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PHYSICOCHEMICAL EVALUATION, QUANTIFICATION OF PHYTOCONSTITUENTS AND TLC PROFILE OF CAREYA ARBOREA LEAVES

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ABSTRACT:

In the present study, physicochemical evaluation and thin layer chromatography of leaf extract of C. arborea (Lecythidaceae) were performed. Water extract, methanolic extract and ethyl acetate extracts of C. arborea leaves were used for quantification of phytoconstituents. Careya arborea leaves were tested for total phenolic, flavonoid, total alkaloid, and total saponin content. The Folin-Ciocalteau reagent and the colorimetric method were used to determine the total phenolic and flavonoid content, respectively. Using diosgenin as a reference, the total saponin content was calculated. It has been found that powdered C. arborea leaves contain anisocytic stomata and crystals of calcium oxalate. The ethyl acetate extract of C. arborea leaf showed highest concentration of phenolic, flavonoid content than the methanolic and water extract. Methanolic extract contain more amount of saponin than methanolic extract of leaves of C. arborea. Leaves were reported to contains 0.2 % total alkaloid. This experimental finding can be useful for quality control of C. arborea leaf.

Keywords: Careya arborea, powder study, total alkaloid, total phenolic, total saponin

1. INTRODUCTION

The standardization of herbal drug is a challenging task. As plants contain various phytoconstituents and the accumulation of its in various parts of the plants are depends on various factor like climatic conditions, rainfall, whether the plant is obtained from wild or cultivated, time of collection cultivation nature of soil etc. Thin layer chromatography (TLC) is simple, less expensive, and easy to execute, and its components have low electrical power requirement.

Careya arborea Roxb (Lecythidaceae) is a large deciduous that tree grows up to 20 m, commonly known as "wild Guava and Kumbhi." It is found in Tropical dry deciduous forests, also in the plains. The flowering and fruiting is in February-August. Common names ; Ceylon Oak Tree, Wild Guava, Kumbhi Tree, Patana oak, Slow match tree, Wild guava. Fruits are large, globose, fleshy, indehiscent, crowned with the calyx limb. Seed numerous, embedded in the fleshy pulp. Leaves are alternating crowned at top.

Leaves are applied as a poultice to promote healing of ulcers. Juice of fresh flowers mixed with honey is used to treat colds and coughs. It is used for cough, skin disease, leprosy, urinary disorder, ulcer, diarrhea, and sinusitis and worm infestation. Leaves reported ash content 6.94%, fibers 22.12%, tannins 19.00%, and fats 5.85%. Leaves were reported triterpenoid careyagenolide. *C. arbore*a leaves reported gastroprotective activity, Wound healing activity[1] and antileishmanial activity.

2. MATERIALS AND METHODS

Material and methods

Plant material

Plant materials of *C. arborea* were collected from Vadodara in the of June 2012. The plant was identified and authenticated by Dr. P. S. Nagar at the Botany Department of The M. S. University, Vadodara. The Voucher specimen (DC-CA-2) was stored in the herbarium of our laboratory. Leaves were dried under shade.

Reagent and Chemicals

All the chemicals and reagents used were of analytical grade. Folin Ciocalteu reagent, Quercetin, Aluminum chloride, Gallic acid, Potassium acetate, Sodium carbonate Ascorbic acid were procured from E. Merck (Darmstadt, Germany), Hi-Media lab. Ltd (Mumbai) and Sigma **(**Chemical Co, St. Louis, MO, USA). All UV–Vis measurements were recorded on a Shimadzu UV–1800.

Powder microscopy

Dried powder of leaf *C. arborea* was decolorized with alcoholic KOH and stained with phloroglucinol: HCl (1:1). A drop of glycerine was placed on a slide covered with a cover slip and observed under a microscope. The powders were also mounted in acetic acid and iodine for observation of calcium oxalate and starch respectively. Diagnostic features were identified and noted.

Phytochemical screening

Air -dried powdered leaves of *C. arborea* weighing 50g were successively extracted in soxhlet apparatus with increasing polarity; petroleum ether, toluene, chloroform, ethyl acetate and methanol. Each time the material was dried, before extracting with the next solvent. Finally, Marc was macerated with chloroform water for 24hours to obtain the aqueous extract. All the extracts were concentrated by distilling the solvent and the extracts were dried in water bath. Consistency, colour, appearance of the extracts and percentage yield were noted.

Extracts were tested for the presence of various types of phytoconstituents i.e. alkaloid, phenolic, flavonoid, saponin and sterols, by employing chemical tests.

