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Pharmacognostic evaluation of the whole plant of Acmella Calva (DC.) R. K. Jansen

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Abstract
Objective: The primary objective of the study is to describe the pharmacognostic nature of the rare medicinal plant which can be considered as anticariogenic agents due to the presence of active constituents in it.
Methods: This was carried by collecting the whole plant of *Acmella calva* (DC.) R.K. Jansen, subjected to successive solvent extraction procedure. Presence of phytochemical constituents, and other pharmacognostic studies likes percentage of moisture content, Ash values, fluorescence analysis and HPTLC analysis were performed.
Results: The crude part of selected medicinal plant was subjected to calculate the percentage of moisture content and ash values. The fluorescence analysis showed the presence of flavonoid (yellow), steroid (Brown) and phenols by colour formation at longer and shorter wavelengths. Ethanolic extract of selected medicinal plant was considered to carry out the study because the plant possessed high yield in solvent ethanol at 23% respectively and showed the presence of active phytochemical constituents like tannins, steroids, phenols and flavonoid in the preliminary phytochemical analysis. Then HPTLC fingerprinting was performed using mobile phase (Toluene: Ethyl acetate: Methanol (7:2:1)) which showed the presence of flavonoid with the Rf value 0.54 at 366nm.
Conclusion: The present study, indicates the capability of selected medicinal plant for anticariogenic activity due to the presence of good flavonoid content and as they are obtained naturally the chance and range of side effects are considered to be less. Yet pharmacological activity has to be performed for further details.

Keywords: *Acmella calva* (DC.) R.K. Jansen, flavonoid, HPTLC fingerprinting, phytochemical constituents, fluroscence analysis

Introduction
The medicinal plants has occupied a major role in the field of Pharmacy and medicine. India throughout its long history has accumulated a rich body of experiential facts of the use of medicinal plants for the treatment of various diseases. In India, around 45,000 medicinal plant species have been recorded recently, but more than 1000 traditional communities use about 800 plants for curing different diseases. According to WHO it was detailed that the people of both developing and developed countries the use of medicinal plants as remedy for many disorders. In developing countries, the usage of economic drug usage has been established a platform which can overcome the adverse effects and limits the drug usage to a great extent [1]. Traditional herbal medicine is a rich source for modern, molecular target specific drug discovery. Herbal medicines prepared from materials of plant origin and they are prone to contamination, deterioration and variation in composition. Plant derived medicine is widely used for human alleviation because it has no side effects. In this context the medicinal plant identification and the extraction of drugs from them is playing a major role in the field of science [2].
Among all these, the generally used anticariogenic agents which are accompanied in all the conditions of diseases are proved to be causative agents of many dental diseases recently [3]. To overcome these effects the medicinal plants are been identified which can be used as a potent alternative for the available drugs in the market [4].
Acmella calva (DC.) R.K. Jansen. (Local name - Toothache plant) is a rare perennial herb belongs to the Aster family, Asteraceae. It is native to America, Pacific islands, Asia and common in Tropical regions. It is used medicine.It is also act as ornamental plant and has insecticidal properties. It acts as an herbal remedy for toothache and oral infections.[5] Various parts of Acmella is used to cure wounds, paralysis of tongue, psoriasis, cardiovascular disease, dysentery, tincture, tumour, thyroid disorder and stimulate secretion of saliva [6, 7].

Materials and Methods
A. *Collection and Authentication of the plant material*
The whole Plant of *Acmella calva* (DC.) R.K. Jansen were collected in the month of October from the Nanjikottai road, Tanjore, Tamil Nadu, India. The whole plant was washed and shade dried under room temperature. The dried plant was powdered coarsely. The plant was identified and authenticated by Dr. S.

Soosairaj, Assiatant Professor, St. Joseph's College, Tiruchirappalli – 02 in accordance with the "Flora of Central and Northern Tamil Nadu" by John Britto S (2019) and the specimen accessed as 3002.

B. *Pharmacognostical evaluation*

1. Physico-chemical Parameters

The Physico-chemical evaluation of a crude drug involves the determination of identity, purity and quality to the concentration of the active constituents in the drug that makes it valuable to medicine [8]. The following standardization parameters were evaluated to obtain the qualitative information about the purity and quality of the whole plant of *Acmella calva* (DC.) R.K. Jansen.

