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Phytochemical Investigations of Flower Drug from Plant *Acmella Paniculata* (Wall. Ex DC.) R. K. Jansen

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Abstract
Objective: *Acmella paniculata* (Wall. Ex DC.) R. K. Jansen, also known as Akkalkadha or toothache plant is an important medicinal herb of the Asteraceae family that grows in the tropics and subtropics of the world.
Material and Methods: The present phytochemical investigation aimed at evaluating the impact of different solvents on extraction yields, ash analysis, fluorescence analysis and phytochemical compounds from flower drug of *A. paniculata* plant. The results indicate that solvents have a significant impact in the yield of extraction.
Results: Resulting in the higher extraction yield in solvent distilled water and ethanol. Flavonoids, alkaloids, tannins, saponins, cardiac glycosides, and terpenoids are found in the crude flower drug of the plant.
Conclusion: The present study is useful in drug standardization, identifying the adulteration in the Ayurvedic medicines from this plant.

Introduction

Alternative medicines are being used by about 60 percent of the world's population. These medicines are not only used by the rural masses for their primary health care in developing countries but are also used in developed countries where modern medicines dominate [1-3]. The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments. Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. In India, about 70 percent of rural population depends on the traditional Ayurvedic system of medicine [1-3]. Due to amplified and unsystematic use of antibiotics for treatment of

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humans and animals develops the antibiotic resistance and multidrug resistance microorganisms which has increased a great deal in developing countries [4]. The requirement of more and more drugs from plant sources is constantly increasing which necessitates screening medicinal plants with promising biological activity [5-6].

The family Asteraceae which is known to be one of the largest vascular plant families, with an overall 30,000 species and 1100 genera, almost whole of these plant types were intended to display antimicrobial activity [7] which was due to the production of principal secondary metabolites, namely sesquiterpene [8]. The well-known genus *Spilanthes* had been found to contain 35 tropical species, of which three of them are reported from India. Probably around the world in tropical and subtropical regions *A. paniculata*, a vital medicinal plant is prominently distributed [8].

A. paniculata is a well-known anti-toothache plant and is used in traditional medicine for many purposes. The different plant parts of *A. paniculata* like flowers heads, leaves, roots, stem and other aerial parts have been used in various health care systems [9]. In the tropics and subtropics, this plant is widely used in traditional medicine. The major use in medicine is for toothache where the fresh flower head and leaves are chewed or placed in tooth cavities to relieve pain. Traditionally, *Acmella* plants are used to treat stammering in children, fungal skin diseases and remedy for snakebite. In India, juice of inflorescence of *A. paniculata* is used to treat mouth ulcers [10]. Ethiopian traditional healers use the crushed aerial parts in a paste dressing for external injuries [11-13].

It is necessary to explore the phytochemical constituents of any medicinal plant to establish a relation between pharmacology and chemistry of the plant. Many studies have been carried out for chemical analysis and structural determination of pungent alkamides from *A. paniculata*. The major pungent constituent reported in this plant *A. paniculata* is "spilanthol," which is an isobutylamide and is well known for its insecticidal properties [2-3, 14-15]. The flower head and root part of the plant have been reported to be the rich source of active principles. Triterpenoids have also been found in the plant [16]. Spilanthol is chemically N-isobutylamide which is bitter in taste and could stimulate salivation. The formula of spilanthol was determined as (2E, 6Z, 8E)-N-isobutylamide-2,6,8-decatrienamide [17]. Spilanthol has a strong pungent taste; it may produce local astringency and anaesthetic effects [2-3, 18].

