

Qualitative and Quantitative Analysis of *Clerodendron colebrookianum* Walp. Leaves and *Zingiber cassumunar* Roxb. Rhizomes

S. Majaw* and J. Moirangthem

Department of Biotechnology and Bioinformatics,
North Eastern Hill University, Meghalaya, India

*Corresponding author: Email: smajaw.nehu@gmail.com

Issued May 01, 2009

Abstract

This article describes simple methods for performing qualitative and quantitative analysis of *Clerodendron colebrookianum* leaves and *Zingiber cassumunar* rhizomes. The distribution of the main active principles (phlobatannins, flavonoids, alkaloids, saponins, tannins, terpenoids, steroids, glycosides and anthraquinones) in *C. colebrookianum* leaves and *Z. cassumunar* rhizomes were assessed. Both the plant materials were found to contain all the above components except for the absence of anthraquinones in both *Z. cassumunar* rhizomes and *C. colebrookianum* leaves, and absence of phlobatannin and glycosides in *C. colebrookianum* leaves. The importances of the distribution of these chemical constituents were discussed. This study provides scientific method to investigate the active components of the above mentioned plants.

Key words: Active principles, *Clerodendron colebrookianum*, *Zingiber cassumunar*

Introduction

The detection of active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude plant extracts and is very important in regards to their potential pharmacological effects [1]. The work described here does not consist of a new method of identifying active principles in a given plant extract, since present day techniques are well developed, widely accepted and allowed for adequate drug analysis. What is intended, however, is to perform simple qualitative and quantitative analysis for two plants, *i.e.*, the rhizomes of *Zingiber cassumunar* Roxb. (Zingiberaceae) and the leaves of *Clerodendron colebrookianum* Walp. (Verbenaceae) followed by terpenoids

extraction with the identification of monoterpenes using thin layer chromatography (TLC). Such kind of study for the above plants has not been performed previously. Rhizomes of *Z. cassumunar* possess anti-inflammatory effect with antimicrobial activity and *C. colebrookianum* is a potent hypotensive plant [2-4]. We report common process for both the plant products, irrespective of the kind of active principles they might contain, in contrast to the work of others who started with more selective extractions for each kind of active principle. This allows for a reduction in time and money necessary for the analysis of plant extract of unknown composition.

Materials and methods

Collection and identification of plants

C. colebrookianum leaves and *Z. cassumunar* rhizomes were collected from Meghalaya, India. The specimens were submitted and identified by herbarium curator, Department of Botany, North Eastern Hill University, Meghalaya, India. The leaves and rhizomes were thoroughly washed with water and dried in oven (40 °C) [5]. It was then ground into coarse powder form and was used for further investigation.

Qualitative Analysis

Test for Phlobatannins

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl) to observe the deposition of red precipitate [6].

Test for Flavonoids

A portion of crude powder was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow coloration [6].

Test for Alkaloids

0.5 g of crude powder was defatted with 5% ethyl ether for 15 min. The defatted sample was extracted for 20 min with 5 ml of aqueous HCl on a boiling water bath. The resulting mixture was centrifuged for 10 min at 3000 rpm. 1 ml of the filtrate was treated with few drops of Mayer's reagent and a second 1 ml with Dragendroff's reagent and turbidity was observed [7, 8].

Test for Saponins

0.5 g of crude powder was shaken with water in a test tube and it was warmed in a water bath and the persistent of froth indicates the presence of saponins [9, 10].

Test for Tannins

0.5 g of the crude powder was stirred with 10 ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate was taken as evidence for the presence of

tannin [7].

Test for Terpenoids

0.5 g of crude powder was dissolved in 5 ml of methanol. 2 ml of the extract was treated with 1 ml of 2, 4-dinitrophenyl hydrazine dissolved in 100 ml of 2M HCl. A yellow-orange coloration was observed as an indication of terpenoids [11].

Test for Steroids

0.5 g of crude powder was dissolved in 5 ml of methanol. 1 ml of the extract was treated with 0.5 ml of acetic acid anhydride and cooled in ice. This was mixed with 0.5 ml of chloroform and 1 ml of concentrated sulphuric acid was then added carefully by means of a pipette [11]. At the separations level of the two liquids, a reddish-brown ring was formed, as indication of the presence of steroids.