2. Determination of moisture content

Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. Drying plays a very important role in the quality as well as purity of the material. Moisture will lead to the activation of enzymes and gives suitable condition, to the proliferation of microorganisms [9].

3. Determination of ash values

Ash value aids in determination of quality and purity of crude drug in powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination [10].

4. Fluorescence analysis

About 10 g of powdered drug was taken in Petri dish and treated separately with different reagents viz., methanol, 1N HCl, 50% Sulphuric acid, 1N methanolic sodium hydroxide, methanol (70% v/v), 50% nitric acid, 1N methanolic sodium hydroxide, and 5% potassium hydroxide. These were observed under short UV (254 nm), long UV (365 nm) and visible light [11].

C. Procedure for extraction

The whole part of *A. calva* was collected air dried and pulverized into coarse powder. The powder was stuffed into Soxhlet apparatus and extracted respectively with petroleum ether, ethanol, chloroform and methanol. The solvent is removed through distillation under reduced pressure and preserved in desiccators for further experiment [12]. The percent yield was tabulated.

D. Preliminary Phytochemical studies

Qualitative analysis

A preliminary phytochemical investigation was performed for the different solvent such as (ethanol, methanol, petroleum ether, chloroform, acetone, hexane and water) to identify various secondary metabolites from the whole plant of *A. calva* [13].

Quantitaive analysis

The quantitative analysis was carried out for the ethanolic extract whole plant of *A. calva* under standard procedure of Brain and Turner, 2001 [14, 15].

HPTLC Analysis

TLC plates were prepared with silica gel & activated before sample application. The sample was prepared where the stationary phase was HPTLC silica gel 60F 254 with mobile phase of Toluene: Ethyl acetate: Methanol (7:2:1) while the derivatization was carried out with Vanillin Sulphuric acid reagent and quercetin was used as a standard [16].

Results

Physico-chemical Parametres

Moisture Content

The percentage Moisture content in the whole plant of *A. calva* was found to be 7.0%w/w respectively (Figure - 01). Overall the moisture content of the whole plant is below 10% and it can be considered that the plant material is properly dried and stored well.

Ash values

The percentage of ash values of the selected medicinal plant was subjected to the ash values evaluation parameters like total ash, acid insoluble ash, water soluble ash, and sulphated ash were estimated and total ash values of all the selected medicinal plant were found to be high 7.9% when compared with the other ash values that are stipulated in the (Figure - 2) respectively. High total ash (more than 15%) value indicates that presence of inorganic constituents and very low value of acid insoluble ash denotes the presence of negligible amount of siliceous matter.

