December 2009 Volume 5 Issue 4

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 5(4), 2009 [202-205]

Chemical and biological studies on *Centella asiatica* (Umbelliferae)

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Received: 24th August, 2009 ; Accepted: 3rd September, 2009

ABSTRACT

Centella asiatica (Umbelliferae) is commonly known as Asiatic Pennywort and has a wide range of use in ayurveda and in other traditional system of medicine, starting from brain tonic to antihypertensive, antitubercular etc. The main chemical constituents of the plant are saponins (brahmoside, asiaticoside etc.), alkaloids (hydrocotyline), bitter principle (vellarin) etc. In the present work, we have studied the preliminary phytoconstituents of the plant along with its In vitro antioxidant activity, antimicrobial and wound healing potential. In the all the studies, the ethanolic extract of the plant showed significant positive result as compared with that of standard.

KEYWORDS

Centella asiatica (Umbelliferae);
Antimicrobial;
In Vitro Antioxidant;
Wound Healing.

INTRODUCTION

Centella asiatica (Umbelliferae) is commonly known as Asiatic Pennywort and is found in marshy places throughout India up to 200 m[1]. This plant is very commonly used in our traditional medicine as brain tonic for improving memory and for overcoming mental confusion, stress, fatigue etc. It is also used for obstinate skin diseases and leprosy[1]. The main chemical constituents of the plant are saponins (brahmoside, asiaticoside etc.), alkaloids (hydrocotyline), bitter principle (vellarin), sugars, fatty acids etc[1,2]. Search of earlier literature revealed that, apart from brain tonic it can be used in the treatment of duodenal ulcers, second and third degree burn cases, hypertension, tuberculosis, syphilis, amoebic dysentery, common cold, leprosy etc[1-4]. The plant is also reported to be a weak skin sensitizer, and causes contact dermatitis in some cases[5,6].

In our present study we have investigated the preliminary phytochemistry of the plant, along with its in vitro antioxidant activity, antimicrobial potential and wound healing activity.

MATERIALS AND METHODS

Plant material

The plant for the study was collected from the rural area of Kolaghat, of East Midnapur district, West Bengal. The fresh plants were washed with water and were shade dried. The dried plants were packed in dry plastic bags and were brought to the laboratory. In the laboratory the plants were screened for abnormalities and only healthy plants were used in the study. A voucher specimen of the plant is preserved in the herbarium of Department of Pharmacognosy, Institute of Jalpaiguri for future reference. The plants were cut into pieces and were then grounded to form powder.

As a part of the pharmacognostic study we have performed ash analysis[7,8] and extractive value determination[7,8].

Extraction procedure

The finely powdered plant material was macerated using ethanol for 48 hrs with occasional stirring, the extract was then filtered and the plant material was re-extracted with the same solvent for a further period of 48 hrs in the same manner and was filtered, the filtrates were combined and was evaporated to dryness under reduced pressure at a temperature not exceeding 40^0C to get the crude extract.

Preliminary phytochemical screening

The crude ethanolic extract was subjected to preliminary phytochemical screening using specific reagents[7,9-11]; the extract was also subjected to TLC using different solvent systems and specific derivatizing reagents[10,11] were used to confirm the identity of the phytoconstituents.

In vitro antioxidant activity

The antioxidant activity of the plant Centella asiatica (Umbelliferae) was determined by the assay of reducing power as reported by Naznin Ara and Hasan Nur (2009)[14]. 1 ml of plant extract solution (different concentration) was mixed with 2.5 ml phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide [K3Fe(CN_6)] (10g/l), then mixture was incubated at 50 degree C for 20 minutes. Then, 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml Fecl3 (1g/l) and absorbance measured at 700nm in a laboratory colorimeter. Ascorbic acid was used as standard and phosphate buffer used as blank solution. The absorbance of the final reaction mixture of two parallel experiments was expressed as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power.