Test for Glycosides

0.5 g of crude powder was dissolved in 5 ml of methanol. 10 ml of 50% HCl was added to 2 ml of methanolic extract in a test tube. The mixture was heated in a boiling water bath for 30 min. 5 ml of Fehling's solution was added and the mixture was boiled for 5 min to observe a brick red precipitate as an indication for the presence of glycosides [7].

Test for Anthraquinones

0.5 g of crude powder was shaken with 10 ml of benzene and was filtered 0.5 ml of 10 % ammonia solution was added to the filtrate and the mixture was shaken well and the presence of the violet color in the layer phase indicated the presence of the anthraquinones [8].

Quantitative Analysis

Flavonoid determination

10 g of each plant crude powder was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 m). The filtrate was later transferred into a crucible and evaporated into dryness and weighed to a constant weight [12].

Saponin determination

20 g of crude taken from each plant were put into a conical flask and 100 cm³ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55 °C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated.

60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5%

aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to constant weight and the saponin content was calculated [13].

Total phenol determination

Total phenol content was determined according to McDonald's method using Folin-Ciocalteu reagent (Gallic acid as a standard) [14].

Carbohydrate and Protein Estimation

Carbohydrate content of *C. colebrookianum* leaves and *Z. cassumunar* rhizomes were estimated using anthrone reagent [15]. Total protein content was estimated according to Lowry's method [16].

Terpenoid extraction for Thin Layer Chromatographic (TLC) Analysis

50 g of the powdered leaves and rhizomes were extracted with solvent combination of methanol and water (4:1) at room temperature for 24 h. The solution was filtered using Whatman filter paper No. 1 and the filtrate was then evaporated to 1/10 volume at 40 °C. The evaporated filtrate was acidified with 2M sulphuric acid (pH 0.89) followed by chloroform extraction (three times the volume), stirred and allowed to stand in a separatory funnel. Out of the two layers formed, the non-aqueous layer was taken and evaporated till dryness. The dried extract contained components like terpenoids which were further used for thin layer chromatography analysis [17].

Thin Layer Chromatography

Glass plates (20×20 cm) were coated (0.5 mm) with silica gel (Qualigen fine chemicals) and samples applied correspond to approximately 5 mg/ml of each extract dissolved in methanol. Chromatography was performed in 100% chloroform according to the method described by Harborne [17]. The spots were visualized using concentrated sulphuric acid as a spray reagent followed by heating of plates at 100 °C for 10 min. The spots were identified based on the color, produced on reacting with a spray reagent.

Results

The present study carried out on the plant samples revealed the presence of medicinally active constituents. Alkaloids, flavonoids, saponins, tannins, terpenoids and steroids are present in both the plant materials. Glycosides and phlobatannins were found to be absent in *C. colebrookianum* leaves while anthraquinones was absent in both *C. colebrookianum* leaves and *Z. cassumunar* rhizomes (Table 1).

Table 1. Qualitative analysis of the phytochemicals of *C. colebrookianum* and *Z. cassumunar*.

Serial Number	Plant	<i>C. colebrookianum</i> Leaves	<i>Z. cassumunar</i> Rhizomes
1	Phlobatannins	—	+
2	Flavonoid	+	+
3	Alkaloid	+	+
4	Saponin	+	+
5	Tannin	+	+
6	Terpenoid	+	+
7	Steroid	+	+
8	Gylcoside	—	+
9	Anthraquinone	—	—

+ indicate the presence of constituents and — indicate the absence of constituents.

Table 2. Qualitative analysis of investigated plants.

Serial Number	Components	<i>C. colebrookianum</i> Leaves	<i>Z. cassumunar</i> Rhizomes
1	Flavonoid content mg/g Dry weight material	32.83 ± 0.49	54 ± 0.942
2	Saponin content mg/g Dry weight material	88 ± 0.942	69.03 ± 0.633
3	Phenol content g/L	2.52 ± 0.041	0.42 ± 0.009
4	Carbohydrate content µg/ ml	131.33 ± 1.962	387.33 ± 6.688
5	Protein content µg/ml	297.66 ± 13.468	851 ± 10.198

Quantitative estimation of the chemical constituents in the studied medicinal plants is summarized in Table 2. *Z. cassumunar* rhizomes contained higher content of flavonoids, carbohydrates, proteins with lesser amount of saponins and phenols than the *C. colebrookianum* leaves. Thin layer chromatography technique was used to separate different monoterpenes from the extracted terpenoids. With chloroform (100%), it revealed, three spots in *C. colebrookianum* leaves and in case of *Z. cassumunar* rhizomes, it

showed two spots.