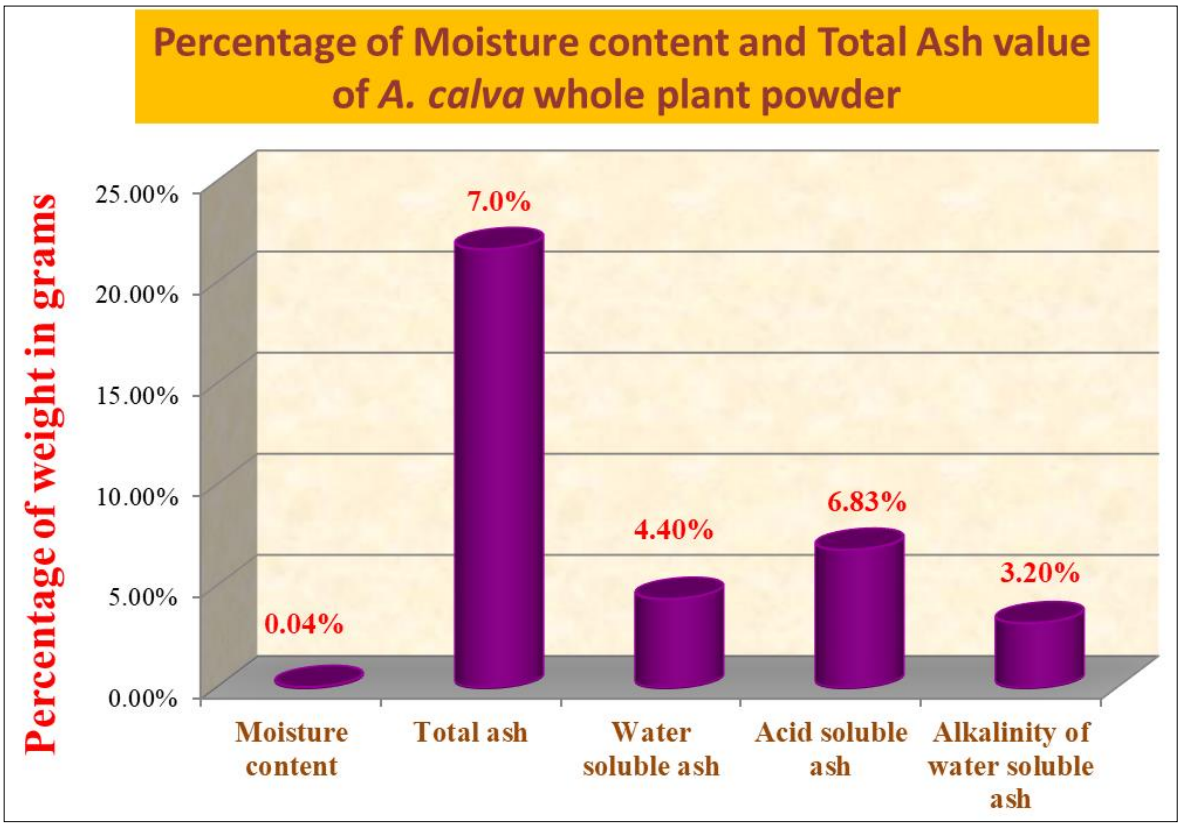


Fig 1: Percentage of Moisture content and Total Ash value of *A. calva* whole plant powder

