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EVALUATION OF THE PHYTOCHEMICAL CONSTITUENTS OF ACMELLA CALVA (DC.) R.K.JANSEN

P. SHANTHI* AND P. AMUDHA

Department of Botany, Holy Cross College (Autonomous), Tiruchirappalli - 620 002, Tamil Nadu, India.

Corresponding Author shanthivaishali@yahoo.co.in

ABSTRACT

Acmella calva (DC.) R.K.Jansen., commonly known as toothache plant was analyzed for their chemical composition and minerals. The various extracts of the plant parts (leaves, inflorescence and whole plant) possessed the presence of active phytochemical constituents such as alkaloids, glycosides, flavanoids, reducing sugar, tannins, anthraquinone, saponins and cardiac glycosides. The amount of total soluble protein (0.78±0.09 mg/g) and the total free amino acids (14.95±1.00 mg/g) was remarkably high in the inflorescence. In the whole plant, the carbohydrates content was (6.66±0.35 mg/g) estimated in high quantity. The secondary plant products such as phenols (6.14±0.46 mg/g) and tannins (2.63±0.06 mg/g) were found maximum in the leaves. The herb is a good source of minerals. The maximum amount of nitrogen (0.24±0.15 mg/g) and phosphorus (0.06±0.07 mg/g) was obtained in the inflorescence and the iron (2.67±0.05 mg/g) content was recorded high in the leaves.

KEYWORDS

Acmella calva, medicinal plant, phytochemicals, minerals

INTRODUCTION

Medicinal plants including species as a group comprise approximately 8000 species and account for around 50 percent of all the higher flowering plant species in India. India has a rich biodiversity; the growing demand places a heavy strain on the natural resources. Drugs obtained from plants are believed to be much safer and exhibit a remarkable efficacy in the treatment of various ailments[1].

Acmella calva (DC.) R.K.Jansen (Akarkra, toothache plant) is a valuable medicinal plant belongs to the family *Asteraceae*. It is a rich source of therapeutic constituents and is also reported to have medicinal as well as pesticidal properties. The flower heads are used to relieve toothache. The roots, flower heads and the whole aerial parts contain spilanthol, which are a powerful stimulant, sialogogue and local anesthetic[2]. The plant extract was also used for stimulating, reorganizing and strengthening the collagen network in anti-age applications, e.g. in anti-wrinkle cream formulations[3,4]. The plant possesses bio-insesticidal[5], anti-inflammatory[6], anibacterial[7], antifungal[8], antinociception and antihyperalgessic activity[9]. The decoction is taken for dysentery. An ether extract of fresh flower tops is effective against anopheles mosquito larvae. Tincture made from flower heads is reported to be used as a substitute for tincture of pyrethrum to treat inflammation of

jaw-bones and caries[10]. The plant is being over-exploited in Nilgiris[11]. The plant contains several bioactive compounds. The present study was designed to evaluate the primary, secondary metabolites and mineral constituents of *A. calva* with respect to the role of this herb in ethnomedicine.

MATERIALS AND METHODS

Plant material collection

Healthy plants parts (leaves, inflorescence) and whole plant of *Acmella calva* (DC.) R.K.Jansen were collected from the Herbal Garden of Department of Botany, Holy Cross College, Tiruchirapalli, Tamil Nadu, India. It was authenticated by Rapinat Herbarium and Center for Molecular Systematics, St.Joseph's College (Campus), Tiruchirappalli, Tamil Nadu, India under the voucher code number: PA-001.

Extraction of plant material

The collected plant parts were cleaned, shade dried and powered by a mechanical grinder. Fifteen grams of pulverized plant materials were soaked in 100 ml of solvents (aqueous, chloroform, acetone, petroleum ether and ethanol) and incubated for 24 hrs. They were filtered using standard Whatman No.1 filter paper and the filtrate was allowed to evaporate at low temperature 10°C using Buchi Rotavapor. The extracts were stored in refrigerator and used for further analysis.

Primary phytochemical screening

The aqueous, chloroform, acetone, petroleum ether and ethanol extracts of leaves, inflorescence and whole plant of *A. calva* were subjected to qualitative test using standard procedures to identify various plant constituents as described by Harborne[12], Trease and Evans[13].