Herbs, annual or perennial. Leaves opposite and/or basally rosulate. Capitula solitary or in few headed cymes, radiate, disciform, or discoid. Involucres ± hemispheric to ovoid; phyllaries 1-3- seriate, subequal or with outer row spreading and longer, entire or irregularly dentate; receptacles conical; paleae falling with achene, ± navicular, membranous to scarious, each ± equaling subtended floret. Ray florets, when present, 2- or 3-lobed, variously colored. Disk florets: corolla yellow or orange, 4- or 5-lobed. Achene margin ciliate, glabrous, or sometimes corky; ray achenes broadly ovate or elliptic, 3-angled; disk achenes ellipsoid, strongly compressed; pappus absent or of up to 10 awn like bristles [19-20].

Herbs, annual. Stems branched, erect or ascending, to 30 cm or taller, rarely rooting at nodes. Petiole 1-2 cm; leaf blade ovate to ovatelanceolate, 2-4 × 1-2.5 cm, 3-veined, base cuneate, margin entire or coarsely or crenately serrate, apex acute. Capitula discoid, solitary, terminal or axillary, 8.4-12.5 × 6.9-10 mm; peduncles 2.5-16 cm, sparsely pilose; phyllaries 9-12, 2-seriate, ovate-lanceolate, ca. 6 mm, herbaceous, glabrous; receptacle 5-8 × 1.1-3 mm, apex acuminate. Florets 90-200; corollas tubular, minute, 4- or 5-lobed. Achenes obovoid, 3- angled, ca. 3 mm, margin scabrid, apex slightly depressed; pappus of 2 subequal bristles, longer one 0.5-1.1 mm, shorter one 0.4-0.9 mm. Flowering – fruiting: - October to April [19-21].

Phytochemical estimation of medicinal plants plays a significant role in revealing the new sources for therapeutically important compounds [22]. Bioactive compounds that are isolated from the plant materials have proven to be a beneficial source of metabolites which are difficult to get from other sources [23]. Analysis based on previous scientific documentation reveals that not much significant work has been done on the chemical composition and biological activities of *A. paniculata* from India. However, one report has been documented for the phytochemical composition of leaves of the plant from Indonesia [24-25]. The current study analyses the phytochemical composition, ash and fluorescence analysis of the whole flower drug of *A. paniculata*.

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Materials and Methods Collection and authentication of plant material

The whole plant of *A. paniculata* was collected from rural areas of Surgana Tehsil of Nashik District, Maharashtra in the month of March and June. The plant was authenticated and the herbarium specimen was deposited at Department of Botany, MGV's Arts, Science and Commerce College Surgana, District Nashik, Maharashtra [26]. The flowers were collected, washed with running tap water, air dried under low temperature for 48 h and later in hot air oven at low temperature for a week. Dried flowers were crushed into smaller pieces and stored for further study [27].

Preparation of extracts Preparation of flower Extract

Solvent extraction of crude whole flower extract was made ready by Soxhlet extraction techniques. About 20 gm of fresh flower material was equally packed into a thimble and extracted with 250 ml of two solvents one by one. Solvents used were acetone and distilled water as per polarity [28]. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor emerge as colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40℃ till all the solvent got evaporated. Dried extract was kept in refrigerator at 4℃ for their further use in phytochemical evaluation [28-31].

Extract recovery percentage

The amount of *Acmella paniculata* extract obtained after successive extractions was weighed and the percentage yield calculated according to the following formula [32],

Extract Recovery Percentage = Amount of extract/Amount of plant sample (G) X 100

Ash analysis and fluorescence analysis

Total ash and acid insoluble ash contents are important indices to determine quality and purity of herbal medicines. Total ash values of floral buds were determined by igniting the powdered sample gradually between 500°C and 600°C until it turned white, and the resultant powder was desiccated and weighed. Acid insoluble ash content was measured by solubilizing the part of total ash in hydrochloric acid with boiling followed by collecting and washing on filter paper, cooling in desiccator and weighing. Similarly, water soluble ash content was also measured. For extractive values, 2 g of each finely powdered sample were macerated with solvents of different polarities and was stirred on a shaker for 6 h. The extracts were filtered, concentrated to dryness and weighed. Ash and extractive values were determined as given in the guidelines of WHO [32-34]. The fluorescence analysis of powdered samples was carried out using ultraviolet (UV) lamps of short (254 nm) and long (365 nm) wavelengths. The samples were observed for fluorescence when mixed with different solvents and reagents. The fluorescence analysis was carried out by the method of Chase and Pratt [33-35].

