of the suitable solvent for furthr extraction. Phytochemical investigation was carried out for various solvent extracts as per their polarity. The dried powder of the plant was extracted with alcohol and water. The hydroalcoholic extract was then studied for analgesic and antimicrobial activity. The analgesic activity was carried out by tail immersion method, pentazocine as standard drug. The hydro alcoholic extract of the crude drug was screened for its antimicrobial activity against the gram positive and gram negative bacteria and fungi. The results obtained were compared with the standard drug Ampicilline trihydrate and Amphoterecine B. The hydro alcoholic extract shows the mild to moderate zone of inhibition for the gram positive, gram negative bacteria and fungi (23.5mm, 20mm, and 20.4mm).

Keywords: *Coleous aromaticus*, Lamiacea, Analgesic activity, Antimicrobial activity, Cup Plate Method.

Introduction

The value of medicinal plants to the mankind is very well proven. India harbors about 15 percent of (3000 – 3500) medicinal plants, out of 20,000 medicinal plants of the world. About 90 percent of these are found growing wild in different climatic regions of the country.[1] It is estimated that 70 to 80% of the people worldwide rely on traditional health care system and largely on herbal medicines.[2, 5] *Coleus aromaticus* known as Patrachur (Bengali), Paterechur(Hindi), Patrachura (Oriya), Panova (Marathi) is a perennial herb that belongs to the family of Lamiaceae. *Coleus aromaticus* (Benth) is a commonly available medicinal herb in India. The plant chosen for study, *Coleus aromaticus* (Benth), belonging to the Family: Lamiaceae is grown as a household herb in Tamilnadu (vernacular Tamil name: Ommam or Ommavalli) which is native to East Indies and is widely cultivated in Africa and almost all tropical countries. It is also popularly known as "Indian Oregano". It grows to a maximum of 1.5 to 2 meters and has a thick green stem. The leaves of the plant are thick, succulent and juicy Fig 1. The plant emanates mild, pleasant odour which increases when it is cut or crushed.[7] The leaves of this plant are traditionally used for the treatment of severe bronchitis, asthma, diarrhea, epilepsy, diuretic and nephroprotective activities[8], vesicle calculi, fever and also to exhibit antilithiotic[9] chemopreventive[10], antiepileptic and antioxidant properties.[11, 12]

It is also used for the treatment of cancer as it has anti tumor and cytotoxic activities.[13, 14] Ethanolic extract of Coleus aromaticus is reported to posses anticlastogenic potency against anticancer drugs.[15] This plant also serves as diuretic[16] and nephroprotective activities.[17] Aqueous extract of Coleus aromaticus increases phagocytosis capacity of neutrophil cells and mast cell stabilization.[18]

Fig: 1 Plant of *Coleus aromaticus*

Material and method

Reagent and chemical use

Pantazocine, Ampicilline Trihydrate, Amphotericine B from Fortwin (Ranbaxy) and Tween 80 from BHD chemical limited.

Equipment

Sterile disposable syringe (1ml, 100 divisions): Dispovan BD insulin syringe, Electronic and digital Balance: Citizen

Collection and preparation of the plant sample

The fresh whole plant were collected from the herbal garden of Regional Research Laboratory, Bhubaneswar, during September and authenticated by Dr Satyabrata Sahoo by comparing with authentic specimen. After authentication, fresh leaves were collected from young and mature plant. The leaves were sprayed on aluminium trays as a thin layer and dried under shade. After drying, leaves were pulverized into coarse powder with the help of a mechanical grinder. The coarse powder was allowed to pass through sieve No 40 and further used for extract preparation. The successive extraction was carried out for finding out the extractive value of each extracts as mentioned in Table 1.

Table 1: Successive extract values of *Coleus aromaticus* leaves

Solvent	Petroleum Ether	Benzene	Chloroform	Ethyl Acetate	Methanol	Water
Extractive value %w/w	3.2	4.7	2.2	1.5	22.3	17.5

The dried leaves (81.8gm) were extracted with the mixture of alcohol and water in the ratio of 3:2 for 72 hours by maceration process. After extraction, the marc was separated out from the liquid extract by filtration. The marc was rejected. The liquid extract was concentrated under vacuum to a semisolid residue. The vacuumed concentrated residue was a sticky mass with black–green in color. The extract was dried in a Desiccator. The yield of the extract was found to be 19.88%w/w.

Preliminary Phytochemical investigation

The plant extract was exposed to various phytochemical investigations for the absence or presence of various constituents which are tabulated in Table 2.