***Test for alkaloids (Mayer's test*)**: About 0.5 ml of extracts were separately treated with few drops of 1 ml 2N HCl and filtered. (Mayer's reagent: 1.36 g of mercuric chloride was dissolved in 60 ml distilled water and 5 g of potassium iodide was dissolved in 10 ml of distilled water. The two solutions were mixed and made up to 100 ml of distilled water). Few drops of reagent were added to 1.0 ml of acidic aqueous solution of samples. Formation of white or pale precipitate showed the presence of alkaloids.

***Test for glycosides*:** A small amount of alcoholic extract of sample was dissolved in 1.0 ml of water and then aqueous solution of sodium hydroxide was added. Formation of a yellow color indicates the presence of glycosides.

***Test for flavanoids*:** In a test tube containing 0.5 ml of extract of the samples, 5-10 drops of dilute hydrochloric acid and a small piece of Mg were added. The solution was boiled for few minutes. In the presence of flavanoids, the reddish pink or dirty brown color was produced.

***Test for reducing sugars*:** A known quantity of extract was dissolved in 5ml of distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

***Test for tannins (Lead acetate test*):** In a test tube containing about 5.0 ml of an aqueous extract, a few drops of 10% solution of lead acetate was added. Formation of white precipitate indicates the presence of tannins.

***Test for anthraquinones*:** The extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 solution were added to the mixture and heated. Formation of rose-pink color indicates the presence of anthraquinones.

***Test for saponins*:** The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. One cm length of foam was formed indicating the presence of saponins.

***Test for cardiac glycosides*:** About 2 ml of extract, 1 ml of glacial acetic acid, $FeCl_3$ and Conc. H_2SO_4 were added. Formation of blue precipitate indicates the presence of cardiac glycosides

Quantitative estimation of the phytochemicals and minerals:

***Determination of total protein content*:** Hundred milligram of sample was homogenized with 5 ml of ice-cold phosphate buffer and centrifuged at 2000 rpm for 5 minutes. To the supernatant solution, equal volume of 10% ice-cold TCA was added and incubated for 10 min. at 4°C for an hour. The precipitated protein was centrifuged and the pellet was dissolved in one ml of 0.1N NaOH. 0.5 ml of the protein solution was mixed with 5 ml of alkaline copper reagent. It was shaken well and allowed to stand at room temperature for 10 min. Then, 0.5 ml of folin – ciocalteau reagent was added and the volume was made upto a known quantity using distilled water. Blank was prepared without the sample extract. After 30 minutes the optical density of the solution was read at 660 nm in Spectronic – 20 D[14].

***Determination of total free amino acid*:** Hundred milligram of dried sample powder was homogenized with 5 ml of 80 % ethanol and centrifuged at 2000 rpm for 10 min. The pellet was re-extracted with the same solvent and centrifuged again. The supernatant were pooled. To the supernatant equal volume of petroleum ether was added to remove the chlorophyll pigments using separation funnel. The lower layer was taken as sample. 0.5 ml of acetate buffer was added to the 1 ml of alcoholic extract, followed by 1 ml of 1% ninhydrin. The reaction mixture was heated for 15 min. in a boiling water bath at 100ºC for colour development. It was then cooled and the volume was diluted to 10 ml with distilled water. For blank, 0.5 ml distilled water was taken and all the reagents were added and carried out as above. The color intensity was measured at 570 nm[15].

***Determination of total carbohydrates*:** Hundred milligram of dried sample powder was homogenized with 5 ml of 80 % ethanol and centrifuged at 2000 rpm for 10 min. The pellet was re-extracted with the same solvent and centrifuged again. The supernatant were pooled. To the supernatant, equal volume of petroleum ether was added to remove the chlorophyll pigments using separation funnel. The lower layer was taken as sample. 1 ml of protein free carbohydrate solution was mixed with 4 ml of the anthrone reagent (0.2% in Conc.H_2SO_4). The reaction mixture was heated for 5 min. in a boiling water bath at 100ºC with the marble on the top of the test tube to prevent loss of water by evaporation. Suitable reagent blank was prepared. The colour intensity was measured at 620 nm in a Spectronic -20[16].