Phytochemical screening

A stock concentration of 1 % (W/V) was prepared using the respective solvent in each case. These extracts along with positive and negative controls were tested for the presence of active phytochemicals. Preliminary phytochemical analysis was carried out to find the presence of the active chemical constituents in extracts such as amino acids, carbohydrates, alkaloids, steroids, Cardiac Glycosides, flavonoids, saponins, tannins, terpenoids and phenols. In general, tests for the presence of phytochemical compounds involved the addition of appropriate chemical reagent(s) to the extract in test tubes [29, 36-44].

Test for Amino Acids

2 ml of solvent extract was mixed with 2ml ninhydrin reagent and kept in hot water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Carbohydrates

2 ml of methanolic extract was mixed with 2 drops of Molisch's reagent and shake well. Add 2 ml of concentrated sulphuric acid in the sides of the test tube. A reddish violet color ring appeared at the junction of the two layers immediately indicated the presence of carbohydrates in the sample.

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Test for Alkaloids

1 ml extract was mixed with 1% HCl and 6 drops of Mayer's reagent and Dragendorf's reagent. A turbidity or precipitation indicated the presence of alkaloids in the sample.

Test for Steroids

2 ml of acetic anhydride was mixed with 0.5 ml solvent extract and further added with 2 ml concentrated sulfuric acid. The color change from violet to blue or green indicates the presence of steroids.

Test for Cardiac Glycosides

5 ml of solvent extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution already. This solution is further under layered with 1ml conc. H_2SO_4 [42-44]. A brown ring on the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer [26].

Test for Flavonoids

Aqueous extract was added with 5 ml ammonia solution and conc. H_2SO_4. A yellow coloration confirms the presence of flavonoids which disappears on standing long.

Test for Saponins

Take small amount of extract with 20 ml of distilled water. Agitate the mixture for 15 minutes in graduated cylinder. The formation of 1cm layer of foam indicated the presence of saponins.

Test for Tannins

Take 5 ml of extract with few drops of lead acetate. A yellow precipitate confirms the presence of tannins.

Test for Terpenoids

Take 2 ml solvent extract with 2 ml of chloroform and 3 ml of conc. H_2SO_{40} [26] to form a monolayer of reddish brown coloration of the interface revealed presence of terpenoids.

Test for Phenols

2 ml of extract was mixed with 3 ml of ethanol and a pinch of $FeCl_3$ to form greenish yellow color showing presence of phenols [42-44].

Results

Extract yield percentage of the plant extracts

The *Acmella paniculata* flowers shows maximum extract yield in hot distilled water extract (13.4 %) and shows the least yield in chloroform extract (7.2%). Ethanol extract has an extract yield percentage of 10.1% and acetone has a yield of 9.4% (Table 1).

Ash analysis and fluorescence analysis

Ash analysis of powered flower drug of plant *A. paniculata* was performed and total ash, acid insoluble ash and water soluble ash values were calculated. The total ash value found was to be 9.35%, acid insoluble ash was 5.75% while the water soluble ash was 3.12% (Table 1).

Table 1. *Ash analysis and extractive values of powdered drug Acmella paniculata flowers.*

Ash analysis of flower drug	
Parameter	**Value**
Total ash	9.35 %
Acid insoluble ash	5.75 %
Water soluble ash	3.12 %
Extractive values with organic solvents	
Distilled water	13.4 %
Ethanol	10.1 %
Acetone	9.4 %
Chloroform	7.2 %

Fluorescence showed by the powdered flower drug of *A. paniculata* plant is showed in table 2. The day light, short ultra violet light (UV-254) and long ultra violet light (UV-365) were used [45] with power as such and with chemicals like HCl, HNO_3, H_2SO_4, NaOH, chloroform and distilled water.

