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Comparitive phytochemical screening of *Acmella calva* (dc.) r. k. Jansen and *crotalaria ovalifolia* wall: Potential medicinal herbs

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Abstract
The study was aimed at analysis of potential bioactive constituents of aerial parts of *Acmella calva* (DC.) R. K. Jansen and *Crotalaria ovalifolia* Wall. The qualitative phytochemical screening was done using ethanol and water as solvents. Alkaloids, flavonoids, tannins, phenols, glycosides, proteins and amino acids, carbohydrates, terpenoids, saponins, quinones, anthroquinones, gum and mucilage were qualitatively analysed. The aqueous extract gave better result which indicated the presence of alkaloids, phenols, tannins, flavonoids, saponins, terpenoids, carbohydrates, coumarins and quinones. Terpenoids were absent in *Crotalaria ovalifolia*. Anthraquinones, protein and aminoacids, fixed oil and glycosides were not deducted in both the extracts of study plants.

Keywords: Phytochemicals, *Acmella calva, Crotalaria ovalifolia*, Asteraceae

1. Introduction
Medicinal plants are the oldest known health-care source. They are also important for pharmacological research and drug development and are used as basic components for the synthesis of drugs or as models for pharmacologically active compounds. Phytochemical investigation is the primary step to discover the medicinal importance of such plants. It reveals the presence of bioactive components which forms the source of drugs.
Acmella calva (DC.) R. K. Jansen belonging to the family Asteraceae is an erect annual herb attaining a height of 50-60 cm and it has yellow cone like flowers. It is commonly known as Akarkara or toothache plant. It has been used as folk medicine since ancient times to cure severe toothache, affections of throat and gums, stomatitis, paralysis of tongue and psoriasis. *Crotalaria ovalifolia* Wall. Also belongs to the family Asteraceae. It is a prostrate herb, stem wiry, leaves simple, ovate, orbicular, sparsely pubescent, up to 1.2 inch long, 0.7 inch broad, herbaceous, with pubescence hair all over the body. Flower arranged in racemes, few flowered, flowers 0.6 inch long, pod 1 inch long pod stalked, oblong, inflated.
However, very little information is available on such activity of medicinal plants. Hence, in the present study phytochemical screening of 2 important medicinal plants *Acmella calva* and *Crotalaria ovalifolia* was carried out.

2. Materials and Methods
2.1 Collection of the plant material
Healthy whole plants of *Acmella calva* and *Crotalaria ovalifolia* were collected near Kandhara falls of Wayanad, Kerala and Thindal, Erode, Tamil Nadu respectively.

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

2.3 Preliminary Phytochemical Screening
The aqueous and ethanol extracts of whole plant of *Acmella calva* and *Crotalaria ovalifolia* were subjected to qualitative test using standard procedures to identify various plant

constituents. Carbohydrates, glycosides, proteins, amino acids, fixed oils, steroids, alkaloids, phytosterols, flavonoids, tannins, phenolic compounds, saponins, triterpenoids etc. were qualitatively analysed.

2.4 Detection of Alkaloids Wagner's test [1]
Filtrate was prepared by adding 3 ml of HCl to 50mg of plant extract. To 1 ml of the filtrate, few drops of Wagner's reagent were added by the side of the test tube. The colour was observed. A reddish-brown precipitate confirms the presence of alkaloid.

2.5 Detection of Phenolic Compounds Lead Acetate test
5 mg of extract was mixed with 5 ml of distilled water. To this mixture, 3 ml of 10% lead acetate solution was added. Formation of bulky white precipitate indicates the presence of phenols.

2.6 Detection of Tannins Ferric Chloride test
The extract (5ml) was dissolved in 5 ml of distilled water and to this few drops of neutral 5% ferric chloride solution was added. The formation of blue green colour indicates the presence of tannins.

2.7 Detection of flavonoids Ammonium hydroxide test
An aqueous solution of the extract was treated with ammonium hydroxide solution. The yellow fluorescence indicates the presence of flavonoids.

2.8 Detection of Glycosides Borntrager's test
2ml of the extract was treated with 3 ml of chloroform and the chloroform layer was separated. To this dilute 10% ammonia solution was added. Pink colour in the ammonia solution indicates the presence of glycosides.

2.9 Detection of Terpenoids
Chloroform (2ml) and 1ml of concentrated sulphuric acid was added carefully to 0.05ml of the plant extract. Formation of reddish brown colour indicates the presence of terpenoids.

2.10 Detection of Carbohydrates
(i) Barfoed's test
1 ml of Barfoed's reagent was added to 1 ml of plant extract and the mixture was heated on a boiling waterbath for 2 minutes. The formation of red precipitate indicates the presence of carbohydrates.

(ii) Fehlings test [2]
1 ml of the extract was boiled on water bath with 1 ml of Fehlings solution A and B. The colour change was observed. A red precipitate formation indicates the presence of sugars.

2.11 Detection of Protein and Aminoacid
(i) Biuret test [3]
An aliquot of 2 ml of the filtrate was treated with a drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added followed by excess of potassium hydroxide pellets. The formation of pink colour in ethanol layer indicates the presence of proteins.

(ii) Ninhydrin test [4]
2 drops of ninhydrin solution (5mg of ninhydrin in 200 ml of acetone) was added to 2 ml of aqueous filtrate. The colour change was observed. The formation of characteristic purple colour indicates the presence of amino acid.