***Tannin determination*:** 500 mg powdered sample material was transferred to 250 ml conical flask containing 75 ml of distilled water. The contents in the flask were boiled for 30 min, centrifuged for 2000 rpm for 20 min. The supernatant was collected in 100 ml volumetric flask and made up to a known volume. One ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml of distilled water. To this 5 ml of Folin-Denis reagent and 10 ml of sodium carbonate solution were added and diluted to 100 ml. It was shaken well and left for 30 min. and the absorbance was read at 700 nm against a reagent blank (water) [17].

***Determination of total phenols*:** 100 mg of the sample was extracted with 5 ml of 80% ethyl alcohol and centrifuged at 2000 rpm. The supernatant was taken for assay. One ml of folin–ciocalteau reagent was added to 1 ml of the alcoholic extract of the sample. 2 ml of 20 % sodium carbonate was added and heated for one min. After cooling, the solution was made up to 10 ml with distilled water. A blank was prepared by adding all the reagents except the sample. The absorbency was read at 650 nm in spectrophotometer[18].

***Determination of nitrogen*:** For the estimation of total nitrogen, 50gm of sample was transferred to a micro-kjeldahl digestion flash. One ml of concentrated H_2SO_4 (AR) was added to the flask and then 60 mg of catalyst mixture [(copper sulphate, ($CuSO_4.5H_2O$): potassium sulphate, (K_2SO_4) and selenium dioxide, (SeO_2) in ratio of 1:8:1, were ground separately and mixed together)] was also added. The flask was gently heated on a digestion rack until foams of H_2SO_4 were evolved and then heated strongly until the digests in the flasks turned apple green. Reagent blank (all reagents except sample) was run simultaneously. The digest was cooled and then made upto 10 ml with glass distilled water. To a suitable aliquot of this (1.0 ml), 2.0 ml of water Nessler's reagent and 3.0 ml of 2N NaOH were added in the order given and mixed by shaking. After 15 minutes, the absorbance was read at 490 nm against a reagent blank. A standard graph using known quantities of "Analar" NH_4Cl was used for reference to determine the quantities of total nitrogen in the sample[19].

Preparation of dry ash

One gram of sample was taken in silica crucible and heated over a low Bunsen flame to volatilize as much of the organic material as possible. The crucible was then transferred to a temperature controlled muffle furnace. The temperature was maintained at about 300 ºC for 5-7 hours. It was removed from the muffle furnace and allowed to cool. After cooling, the weight of the ash was noted down. The ash was dissolved in 50% HCl (Con. HCl: water (1:1)) and filtered using Whatman No.44 filter paper. The filtered solution was made upto a known volume and stored for the analysis of mineral constituents[20].

***Determination of phosphorus*:** To the 1ml of ash solution, 5 ml of molybdate reagent was added along with 2.0 ml of amino naphthol sulphonic acid solution. The reagent mixtures were shaken well and made upto 50 ml distilled water in a standard flask. The blank solution was prepared simultaneously without the addition of ash solution. It was allowed to stand for 10 min. The intensity was measured at 650 nm against a reagent blank at Spectronic - 20 D^{20}.

***Determination of iron*:** To the 2ml of the digested ash solution, 10 ml of acetate buffer and 2 ml of phenanthroline reagent were added and the volume was made upto 50 ml. The blank solution was prepared without the addition of ash solution. Reading was taken at 460 nm in a Spectronic - 20 D^{20}.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Preliminary phytochemical investigation showed that saponins were present in all the extracts of whole plant, leaves and inflorescence. Aqueous extract of whole plant and inflorescence showed positive test for reducing sugar, anthraquinone, flavanoids, saponins, tannins, alkaloids and glycosides. Alkaloids were present in chloroform extract of whole plant, leaves and inflorescence. Cardiac glycosides were present in chloroform, petroleum ether extract of inflorescence (Table 1). The phytochemical screening of chemical constituents of the plant in different solvents studied showed that the leaves, inflorescence and whole plant were rich in alkaloids, glycosides, flavanoids, reducing sugar, tannins, anthraquinone, saponins and cardiac glycosides. Our results are in agreement with the report of Edeoga et al.[21] in several plant samples. In the present investigation, aqueous extract showed the presence of flavanoids which may be accounting for the anti-inflammatory and analgesic activites[6]. The presence of alkaloid and saponins in the plant indicates that the plant extracts could be used for the antifungal activity[24]. Thus the preliminary screening test indicates the presence of the above mentioned bioactive compounds. Subsequently it may be used for the preparation of drug in a systematic way which may lead to the cure of many ailments in the future.