Table 2. *Fluorescence analysis of powdered drug of Acmella paniculata flowers in day light, UV-254 and UV-365 light after treated with different reagents.*

Fluorescence analysis of flower drug

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Treatment	Day Light	UV-254	UV-365
Normal/ powder as such	Brown	Dark brown	Grey
HCL	Greenish brown	Dark brown	Light green
HNO_3	Red brown	Brown red	Brown red
H_2SO_4	Dark brown	Dark brown	Brown
NaOH	Yellowish brown	Blue	Light blue
Chloroform	Dark brown	Blue	Light blue
Distilled water	Light brown	Blue	Light blue

Phytochemical investigation

Phytochemical investigations of *Acmella paniculata* flowers were conducted using distilled water, ethanol, acetone and chloroform extracts. The distilled water extracts showed presence of alkaloids, flavonoids, saponins and terpenoids. The ethanol extracts showed the presence of alkaloids, cardiac glycosides, flavonoids, saponins, tannins and terpenoids. The acetone extracts contain amino acids, alkaloids, cardiac glycosides, tannins and terpenoids. The chloroform extracts showed the presence of alkaloids and saponins only. The alkaloids were found to be present in almost all the four extracts while carbohydrates, steroids and phenols were totally found to be absent in all the four extracts [26].

Table 3. *Phytochemical investigations of Acmella paniculata flower drug extracts.*

Variable	Distilled Water Extract	Acetone Extract	Chloroform Extract	Ethanol Extract
Alkaloids	+	+	+	+
Amino acids	-	+	-	-
Carbohydrates	-	-	-	-
Cardiac Glycosides	-	+	-	+
Flavonoids	+	-	-	+
Phenols	-	-	-	-
Saponins	+	-	+	+
Steroids	-	-	-	-
Tannins	-	+	-	+
Terpenoids	+	+	-	+

(+) = Presence, (-) = Absence

Discussion

The healing properties of plant metabolites generally resulted from the mixture of bioactive compounds. Herbal compounds, as natural compounds or standardized extracts, give limitless scope for the drug development because of the unequalled opportunity of chemical range within the plants [46]. The products of *Acmella paniculata* in market within the areas of medicine, beauty and food products has better destiny, even as further studies are required to determine the iminent applicability and prospects which can also give an explanation for their bioactivity. In order to decide the actual potential of bioactive compounds and to broaden new technologies, greater information is needed to consent by silence or without objection. Lacking the precise information of the species, all of the phytochemical and pharmacological studies might be troublesome. Hence, the present investigation reveals the importance of extraction of secondary metabolites from plant *Acmella paniculata*. Thus it is concluded that the plant *A. paniculata* is an ideal source of secondary metabolites and highlights the phytochemical investigation of *A. paniculata*.

Conclusion

The present investigation reports the extraction, extract recovery percentage, ash analysis and fluorescence analysis using different solvents and reagents. Among the solvents used, ethanol and distilled water were the best solvents for extracting secondary metabolites from flower drug of plant *Acmella paniculata*. Ash analysis is useful in studying the inorganic elemental analysis of the crude drug. The fluorescence analysis was performed because different

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chemical constituents in plant behave differently in different reagents and emits variable colours. Such colours can be used as pharmacognostic standards for evaluation of any crud drug. The phytochemical investigation of flower drug of plant *A. paniculata* was performed for qualitative analysis of bioactive secondary metabolites. The present study is useful in drug standardization, identifying the adulteration in the Ayurvedic medicines from this plant. Further investigation should be under taken to evaluate and analysis of quantitative phytochemicals, antimicrobial properties and antioxidant properties of this crude drug.

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Conflicts of interest

None of the authors have any conflict of interest to declare.

Consent for publications

All authors approved the final manuscript for publication.

Availability of data and material

Data are available on request from the authors.

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