Animals

Whister albino rats of either sex were procured from North Bengal Medical College, Shiliguri and were maintained at standard housing condition (12 hour light and dark circle, temperature 25^0C±2^0C) for a couple of week for acclimatization. The animals were fed with standard pallet diet and water ad libitum; through out the study period. A clearance from the institutional animal ethical committee has been obtained for the study.

Wound healing activity

For determination of the wound healing activity excision wound model was used[12,13]. The animals were divided into four groups (N = 6); group I served as control and received only the ointment base (Paraffin ointment base)[13], the animals of group II were treated with standard Povidone Iodine ointment, the animals of group III received 1%w/w ethanolic extract ointment and group IV animals received 2%w/w ethanolic extract ointment. All the animals were treated with twice a day tropical application of the ointments.

For development of the wounds, the animals were anesthetized using stabilized diethyl ether and under mild anesthesia the skin of the impressed area were excised to full thickness (about 2 mm) to obtain a wound area of about 500 mm^2. The application of the ointments started from the day after the operation and was continued until the full epithelialization. The areas of the wounds were measured periodically to obtain the percent wound closure. The total time required for the complete epithelialization was also measured.

Antimicrobial study

The antimicrobial study was conducted by cup-plate agar diffusion method[10,11]. The different antibacterial stains used in the study were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacilus subtilis*. Culture media was prepared and after sterilization, was taken in sterilized Petri dishes and the microorganisms were grown by pouring microbial suspension on the solidified media in Petri dishes and incubating them at 30^0C temperature for 24 hrs. After growth of the microorganism, pours were made using borer and different concentration of the extracts were placed in each hole, 10 ppm standard ciprofloxacin solution was used as standard. The Petri dishes were further incubated at 30^0C for a period of 24 hrs and the diameter of the zone of inhibition was measured. The diameter of zone of inhibition for each concentration was measured thrice

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against each microbial strain and the result shows the average diameter of zone of inhibition.

RESULTS AND DISCUSSION

The results of the preliminary pharmacognostic study and phytochemical analysis are shown in TABLE 1. The result of the preliminary phytochemical studies showed the presence of saponins, alkaloids, glycosides, sugars etc.

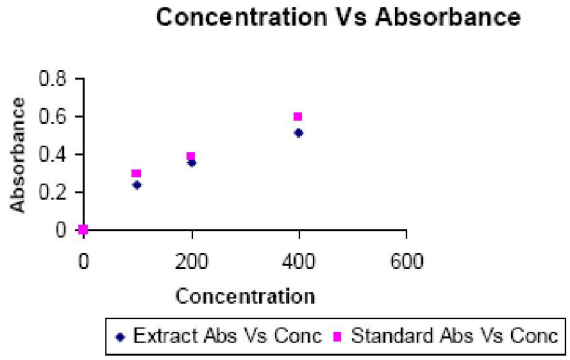
TABLE 1 : Ash analysis and extractive values of *Centella asiatica*

1. Ash Analysis	
i) Total Ash	13.87% of Dry Weight
ii) Acid Insoluble Ash	14.60% of Total Ash
iii) Water Soluble Ash	23.07% of Total ash
iv) Sulphated Ash	28.26% of Total Ash
2. Extractive Values	
Pet. Ether Soluble Extractive	1.97% w/w
Chloroform Soluble Extractive	0.87% w/w
Ethanol Soluble Extractive	20.70% w/w
Water Soluble Extractive	15.92% w/w

The results of the antioxidant activity are shown in TABLE 2 and Curve 1; and the results are in agreement with that reported earlier[14]; the antioxidant activity of the plant is very good as compared with that of standard.