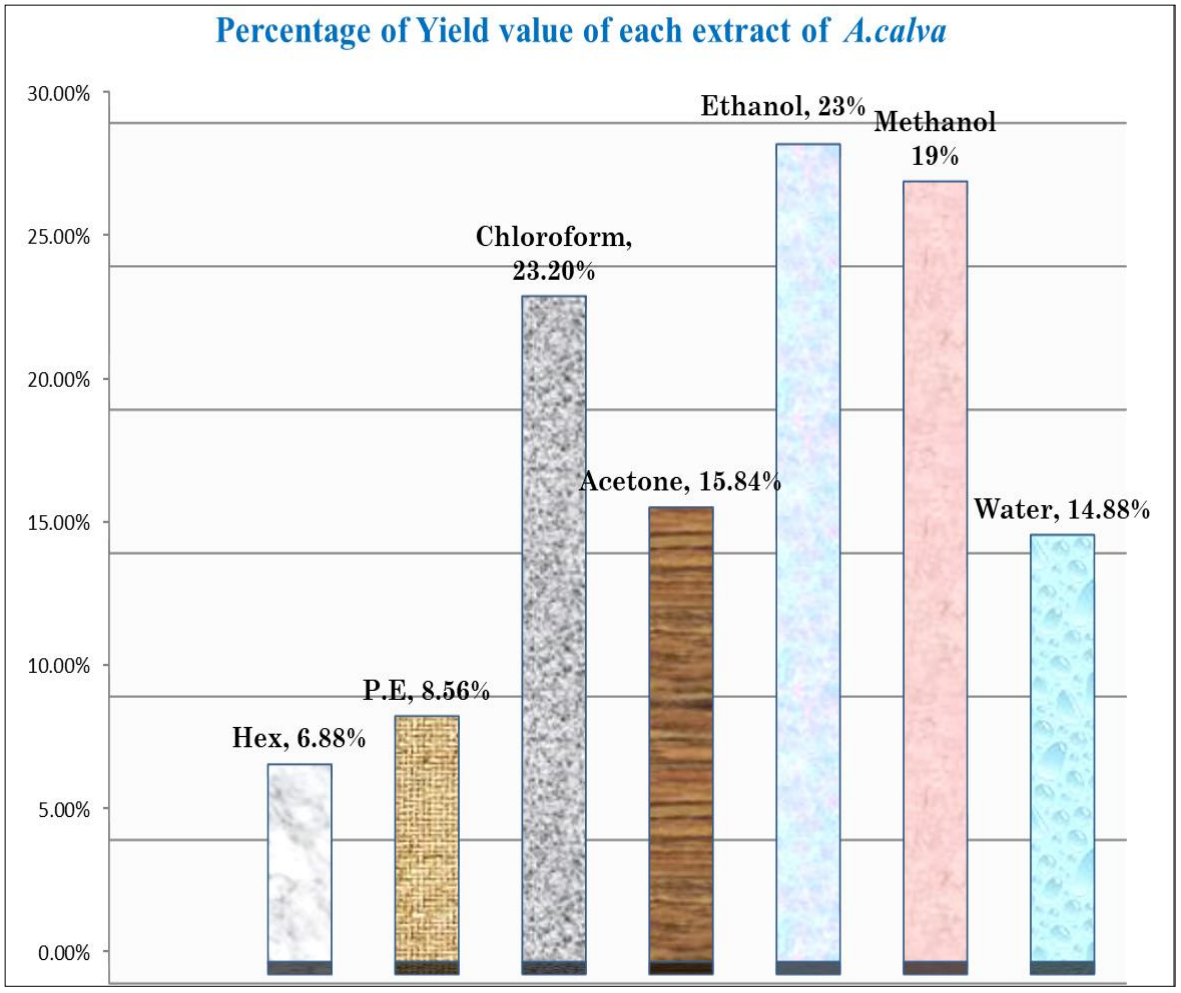


Fig 2: Percentage yield of the different solvent of *Acmella calva* (DC.) R. K. Jansen

Fluorescence analysis

A. calva was made into courses powder and treated with following chemical reagents (1N Methanolic sodium, methanol, 50% sulphuric acid, nitric acid and 5% potassium hydroxide) and observed under shorter (254nm) and longer wavelenght (265nm). The results revealed the presence of flavoinds, phenols and steroids in higher amount by appearance of yellow, brown and green colour at both wavelengths (Table - 1).

Table 1: Flurosecence analysis of the whole plant of *Acmella calva* (DC.) R. K. Jansen

Treatment	Ordinary light	Shorter Wavelength (254nm)	Longer Wavelength (265nm)
5% NaoH	Yellowish red	Brown	Yellow
50% H_2SO_4	Dark brown	Red	Green
50% HNO_3	Orange	Light green	Light green
5% $FeCl_3$	Yellowish brown	Orange	Yellow
Water	Light green	Green	Green
Aniline	Brown	Light brown	Dark red
Conc. KOH	Reddish yellow	Yellow	Yellow
66% H_2SO_4	Green	Green	Yellow
Powder	Light green	Yellowish red	Yellowish red
Chloroform	Brown	Red	Green

Table 2: Qualitative phytochemical analysis of *Acmella calva* (DC.) Jansen

S.No	Tests	Water	Methanol	Ethanol	Acetone	Chloroform	Petroleum ether	Hexane
1.	Carbohydrates	+	+	+	-	+	+	+
2.	Reducing sugar	-	+	+	+	+	-	+
3.	Alkaloids	-	-	+	-	+	+	-
4.	Flavonoid	-	+	+	+	+	-	+
5.	Glycosides	+	+	-	-	-	-	-
6.	Phenols	+	+	+	+	-	+	+
7.	Tannins	+	+	+	-	+	-	-
8.	Steroids	-	+	+	-	-	-	+
9.	Saponins	-	+	+	+	-	-	+

Preliminary investigation of selected medicinal plants

A. Percentage yield

The percentage yields of the selected plant were calculated in different solvents and are tabulated. The plant gave high yield in solvent ethanol at 23% (Figure – 2) for the Whole Plant of *A. calva.* The plant showed a very less yield in petroleum ether solvent which indicates there is very less amount of fats, fixed oils or any non-polar compounds.