Discussions

In this study, the results of the investigations showed that the two plant materials possessed almost all the important secondary metabolites. *Z. cassumunar* rhizomes showed positive results for all the constituents analyzed, except for one i.e., anthraquinones while *C. colebrookianum* leaves showed positive results in the constituents analyzed except for three, glycosides, phlobatannins and anthraquinones [Table 2]. Tannins, phenolics, saponins, alkaloids and flavonoids have been linked or suggested to be involved with antibacterial and anti-viral activity while tannins and flavonoids are thought to be responsible for antidiarrheal activity [18]. Investigations of the mode of action indicate that tannins and flavonoids increase colonic water and electrolyte reabsorption and other phytochemicals act by inhibiting intestinal mobility, while some components have been shown to inhibit particular enteropathogens [18]. Steroids in modern clinical studies have supported their role as anti-inflammatory and analgesic agents [19]. This could explain the role of *Z. cassumunar* rhizome as an anti-microbial agent [3]. This also suggests that *C. colebrookianum* leaves may possess anti-microbial activity. The presence of phlobatannin suggests the diuretic property of the plant [20]. This suggests the possible utilization of *Z. cassumunar* rhizomes as a diuretic agent.

Carbohydrates are the main components of the cell wall, protoplasm and cell-sap while others accumulate as insoluble storage products. The carbohydrates produced cannot be stored in the leaf tissue since further input of carbon compounds into leaf cells would lower the nitrogen to carbon ratio below their critical nitrogen content, thus interfering with cell function. Instead of having been incorporated into the leaves, these carbohydrates were translocated to the rhizomes [21]. The rhizome also serves as a storage organ for food reserves and nutrients, such as nitrogen [22]. Nitrogen is one of the "major" nutrients needed to support good plant growth. Plants use nitrogen to form amino acids needed in the formation of protein [23]. This could be the reason for higher content of carbohydrates and proteins in *Z. cassumunar* rhizomes than in *C. colebrookianum* leaves.

In the method of separation by TLC of monoterpenes from *Z. cassumunar* using chloroform (100%) revealed that spot 1 was yellow and spot two was brownish in color suggesting that spot 1 could be Limonene or α -Pinene and spot 2 could be Pulegone [17]. For *C. colebrookianum*, three spots: spot 1–yellowish, spot 2–pinkish and spot 3–greenish in color were obtained. Therefore, spot 1 could be Pulegone, spot 2 could be Carvone and spot 3 could be 1, 8- Cineole [17]. Monoterpenes are typically found and widely distributed in almost all the plants. Monoterpenes in conifer resins such as α - Pinene, β - Pinene, limonene are potential defense chemicals against bark beetles [23]. Pulegone is the major monoterpene constituent of pennyroyal oil. This oil, obtained from the leaves and flowering tops of *Mentha pulegium* and *Hedeoma pulegoides*, has been widely used as a fragrance component, a

flavoring agent and an herbal medicine to terminate pregnancy [24]. Because limonene affects the pathway that produces cholesterol, they can inhibit cholesterol synthesis, thereby eliminating a minor contributor to cancer formation. Limonene also increase the levels of liver enzymes involved in detoxifying carcinogens, an effect that decreases the possibility that carcinogen will cause cellular damage [25]. The research reported 1, 8-cineole as a potential fumigant of wheat [26]. Carvone is widely used to flavor various food products, pharmaceuticals, toothpaste and it also exhibits antimicrobial/antifungal properties [27].

In conclusion, the plants studied here can be seen as a potential source of useful medicinal values. The procedures are simple and the resources required for the experiment are inexpensive. The experimental protocol, although straightforward, includes procedures that must be performed carefully to obtain good results. Further studies are going on to analyze and extract other components of plant including alkaloids, flavonoids.