Qualitative chemical test

The leaf extracts obtained by successive solvent extraction from *C. arborea* were subjected to qualitative chemical tests for phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins, and phytosterols.

Physicochemical Evaluation

Physicochemical study of leaves *C. arborea* was carried out. Various physicochemical constants like ash value, Extractive values, loss on drying and foam index were determined as per WHO guideline.

Thin layer chromatographic profile

The TLC profile of extracts were developed using $CHCl_3$: ethyl acetate: Formic acid (2:8:0.4) as the mobile phase. The plate was sprayed with alcoholic $FeCl_3$. TLC fingerprinting of *C. arborea* leaf extracts were carried out with a solvent system; Chloroform: Toluene: ethyl acetate (6:3:1) and Chloroform: methanol: water (7:3:0.4) using anisaldehyde sulpuric acid as detection reagent reported in figure 2.

Quantification of phytoconstituents

Quantification of total phenolic, flavonoid, total saponin and total alkaloid were determined in leaves of *C. arborea*

Total phenolic content

Total Phenolic content was determined in methanol extract, ethyl acetate extract, and water extract of leaves of *C. arborea*. A solution of 5mg/ml of extract was prepared in methanol. A stock solution of the Gallic acid (100µg/ml) was prepared in methanol. Folinciocaltu reagent was diluted with distilled water in 1:2 dilutions. A solution of 20g/100 ml of sodium carbonate was prepared in distilled water.

From the stock solution of standard gallic acid 0.5, 0.75, 1, 1.25, 1.5, 1.75, and 2ml were taken which denotes 50, 75, 100, 125, 150, 175, 200 µg/ml gallic acid respectively taken in 25ml volumetric flask. To this, 10ml water and 1.5ml Folin-Ciocalteau reagent were added. The above mixture was kept for 5min. And 4ml 20% sodium carbonate solution was added and made the volume up to 25ml with the distilled water. This mixture was kept for 30minutes. and blue colour developed. The absorbance was measured at 765 nm. The calibration curve of gallic acid was prepared using concentration of gallic acid versus absorbance. Similarly 1ml test solution of the extracts was processed as above and the percentage of total phenolics was calculated using calibration curve of Gallic acid and total phenolics were expressed as % Gallic acid.

Flavonoid content

Flavonoids with various biological activities are considered as one of the key components in the plants. Flavonoids content was determined in methanol extract, ethyl acetate extract, water extract leaves of *C. arborea* using aluminum chloride method.

A stock solution of Quercetin 1mg /ml was prepared in methanol. A solution of 10%w/v aluminum chloride was prepared in distilled water. 1M potassium acetate solution was prepared in distilled water. One ml stock solution of Quercetin was diluted to 100ml by methanol to produce (10µg/ml). From this solution 0.1, 0.2, 0.3, 0.5, 0.8, 1, 1.5ml were taken and diluted up to 10ml methanol to produce 1, 2, 3, 5, 8, 10, 15 µg/ml concentrations respectively. The solutions were separately mixed with 1.5ml 95% methanol, 0.1ml 10% aluminum chloride, and 0.1ml 1M potassium acetate and 2.8mldistilled water. After incubation at room temperature for 30min.The absorbance of reaction mixture was measured at 415 nm. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. A calibration curve was prepared using concentration of quercetin versus absorbance.

Sample Preparation: A solution of sample 5mg /ml extract was prepared in methanol. Similarly, the sample solutions were reacted with Aluminum chloride, 1M potassium acetate and 2.8ml distilled water, after keeping at room temperature for 30minutes. the absorbance mixture was measured at 415 nm. The percentage of total flavonoids was calculated using calibration curve. Flavonoids were expressed as % quercetin.

Total alkaloids determination

Ten gram powdered drug was treated with 100ml 10% acetic acid in methanol and kept for 4hours. Mixture was filtered and concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise until the complete precipitation occurs. The solution was centrifuged, the precipitates were collected and washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed

Total saponins determination

Saponin content was determined in aqueous and methanol extract of leaf *C. arborea.*