B. Preliminary phytochemical analysis of *A.calva*

Qualitative analysis on different phytochemical constituents was carried out using the whole plant powder of *A.calva.* The dried plant powder was extracted with different solvents such as methanol, ethanol, acetone, petroleum ether and hexane. The ethanol extract of *A.calva* showed the positive response for the maximum of 8 phytocompounds except glycosides. The methanol extract proved positive response for seven phytocompounds followed by petroleum ether and hexane which showed positive response for six phytocompounds. The acetone extract showed positive response for phenols, reducing sugar, saponins and flavonoid. of these five solvent tested the maximum number (8/9) of response was detected in ethanol, in which carbohydrates, reducing sugar, alkaloid, flavonoid, glycosides, phenol, tannins, steroids and saponins. Carbohydrates are present in methanol, ethanol, petroleum ether and hexane (Table - 2)

C. Quantitative analysis of phytochemicals of *A.calva*

The quantitative estimation was carried out for the secondary metabolites such as phenol, flavonoid, alkaloids, tannins and steroids using ethanolic extract. The results were presented in figure – 3.

The ethanol extract of *A. calva* showed a maximum of (45.32 mg/g) of phenol. Estimation of tannin showed a maximum of 40.54mg/g. The quantitative study on the alkaloid estimation showed a maximum of 11.07mg/g. The ethanol extract of *A.calva* showed a maximum of 87.45mg/g flavonoid. The steroid estimation showed maximum of 63.56mg/g in *A. calva*.

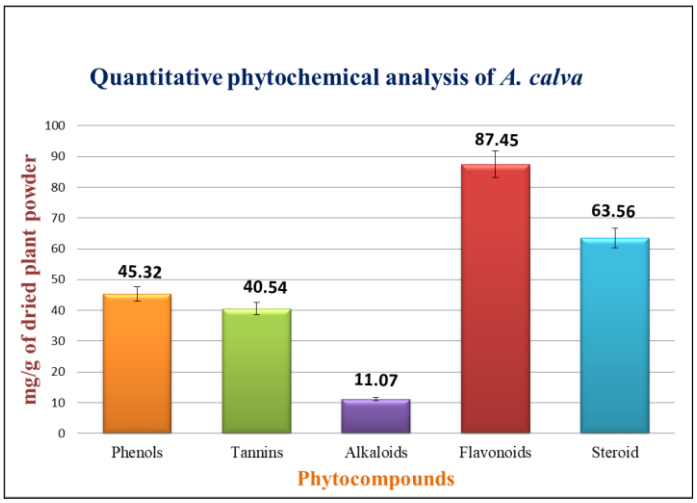
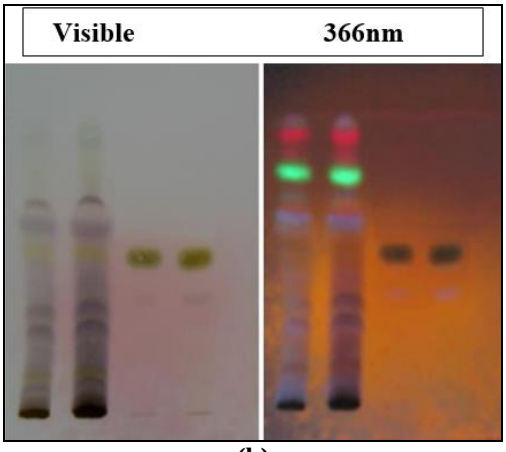


Fig 3: Quantitative phytochemical analysis of A. calva

Screening of Flavonoid by HPTLC Analysis

HPTLC fingerprinting was performed using quercetin as standard with mobile phase (Toluene: Ethyl acetate: Methanol (7:2:1)) which showed the presence of flavonoid in the highest peak under 366nm with the Rf value 0.54 (Fig - 4).