Table 2: Phytochemical Investigation of various solvents

S. No.	Tests	Results					
		Petroleum Ether	**Benzene**	**Chloroform**	**Ethyl Acetate**	**Methanol**	**Water**
1.	Alkaloid	-ve	-ve	+ve	-ve	+ve	-ve
2.	Glycoside	-ve	-ve	-ve	+ve	+ve	-ve
3.	Carbohydrates	-ve	-ve	-ve	-ve	+ve	+ve
4.	Gum and mucilage	-ve	-ve	-ve	-ve	-ve	+ve
5.	Proteins and amino acid	+ve	-ve	-ve	-ve	-ve	+ve
6.	Fixed oil and volatile oil	-ve	+ve	-ve	-ve	-ve	-ve
7.	Tannin and phenolic compounds	+ve	-ve	-ve	+ve	+ve	+ve
8.	Steroid and Sterols	+ve	-ve	-ve	-ve	+ve	+ve
9.	Tri-terpenoid	+ve	+ve	+ve	-ve	+ve	+ve
10.	Saponins	-ve	-ve	-ve	-ve	+ve	+ve
11.	Flavonoids	-ve	-ve	-ve	+ve	-ve	+ve

+v =presence of active constituent,-ve =Absence of active constituent

Analgesic activity by tail immersion method

A total of 16 healthy albino mice (20-35g), age 4-5 weeks of either sex, obtained from the animal house of Institute of Pharmacy and Technology Salepur, Cuttack, Orissa. They were housed under controlled conditions of temperature of 23±20C, relative humidity of 30–70% and 12 hours light–12 hours dark cycle. The animals were housed individually in acrylic cages containing sterile paddy husk (procured locally) as bedding throughout the experiment. All animals were fed with sterile commercial pellet. Animals were kept under fasting for overnight and weighed before the experiment. The study was undertaken after obtaining approval of Institutional Animal Ethics Committee.[19]

Experimental Design

16 experimental animals were randomly selected and divided into four groups denoted as group I group II, group III, group IV containing 4 mice each group. Each group received particular treatment i.e. control, positive control, and two dose of extract. Prior to any treatment, each mouse were weighted properly and the dose of the test sample and control material were adjusted according to the body weight.. The extracts at dose level of 100mg/kg and 200mg/kg were prepared by suspending the extract in 1% w/v gum acacia in distilled water.

Method of identification of the animal

Each group consider of six mice. 10% picric acid was applied to the back of the animals by means brush. The back was marked on the three areas. A spot on the left side of the back was marked to no1, far right side of the back was applied by brush to designate mice no.2, spot on the middle field of the back was made to identify mice no.3 and finally the tail was marked to designate mice no. 4.

Tail immersion method

Before study, Swiss albino mice were screened for sensitivity test by immersing the tail of the mice gently in hot water maintained at 55°C -55.5°C. The mice which left the tail from the hot water with in 5 second were selected for the study. The selected mice were then divided in four groups of 6 mice in each

Group I received vehicle (2 mg /kg)

Group II received Pentazocine (30mg/kg)

Group III received Hydroalcoholic extract at dose 100mg/kg

Group IV received Hydroalcoholic extract at dose 200mg/kg

The test substances were injected intraperitoneally. After administration of the test sample, the reaction was measured at the 0, 15, 30, 45 and 60 minute. The cut of 10 second was used to prevent tissue damage. Table 3

Table 3: Analgesic effect of hydro alcoholic extract of *Coleus aromaticus* on heat tail-flick response

S. No.	**Group**	**Treatment**	**Dose**	**Reaction time (seconds)**				
				0 min	**15 min**	**30 min**	**45 min**	**60 min**
1	I	Control	2mg/kg	3.66±0.33	4.00±0.58	3.66±0.33	3.33±0.33	3.66±0.33
2	II	Pentazocine	20mg/kg	2.33±0.42	4.33±0.42	5.33±0.21	7.00±0.58	8.33±0.67
3	III	Hydro Alcoholic	100mg/kg	3.00±0.37	3.33±0.42	4.50±0.22	6.33±0.67	6.00±0.37

		Extract						
4	IV	Hydro Alcoholic Extract	200mg/kg	3.33±0.33	4.50±0.62	5.00±0.58	6.83±0.60	9.10±0.66

Screening of Antimicrobial activity

Screening of antimicrobial activity was carried out by the cup plate method the following microorganisms are used for the study.