Table 1
Preliminary phytochemical screening of leaves, inflorescence and whole plant of Acmella calva (DC.) R.K.Jansen using different solvents

S. No	Tests	Aqueous			Chloroform			Acetone			Petroleum ether			Ethanol		
		I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
1	Alkaloids	-	+	+	+	+	+	-	-	+	-	+	+	-	-	-
2	Glycosides	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+
3	Flavanoids	+	+	+	+	+	-	+	-	-	+	-	+	-	-	+
4	Reducing sugar	+	+	+	-	+	+	+	+	-	+	+	-	+	-	-
5	Tannins	+	+	+	+	-	+	+	-	-	+	+	+	+	+	-
6	Anthraquinone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
7	Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Cardiac glycosides	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-

I – Leaves, II – Inflorescence, III – Whole plant
+ = Presence, – = Absence

Estimation of phytochemicals and minerals

Quantitative estimation of phytochemicals of different parts (leaves, inflorescence and whole plant) of this medicinal plant were analyzed and summarized in Table 2. The quantity of total soluble protein (0.78±0.09 mg/g) and total free amino acids (14.95±1.00 mg/g) were found higher in inflorescence than in leaves and whole plant. Carbohydrates contents were found maximum (6.66±0.35 mg/g) in whole plant. Leaves showed considerably more amount of phenols (6.14±0.46 mg/g) and tannins (2.63±0.06 mg/g) than the whole plant and inflorescence. Similar findings were recorded by Tanwer et al. (2010), they reported that the *in vitro* and *in vivo* comparative study of primary metabolites in *Spilanthes acmella* Murr[22].

Table 2
Estimation of chemical constituents in different parts and whole plant of Acmella calva (DC.) R.K.Jansen.

S. No	Phytochemicals in mg/g	Leaves	Inflorescence	Whole plant
1.	Protein	0.56±0.03	0.78±0.09	0.59±0.02
2.	Free amino acid	6.81±4.99	14.95±1.00	7.15±0.15
3.	Carbohydrates	3.93±0.25	2.83±0.04	6.66±0.35
4.	Phenol	6.14±0.46	6.12±0.42	6.04±0.10
5.	Tannin	2.63±0.06	1.85±0.03	2.09±0.04

(mg/g) – milligram per gram dry weight
Data are Mean±SD of the treatment and repeated three times.

The mineral contents of the plant parts are shown in Table 3. Nitrogen and phosphorus content were estimated to be remarkably high in inflorescence at 0.24±0.15 mg/g and

0.06±0.07 mg/g respectively. While, iron content was found maximum (2.67±0.05 mg/g) in the leaves. The present study has shown that the high content of phytochemicals of *A. calva* possesses antimicrobial activity. The presence of bioactive compounds indicated that the plant extracts were found active against *Staphylococcus aureus*, *Pseudomonous aeruginosa*[23], *Aspergillus niger, aspergillus parasiticus, Fusarium oxysporium* and *Fusarium moniliformis*[24]

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Table 3
Estimation of minerals in different parts and whole plant of Acmella calva (DC.) R.K.Jansen.

S. No	Minerals in mg/g	Leaves	Inflorescence	Whole plant
1.	Nitrogen	1.11±0.27	0.24±0.15	1.15±1.38
2.	Phosphorus	0.02±2.15	0.06±0.07	0.03±0.02
3.	Iron	2.67±0.05	2.29±0.28	2.57±0.05

(mg/g) – milligram per gram dry weight.
Data are Mean±SD of the treatment and repeated three times.