(b)

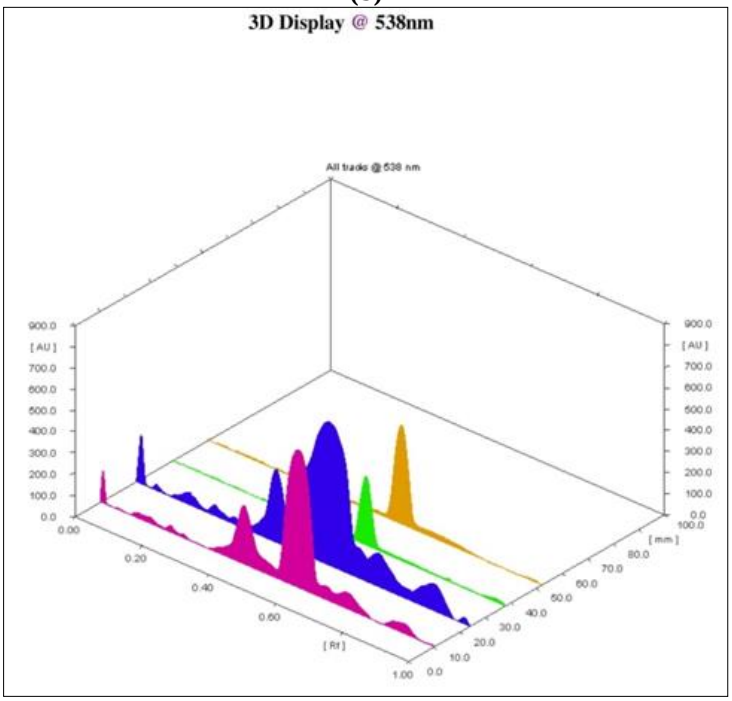


Fig 4: HPTLC finger print analysis of whole plant of *A. calva* (a) Screening of flavonoid using standard (b) Peak display of flavonoid.

Discusion

The total ash and fluorescence analysis is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts or silica. The drug was found to contain an average of total ash 7.90%.Similarly ash value of different plant parts were studied from pharmacognostical point of view in different plants such as *Cardiospermum halicacabum* Linn. [18], *Nigella sativa* Linn. [19], *Berginia ligulata* and *Ammania buccifera* Linn. [20], *Persea macrantha* (Nees) Kosterm [21], *Leucas cephalotes* Spreng [22]. Phytochemical screening of the plant is preliminary and important aspect of the process of establishing herbal medicine quality. Preliminary phytochemical revealed the presence of alkaloids, terpenes, flavonoid, tannins and saponins in whole palnt of *A.calva*. The presence of flavonoid compounds in the plants indicates that this plant may possess anti-microbial properties. This agrees with the findings of Seow *et al.* [18]. Aguinaldo *et al* [19] reported that tannins may possess potential value such as cytotoxic and antineoplastic agents. Flavonoid are potent water-soluble antioxidants and free radical scavengers, which can prevent oxidative cell damage and exhibit anti-cancer effect [20]. Saponins are used in hyperglycemia, antioxidant, anticancer and anti-inflammatory, etc [21].

Recent studies by Seow *et al*. [22] show that the leaf extracts of *G. segetum* reduced the growth of blood vessels and exhibited potent antiangiogenic activity. The inhibition of angiogenesis may lead to control of tumor growth. Similar findings were reported by Patil and Wanjare (2017) [23] in acetone and ethanol stem extract of *F. hispida.* They inferred the presence of saponin may possess a good antimicrobial agent and it has the property to lower cancer risks and lower blood glucose response.

Conclusion

The present investigation evaluated the phytochemistry, standards to the plant powders and the extractive values in different solvents. The plant showed highest extractive value in ethanol supporting a primary assumption that they contain similar constituents and confirms the presence of glycosides, flavonoid, saponins, terpenoids and polyphenols. Since most of the active chemical constituents were extracted in ethanolic solvent it was selected for the further use. The fluorescence studies were further supported by the phytochemical screening using chemical tests. The presence of those phytochemicals may be liable to display various biological activities in the ethno pharmacological uses of the plant for the treatment of assorted diseases. Further, isolation and biological evaluation of these compounds are assured for the drug discovery as well as to authorize the traditional use of this plant. From this result, it is concluded that, the ethanolic extract of *A. calva* having the effective potential compounds, which may leads to the formation of effective drug for cariogenic disease.

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