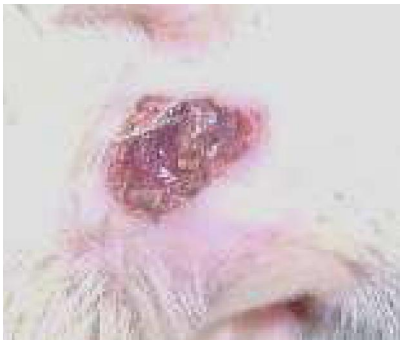
TABLE 2 : Antioxidant activity of ethanolic extract of *Centella asiatica*

Extract/Standard	Concentration (ppm)	Absorbance
Ethanolic Extract	100	0.240
	200	0.356
	400	0.513
Ascorbic Acid	100	0.293
	200	0.386
	400	0.596

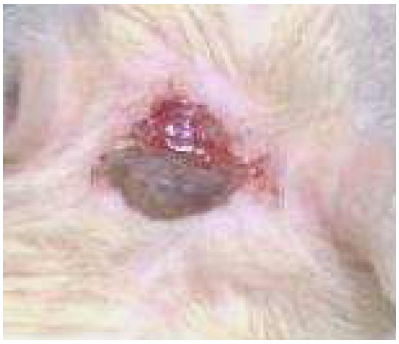


Curve 1 : Antioxidant activity of extract and that of standard ascorbic acid

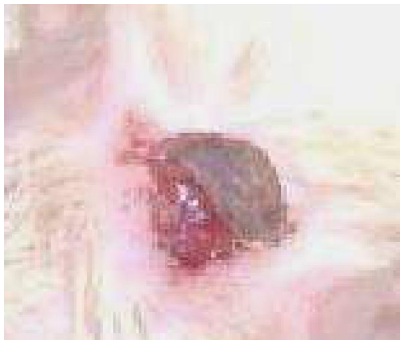
The results of the wound healing activity are shown in TABLE 3; and the wound healing potential of the



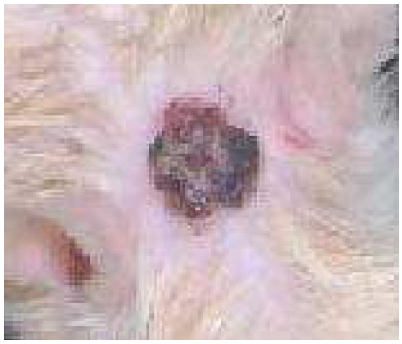
Control Group



Standard Drug Treated Group



1% Extract Treated Group



2% Extract Treated Group

Figure 1 : Typical Wounds in Different Groups of Animals on 8th Day of Treatment

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TABLE 3 : Wound healing Activity of Ethanolic Extract of *Centella asiatica*

Treatment	Closure of Wound Area (%)				Epithelialization in Days
	Day 4	Day 8	Day 12	Day 16	
Control Group	16.23	26.41	59.66	85.27	22
Std. Drug Treated	22.04	42.13	68.2	98.59	18
1% Extract Treated	21.73	42.08	66.51	97.84	19
2% Extract Treated	31.07	66.35	89.61	100*	15

***Showed better activity than standard.**

ethanolic extract is evident from the results and is in agreement with the earlier report[14]. Figure 1 shows typical wounds on different groups of animals on day 8.

The antimicrobial activity of the plant extract is also very potent in the etanolic extract of the plant as compared with standard ciprofloxacin; the results of the anti microbial study are shown in TABLE 4.

TABLE 4 : Antimicrobial Activity of Ethanolic Extract of *Centella asiatica*

Microorganism	Diameter of Zone of Inhibition (mm)			
	10 mg/ml	20 mg/ml	50 mg/ml	Standard
Escherichia coli	18	22	31	29
Pseudomonas aeruginosa	16	19	20	26
Staphylococcus aureus	17	21	29	26
Bacilus subtilis	14	21	27	28

In the present study we have come up with results that are completely in agreement with the earlier reports, so further studies should be undertaken on the plant to identify and isolate the different active constituents responsible for different biological activity.

ACKNOWLEDGEMENT

The authors like to thank the authority of Institute of Pharmacy, Jalpaiguri for providing all necessary facilities to undertake the study. Further, authors like to thank Mr. S.K. Pathak, of Pharmaceutical Microbiology laboratory, of Institute of Pharmacy, Jalpaiguri for his help in the antimicrobial study.

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