10g powdered drug was extracted with 80% methanol. 10mg diosgenin was dissolved in 8ml methanol and 2ml distilled water. Vanillin reagent was prepared by dissolving 8g vanillin in 100ml methanol. 72%v/v H_2SO_4 were prepared by addition of 72ml H_2SO_4 (analytical grade, 95%, w/w) in 28ml distilled water. From the stock solution of diosgenin (1mg/ml), this solution 0, 80,100, 180, 250 µg/ml were taken. 0.5ml the vanillin reagent and 5ml of 72% (v/v) sulphuric acid was added slowly on the inner side of the wall. Mix the solution well and transfer the tubes to a water bath adjusted at 60°C for 10min, cool the tubes in ice-cold water for 3 to 4min, and measure absorbance at 544nm against the reagent blank (0µl of the diosgenin standard solution). Calibration curve was plotted using concentration of diosgenin versus absorbance. A known amount of extract was dissolved in methanol. Similarly 0.4 ml test solution of the extracts was processed and the percentage of total saponins was calculated using calibration curve of diosgenin and total saponin was expressed as % diosgenin.

3. RESULT AND DISCUSSION

Leaves powder microscopy were performed and leaves powder subjected to powder microscopy, Phytochemical screening, TLC profile and quantification of total phenolic, flavonoid, total saponin and total alkaloid were determined in leaves of *C. arborea*

Leaf powder characteristic:

It is a greenish color, astringent taste and characteristic odour. Leaf powder microscopy shows the presence of anisocytic stomata, calcium oxalate cluster, epidermal cells, spiral xylem vessels and lamina fragments are reported in Figure 1.

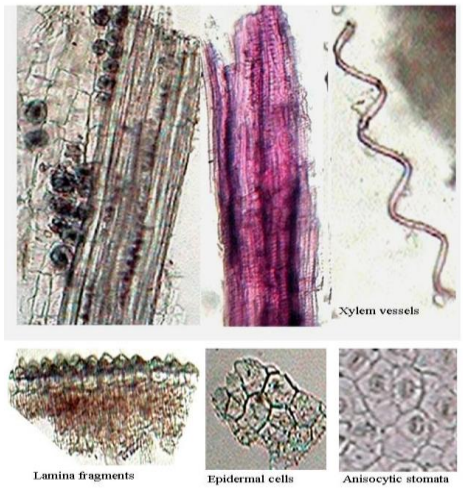

Figure 1 Powder characteristic of *Careya arborea* leaf

Phytochemical screening

Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive chemicals by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity. Preliminary screening is helpful in to identifying the amount chemical constitutes of plant extracted in a particular solvent. Their % yield, color and consistency are recorded in Table 1.

Table 1.The percentage yield, color and consistency of leaf extracts of *Careya arborea*

Extracts	Color and consistency	%Yield (w/w)
petroleum ether	Blackish green, semisolid and sticky	3.1
Benzene	Dark greenish black, sticky solid	3.46
Chloroform	Green, solid, non-sticky	2.30
acetone	Light brown, solid, glassy, non-sticky	5.0
Methanol	Reddish brown, shiny solid, non-sticky	8.84
Water	Dark brownish red, solid, non-sticky	7.64

Qualitative chemical test

Plants considered biosynthetic factory contains primary metabolites and secondary metabolites alkaloids, glycosides, tannins, phenolics and triterpenoids. A successive solvent extracts of leaves were studied for their phytochemical profile reported in Table 2.

Table 2. Qualitative chemical test on extracts of leaves of Careya arborea

Chemical constituent	Petroleum ether extract	Benzene extract	$CHCl_3$ extract	Acetone extract	Methanol extract	Water extract
Carbohydrates	-	-	-	-	+	+
Proteins	-	-	-	-	-	+
Saponins	-	-	-	-	+	+
Alkaloids	-	-	+	+	+	-
flavonoids	-	-	-	+	+	+
Tannin & phenolics	–	–	–	+	+	+
Steroids & triterpens	+	+	+	+	-	-

A leaf showed the presence of carbohydrates, Saponins, steroids, flavonoids, phenolics compound.

Physicochemical evaluation of leaf C. arborea

Physicochemical parameters are useful for the standardization of plant materials. E.g. Extractive value determines the amount of soluble matter in a particular solvent from a given amount of plant material. This also gives preliminary idea about nature of the phytoconstitute present in plant materials. E.g. water soluble phytoconstitute are extracted in aqueous solvent. The results obtained from various determinations are compiled in Table 3.

Table 3. Physicochemical evaluation of leaf of C. *arborea*

Parameter	Leaf
Water soluble extractive value	17.1
Alcohol soluble extractive value	13.0
Loss on drying	0.6
Foaming index	More than 100
Foreign matter	0.3

The values given here are expressed as percentages of air- dried material. Each value is average of three determinations.