2.12 Detection of Saponins
Distilled water (2ml) was added to 1ml of plant extract and shaken in graduated cylinder for 15 minutes lengthwise. Formation of 1 cm foam indicates the presence of saponins.

2.13 Detection of Coumarins
10% of NaOH (1ml) was added to 1 ml of the plant extract. The formation of yellow colour indicates the presence of coumarins.

2.14 Detection of Quinone
Concentrated sulphuric acid (1ml) was added to 1 ml of plant extract. The formation of red colour indicates the presence of quinone.

2.15 Detection of Anthraquinones
Few drops of 2% HCl were added to 0.5 ml of extract. Appearance of the red colour indicates the presence of anthraquinones.

2.16 Detection of fixed Oil [5]
A small quantity of extract was pressed between 2 filter papers. The presence of oil stain on the filter paper indicates the presence of fixed oil.

2.17 Detection of Gum and mucilage [6]
The plant extract was diluted with 5 ml of distilled water and to this 25 ml of absolute alcohol was added with constant stirring. The formation of white or cloudy precipitate indicates the presence of gums and mucilage.

3. Results and Discussion
3.1 Results
The results of the preliminary phytochemical screening are summarized in Table – 1. The preliminary phytochemical screening of ethanolic extract of aerial parts of *Acmella calva* showed positive results for alkaloids, phenols, terpenoids, carbohydrates and quinone and negative results for tannins, flavonoids, glycosides, saponins, coumarins and anthroquinone.

Aqueous extract showed positive results for alkaloids, phenols, tannins, flavonoids, saponins, terpenoids, carbohydrates, coumarins and quinone.

The phytochemical screening of chemical constituents of *Crotalaria ovalifolia* with ethanol extract showed that the whole plant was rich in alkaloids, tannins, flavonoids, carbohydrates, saponins, coumarins and quinone and negative results for phenols. In aqueous extract most of the constituents were present and phenols were also reported in aqueous extract. Anthraquinone, protein and aminoacids, fixed oil and glycosides were not deducted in both the extracts of study plants. Presence of gum and mucilage was reported in both the extracts of *Acmella calva* and *Crotalaria ovalifolia.* According to our results aqueous extract was reported to be potent solvent in extracting phytochemicals than ethanol.

Table 1: Phytochemical Analysis of *Acmella Calva* and *Crotalaria Ovalifolia*

S. No.	Test	*Acmella calva*		*Crotalaria ovalifolia*	
		Ethanol	**Aqueous**	**Ethanol**	**Aqueous**
1	Alkaloids (i) Wagner's Test	+	+	+	+
2	Phenols (i) Lead acetate test	+	+	-	+
3	Tannins (i) Ferric chloride test	-	+	+	+
4	Flavonoids (i) Ammonium hydroxide test	-	+	+	+
5	Glycosides (i) Borntrager's test	-	-	-	-
6	Terpenoids	+	+	-	-
7	Carbohydrates (i) Borfoed's test	+	+	+	+
	(ii) Fehlings test	+	+	+	+
8	Proteins and amino acids (i) Biuret test	-	-	-	-
	(ii) Ninhydrin test	-	-	-	-
9	Saponins	-	+	+	+
10	Coumarins	-	+	+	+
11	Quinone	+	+	+	+
12	Anthraquinone	-	-	-	-
13	Fixed oil	-	-	-	-
14	Gum and mucilage	+	+	+	+

4. Discussion

The phytochemical screening and qualitative study of *Acmella calva* showed that the aqueous and ethanolic extracts of whole plant was rich in alkaloids, phenols, tannins, terpenoids, carbohydrates, coumarins and quinone. Coumarins and tannins were present only in aqueous extract. This was in agreement with previous findings [7-9]. As water is believed as universal solvent, examination with whole plant aqueous extract, the sample showed positive result for all the phytoconstituents except flavonoids and saponins. This was in consonance with the previous work [9].

The medicinal values of plants lie in some chemical substances that have a definite physiological action on the human body. Alkaloids protect the body against chronic diseases. Presence of flavonoids which may be accounting for the anti-inflammatory and analgesic activities. The importance of alkaloids, flavonoids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported [7, 8].

The phytochemical screening carried out with *Crotalaria ovalifolia* revealed the presence of alkaloids, phenols, tannins, flavonoids, saponins, coumarins and quinone. This corroborates the previous work [10].

The presence of some of these secondary metabolites suggests that the plant might be of medicinal importance and supports the bases for some of the ethno uses. Presence of phenolics will protect cardio vascular diseases, inflammation and cancer. Many papers and reviews describe the activity of phenolics. In the present investigation aqueous and ethanol extracts of *Crotalaria ovalifolia* and *Acmella calva* showed the presence of flavonoids, phenols, alkaloids, tannins and saponins which may be accounting for many ailments. Our results are in agreement with the previous reports [11, 12]. 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. The total phenol and flavonoid content of extracts of study plants have been reported as promising medicinal and nutritional ingredients.

5. Conclusion

The present investigation of *Acmella calva* and *Crotalaria ovalifolia* can be concluded that this study yielded a set of qualitative parameters or standards that can serve as to identify and determine the quality and purity of the plant material in future studies.

6. References

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