References

1. Pascual ME, Carretero ME, Slowing KV, Villar A (2002). Simplified screening by TLC of plant drugs. *Pharmaceutical Biology*, 40(2): 139-143.
2. Ozaki Y, Kawahara N, Harada M (1991). Anti-inflammatory effect of *Zingiber cassumunar* Roxb. and its active principles. *Chemical & Pharmaceutical Bulletin*, 39(9): 2353-2356.
3. Pithayanukul P, Tubprasert J, Wuthi-udomlert M (2007). In Vitro antimicrobial activity of *Zingiber cassumunar* (Plai) oil and a 5% plai oil gel. *Phytotherapy Research*, 21: 164-169.
4. Kotoky J, Dasgupta B, Deka N (2005). Pharmacological studies of *Clerodendron colebrookianum* Walp, a potent hypotensive plant. *Indian Journal of Physiology and Pharmacology*, 49: 289-96.
5. Harborne JB (1984). *Phytochemical Methods*. 2nd ed. Chapman and Hall, New York, 1: 4-7.
6. Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian Medicinal plants. *African Journal of Biotechnology*, 4: 685-688.
7. Harborne JB (1973). *Phytochemical Methods: A guide to modern techniques of plant analysis*. Chapman and Hall, New York, pp. 279.
8. Trease GE, Evans WC (1996). *A textbook of Pharmacognosy*. 14th ed. Bailliere Tindall Ltd, London, pp. 832.
9. Smolenski SJ, Silinis H, Farnsworth NR (1974). Alkaloids screening. V. *Lloydia*, 37: 506-536.
10. Kapoor LD, Singh A, Kapoor SL and Shrivastava SN (1969). Survey of Indian medicinal plants for saponins, alkaloids and flavonoids. *Lloydia*, 32: 297-302.
11. Kolawole OM, Oguntoye SO, Agbede O and Olayemi AB (2006). Studies on the efficacy of *Bridelia ferruginea* Benth. bark extract in reducing the coliform load and BOD of domestic

- waste water. *Ethnobotanical Leaflets*, 10: 228-238.
12. Boham BA, Kocipal- Abyazan R (1974). Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinum*. *Pacific Science*, 48: 458-463.
 13. Obadoni BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure Applied Sciences*, 8: 203-208.
 14. McDonald S, Prenzler PD, Autolovich M, Robards K (2001). Phenolic content and anti-oxidant activity of olive extract. *Food Chemistry*, 73: 73-84.
 15. Yemm EW and Willis AJ (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal*, 57: 508-514.
 16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193: 265-275.
 17. Harborne JB. *Phytochemical Methods* (1984). 2nd ed. Chapman and Hall, New York, 3: 100-117.
 18. Enzo AP (2007). Traditional plants and herbal remedies used in the treatment of diarrheal disease: Mode of action, quality, efficacy and safety considerations. In: Ahmad I, Aqil F, Owais M, editors. *Modern Phytomedicine Turning Medicinal Plants in to Drugs*. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 248-260.
 19. Singh AP (2006). Short Review: Distribution of Steroid like Compounds in Plant Flora. *Pharmacognosy Magazine*, 2(6): 87-89.
 20. Awoyinka OA, Balogun IO, Ogunnowo AA (2007). Phytochemical screening and in vitro bioactivity of *Cnidocolus aconitifolius* (Euphorbiaceae). *Journal of Medicinal Plants Research*, 1(3): 063-065.
 21. Wijte A (2005). Combating *Arundo donax* and other rhizomatous aquatic and estuarine nuisance grasses by exploiting their ecophysiological characteristics. Research completion reports, California sea grant college program, paper coastal 03_06.
 22. Smagula JM, DeGomez T (1987). Lowbush Blueberry Nutrition Series: N-P-K. Fact Sheet No. 223, UMaine Extension No. 2093. The University of Maine, Orono, ME 04469.
 23. Paine TD, Stephen FM, Cates RG (1988). Moisture stress, tree suitability and southern pine beetle population dynamics. In: Payne TL, Saarenmaa H, editors. *Integrated control of Scolytid Bark Beetles*. Virginia Polytechnique Institute and State University, Blacksburg, pp. 85-103.
 24. Frohlichl O and Shibamoto T (1990). Stability of pulegone and thujone in ethanolic solution. *Journal of Agricultural and Food Chemistry*, 38: 2057-2060.
 25. Gould MN (1997). Cancer chemoprevention and therapy by monoterpenes. *Environmental*

Health Perspectives, 105: S977-9.

26. Lee BH, Annis PC, Tumaalii F (2003). The potential of 1, 8-cineole as a fumigant for stored wheat. In: Wright EJ, Webb MC and Highley E, editors. *Stored grain in Australia 2003*. Proceedings of the Australian post harvest technical conference, CSIRO Stored Grain Research Laboratory, Canberra, June 25–27.
27. Morrish JLE, Daugulis AJ (2008). Inhibitory effects of substrate and product on the Carvone biotransformation activity of *Rhodococcus erythropolis*. *Biotechnology Letters*, 30: 1245–1250.