Gram positive bacteria	**Gram negative bacteria**	**Fungi**
Bacillus subtilies	*Shigella bayedir*	*Aspergillus niger*
Staphylococcus aureus	*Pseudomonas aeruginosa*	

Preparation of nutrient medium for the subculture of bacteria

5 gm of peptone, 2.5 gm of sodium chloride and 5gm of beef extract were weighed and dissolved in 500ml distil water in a conical flask. The flask was kept on the water bath to dissolve the beef extract. the PH was adjusted to 7.4 ±0.2 by caustic soda (NaOH) solution using PH paper the flask was plagued and warped with silver foil and autoclaved for 20 minutes at 15 psi for 121°C. Then the flask was allowed to cool. The media was poured in small qualities in to presterilized plugged containers. The wire loop was sterilized by flaming to red –hot condition to Bunsen flame. Then it was pulled out, cooled and the top of the container containing the culture was flamed and microorganism (bacteria) was inoculated in small container under laminar airflow cabinet under aseptic condition. The microorganism was then incubated at 20-25°C.

Preparation of S.D.A medium for the subculture of fungi

8 gm dextrose and 2 gm of peptone were weight and dissolved in 200 ml of distilled water in conical flask. The PH was adjusted to 5.4±0.2 by caustic soda (NaOH) solution using PH paper. The flask were then plugged and wrapped with silver foil and autoclaved for 15 minutes at 15 psi for 121°C. Then the flask was allowed to cool. The media was poured in small qualities in to presterilized plugged containers. The wire loop was sterilized by flaming to red –hot condition to Bunsen flame. Then it was pulled out, cooled and the top of the container containing the culture was flamed and microorganism (fungi) was inoculated in small container under laminar airflow cabinet under aseptic condition. The microorganism was then incubated at 30-35°C.

Preparation of test sample

The test samples (Hydroalcoholic extract and reference controls) were dissolved in DMSO. The medium were then poured into Petri dishes separately under aseptic technique; previously sterile in hot oven and allowed to cool. The subculture of bacteria and fungi were poured to Petri dishes over the solidified medium. Under each hole every Petri dishes marked as 1, 2, 3and 4 with a marker pen. The Petri dishes were divided into different groups. The Hydroalcoholic extract were studied at concentration of 100 and 200 mg/ml. Ampicilline Trihydrate (1000mcg/ml) and Amphotericine B (1000mcg/ml) were used as reference control.

After that all the petridishes were incubated for 24 hours at appropriate temperature. The diameter of the zone of inhibition was measured with the help of transparent scale. The results are depicted in the Table 4.

Table 4: Microbiological activity of the Hydroalcoholic extract

S. No	Test substances	Conc	Zone of in hibition (mm)				
			Bacillus subtilies	***Staphylococcus aureus***	***Shigella bayedir***	***Pseudomonas aeruginosa***	***Aspergillus niger***
1	Ampiciline trihydrate	1000mcg/ml	32	30	30	29	-
2	Amphotericine B	1000mcg/ml	-	-	-	-	31.8
3	Hydroalcololic extract	50mg/ml 100mg/ml	18.5 21.8	23.5 24.5	22 24	21 22.5	20.4 19.5

Results and discussion

Coleus aromaticus widely is grown plant in the tropical and temperature region. The leaves are reported to be used in the treatment of various diseases by the tribal population of our country. As per the ethanomedical information the leaves are reported to posses' analgesic activity, science they are used in the treatment of the pain.

The present investigation was carried out on the basis of above fact. The shade dried leaves were extracted successively with a series of solvent with increasing order of polarity via Petri ether, benzene, chloroform acetone methanol and water by simple maceration presses. The extractive value calculated for every solvent. The extract were subjected to preliminary photochemical screening to detect the nature of different phytoconstituents present, using standard procedure and qualitative chemical test Table 2 . The extract was screened for analgesic activity and anti microbial activity.

In the present investigation study the extract of *Coleus aromaticus* leaves were screened for analgesic activity (tail immersion method) and they were compare with the standard drug Pentazocine at a dose level 200mg/kg body wt. Table 3

Screening for antimicrobial activity was studied by cup-plate method by measuring the zone of inhibition.[20]

The extract was tested at concentrations of 50mg/ml, and 100mg/ml, which inhibited the growth of the tested microorganism to maximum level. Table 4

Conclusion

From our study entitled the following conclusion can be drawn. After extraction of the leaves with Hydroalcoholic solvent, it was found that the Hydroalcoholic extract contains tannin phenolic compounds, Flavonoids, steroid and sterols and triterpenoid. The extractive value of the tests extract from Hydroalcoholic solvent possess maximum yield.

In analgesic activity study it was found that the Hydroalcoholic extract showed significant activity at tested dose level, which is comparable with that of reference control Pentazocine. This may suggest the presence of phytoconstituents soluble in hydro alcohol, are endowed with potential activity.

From the antimicrobial study, it was found that the Hydroalcoholic extract showed satisfactory results for the tested concentrations.

The above finding suggested significant analgesic and antimicrobial activity of the leaves. Farther investigation may be carried out for the isolation of compounds, which are responsible for the activity and will be beneficial to mankind for treating various diseases.

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