Thin layer chromatographic profile

The different extracts of fruits and leaves were subjected to Thin Layer Chromatography to detect and confirm the presence of phytoconstituents. TLC spots are reported in Figure 2.

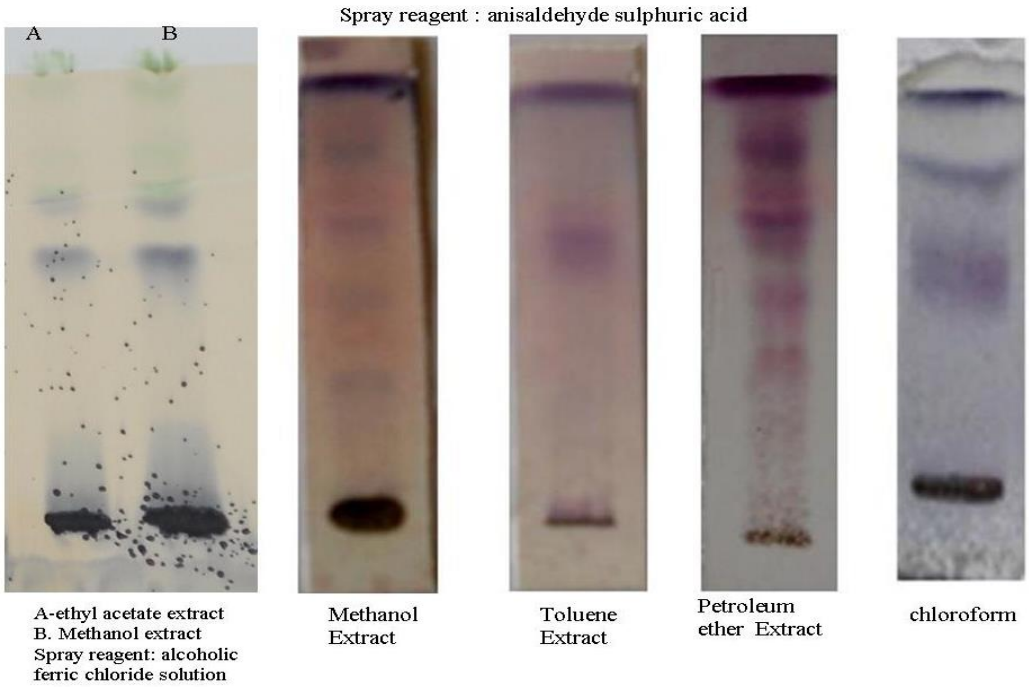

Figure: 1. TLC profile of phytoconstituents of various leaf extracts of *C. arborea*

Determination of total phenolics content

The total phenolic content was expressed in terms of % gallic acid. Total phenolic content in water extract, methanolic extract and ethyl acetate extracts of *Careya arborea* leaves were found 3.892 ±0.2663, 4.298±0.3079 and 4.52±0.5508 respectively. Values represent mean ± standard deviation (n=3). The result shows that ethyl acetate extracts of leaves contains a higher amount of phenolic than methanol and water extract of leaf of *C. arborea*.

Determination of Flavonoid content

Aluminum chloride forms stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with ortho-dihydroxyl groups in the A or B-ring of flavonoids..

Total flavonoid content was calculated as % quercetin**.** Total Flavonoid content in water extract, methanolic extract and ethyl acetate extracts of *Careya arborea* leaves were found 0.196± 0.011, 0.192±0.011and 0.229±0.021respectively . Values represent mean ± standard deviation (n=3). Ethyl acetate extract of leaf contain higher amount of flavonoids than methanol and water extract of leaf of *C. arborea*

Total alkaloid determination

Alkaloids are basic compounds and exist in plants in salt form. Alkaloid is soluble in acidic methanol. In addition to concentrated ammonium hydroxide, alkaloids get precipitated. The precipitate was collected to obtain % yields. *Careya arborea* leaf contains 0.2 % total alkaloid.

Total saponin content

Total saponin was calculated as % Diosgenin. Saponin content in water and methanolic extract *C. arborea* leaves were found 17.79±0.30 and 19.35 ± 0.70 respectively.

4. CONCLUSION

Natural medicines are composed of various phytoconstituents and quality control parameters are crucial for the identification of herbal drugs. TLC profile is a very important parameter of herbal drug standardization for the proper identification of medicinal plants. The present finding reported phytochemical screening and various physicochemical parameters determined as per WHO guidelines. Findings can be useful for identification and to determine the quality and purity of *C.* arborea leaf

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