CONCLUSIONS

This study has provided some biochemical basis for the ethnomedical use of extracts from *A. calva* in the treatment and prevention of infections. The plant studied here can be seen as a potential source of useful drugs. Further studies are going on this plant in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

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REFERENCES

1. Gondes MC and David IN., Natural product drug discovery in the next millennium. Phaom. Biol., 139: 8-17, (2001).
2. Peiris KPP, Silva GK and Ratnasooriya WD., Analgesic activity of water extract of *Spilanthes acmella* flowers on rats. J. Trpo. Med. Plant, 2: 201 (2001).
3. Schubnel L., A different approach to lifting efficacy based on a natural active ingredient. SOFW J., 133: 34-39, (2007).
4. Demarne F and Passaro G., Use of an *Acmella oleracea* extract for its botox-like effect in an antiwrinkle cosmetic composition, FR Pat. 286513, (2009).
5. Ramsewak RS, Erickson AJ and Nair MG., Bioactive N-isobutylamides from the flower buds of *Spilanthes acmella*. Phytochemistry, 51 (6): 729-732, (1999).
6. Chakraborty A, Devi RKB, Rita S., Sharatchandra K and Singh TI, Preliminary studies on anti-inflammatory and analgesic activities of *Spilanthes acmella* in experimental animal models. Indian. J. P. Pharmacol., 36 (3): 148 – 150, (2004).
7. Panthi MP and Chaudhary RP., Antibacterial activity of some selected folklore medicinal plants from west Nepal. Scientific World, 4 (4): 16- 21 (2006).
8. Rani SA and Murty SU., Antifungal potential of flower head extract of *Spilanthes acmella* Linn. African J. of Biomedical Research, 9: 67 -69, (2006).

9. Ratnasooriya WD and Pieris KPP., Attenuation of Persistent Pain and Hyperalgesia by *Spilanthes acmella* Flowers in Rats. Pharmaceutical Biol., 43 (7): 614, (2005).
10. Prajapati NS, Purohit SS, Sharma AK and Tarunkumar., A hand book of medicinal plants. Agrobios (India), 2003, pp. 485.
11. Paulsamy S., Annual Progress Report of Project, "Evaluation of conservation strategies for the sustainable utilization of herbaceous bioresource in the shoals of Nilgiris, the Western Ghats", sponsoresd by the Ministry of Environment and Forests, Govt.of India, New Delhi, (2005).
12. Herborne JB., Phytochemical Methods 3rd Edn. Chapman and Hall Ltd. publishers, London: 135-203, (1973).
13. Trease GE and Evans WC., Trease & Evans'Pharmacognosy 13th Edition. Bailliere Tindale publisher, London: 832, (1989).
14. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ., Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 256-275, (1951).
15. Moore S and Stein WH., Photometric ninhydrin methods for use in the chromatography of amino acids. J. Biol. Chem., 176: 367-388, (1948).
16. Mahadevan A and Sridhar R., Methods in physiological plant pathology, Sivakami publications, Chennai: 121-122, (1982).
17. Sadasivam S and Manickam A., Biochemical methods for agricultural sciences. Wiley Eastern Ltd. publications, Chennai. (1992).
18. Mahadevan A and Sridhar R., Methods in physiological plant pathology, Sivakami publications, Chennai. (1982).
19. Umbreit WW, Burris RH and Stauffer JF., Methods for nitrogen fixation. In : Manometric and biochemical technique 5th edition: 259-260, (1972).
20. Ranganna S., Minerals. In: Manual of analysis of fruit and vegetable precuts. Tata McGraw – Hill publishing company Ltd., New Delhi. 102-111, (1978).
21. Edeoga HO, Okwu DE and Mbaebie BO., Phytochemical constituents of some Nigerian medicinal plants. African J. of Biotech., 4 (7): 685-688, (2005).
22. Tawer SB, Choudhary R and Vijayvergia R., In vitro and in vivo study of primary metabolites and antioxidant activity in *Spilanthes acmella* Murr. International J. of Biotech. And Biochem. 6 (5): 819-825, (2010).
23. Panthi MP and Chaudhary RP., Antibacterial activity of some selected folklore medicinal plants from West Nepal. Scientific World, 4 (4): 16-21, (2006).
24. Rani SA and Murty SU., Antifungal potential of flower head extract of *Spilanthes acmella* Linn. African J. of Biomedical Research, 9 (1): 67-68, (2006).