Research Article

Total Alkaloid and Saponin Content of the Ethanolic Leaf Extracts of *Cassia alata*, *Chrysophyllum cainito*, *Cymbopogon citratus*, *Lantana camara*, and *Terminalia catappa*

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ABSTRACT

Introduction: Alkaloids and saponins are among the phytochemicals with promising medicinal value. Ethnopharmacological studies have shown that *Cassia alata*, *Chrysophyllum cainito*, *Cymbopogon citratus*, *Lantana camara*, and *Terminalia catappa* are traditional herbal plants worth investigating. **Objectives:** The need to generate knowledge about the phytochemistry of these plants, specifically the total alkaloid and saponin content, can be of vital significance for future-related researches and product development. **Materials and Methods:** The determination of total alkaloid and saponin content in the ethanolic leaf extracts of *Cassia alata, Chrysophyllum cainito, Cymbopogon citratus, Lantana camara,* and *Terminalia catappa* were determined spectrophotometrically and gravimetrically, respectively. **Results and Discussion:** The results reveal the presence of alkaloids in the ethanolic leaf extracts. Significant differences is observed in the order of 71.23 (*L. camara*) > 47.16 (*C. alata*) > 36.42 (*C. citratus*) > 6.99 (*C. caimito*) ~ 5.31 mg Atropine Equivalents/g sample (*T. catappa*). For the total saponin content, the ethanolic leaf extracts of *C. cainito, L. camara*, and *T. catappa* are statistically comparable but significantly higher than that of *C. citratus* and *C. alata*. **Conclusion:** The total alkaloid and saponin content in the ethanolic leaf extracts of *C. alata, C. cainito, C. citratus, L. camara,* and *T. catappa* vary depending on their taxonomic classification. Furthermore, the detection of alkaloids and saponins in the leaf ethanolic extracts provide scientific basis to support their medicinal value as potential sources of bioactive compounds.

Keywords: Ethanolic extracts, Taxonomy-dependent, Total alkaloids, Saponin content.

INTRODUCTION

Nature has been the richest source of medicinal plants for thousands of years. Nowadays, the natural product scientists are becoming increasingly aware that secondary metabolites in plants play a major role in their survival in the environment.[1] Moreover, these plant-derived metabolites have been reported to protect humans against diseases.[2] Among these phytochemicals with promising medicinal values are alkaloids and saponins.[3] Plant alkaloids, a cyclic compound containing nitrogen, are known to exhibit multiple activities such as antimalarial, antimicrobial, antihyperglycemic, anti-inflammatory, anesthetics and CNS stimulants. Saponins, on the other hand, are plant secondary metabolites known to possess hypolipidemic, anticancer, anti-inflammatory, and immunostimulatory activity.[2]

Five traditional herbal plants, namely, *Cassia alata*, *Chrysophyllum cainito*, *Cymbopogon citratus*, *Lantana camara*, and *Terminalia catappa* are worth investigating. Ethnopharmacological studies have shown that *C. alata* and *C. cainito* are effective in treating digestive

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problems and infections, and have anti-diabetic and anti-inflammatory activities.[4-5] On the other hand, decoction of leaves of *C. citratus*, *L. camara* and *T. catappa* are effective for treatment against fever, cold,[6] cough, and stomach upset.[7]

The need to generate knowledge about the phytochemistry of these plants, specifically the total alkaloid and saponin content, can be of vital significance for future related researches and development of health products such as food supplements, functional foods and therapeutics among others.

MATERIALS AND METHODS

Sample Collection, Preparation, and Extraction

Healthy and mature leaves of plant samples were collected at Central Mindanao University (CMU), Musuan, Maramag, Bukidnon. Plant samples were submitted to CMU Museum for identification and authentication. The collected leaves were washed thoroughly, air-dried, and pulverized into fine powder. About ninety-gram (90 g) portion of the pulverized leaves were separately extracted with 95% aqueous ethanol for 72 hr. The extracts were concentrated via rotary evaporation at 40°C. The dried extracts were then stored in a tightly closed container under nitrogen and stored in the freezer until use.

Determination of Total Alkaloid Content

A 1000 mg/L stock solution of atropine in ethanol was prepared. From the stock solution, various concentrations of atropine (2, 5, 6, 8, 12, and 13 mg/L) were prepared as working standards for the calibration curve. The alkaloid content of the sample extracts was determined employing the spectrophotometric method.[8] In a separatory funnel, a 2.5-mL aliquot of the reconstituted extract (1000 mg/L in ethanol) were added with 12.5 mL pH 4.7 phosphate buffer and 12.5 mL bromocresol green solution. After shaking the mixture, it was extracted with chloroform. The chloroform layer was collected in a 25-mL volumetric flask and diluted to mark with chloroform. The same steps were done for the working standards and the reagent blank (chloroform). The absorbance of the samples and the standards were determined against the blank at 470 nm using a visible spectrophotometer (CARY 60 UV-Vis Spectrophotometer – Agilent Technologies). The concentration of the alkaloid expressed as mg atropine equivalents (AE) per gram sample was calculated using Equation 1:

$$(\text{mg/g, concentrated extract}) = A \times D/C \quad \text{(Equation 1)}$$

where:

A = Atropine concentration of the sample from the calibration curve, mg AE/L

C = Concentration of the test solution, g/L

D = Dilution factor

Determination of Total Saponin Content

The total saponin content of the leaves samples was determined employing the method of Gracelin *et al.*[9] About 20 g of the ground leaf samples were placed in an Erlenmeyer flask and added with 100-mL of 20% aqueous ethanol. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixtures were filtered and the residue were re-extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over a water bath at about 90°C. The concentrate was transferred into a 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 twice using 60-mL of butanol. The combined butanol extracts were washed twice with 10 mL of 5% aqueous NaCl. The remaining solution were heated in a water bath. After evaporation, the samples were dried in the oven at 105°C until constant weight. The total saponin content were calculated and expressed as percentage saponin using Equation 2.

$$\text{Percentage Saponin, \%} = (W_{EP} - W_{AD})/W_S \quad \text{(Equation 2)}$$

where:

WEP = weight of the aluminum dish with the oven-dried saponin, g

W_S = weight of sample, g

W_{AD} = weight of aluminum dish, g

Statistical Analysis

The determinations were carried out in three (3) replicates. The obtained results were subjected to One-Way Analysis of Variance (ANOVA) at α=0.05. Significant differences among the means were determined using Tukey's Test.

RESULTS

Total Alkaloid Content

Alkaloids were detected in the ethanolic leaf extracts of *L. camara, C. citratus, C. alata, T. catappa*, and *C. caimito*

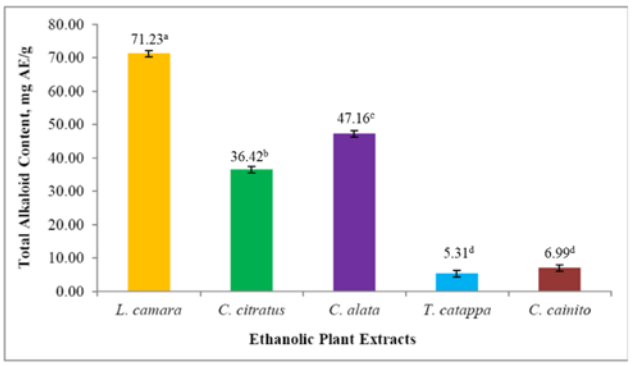

Figure 1: Total alkaloid content, expressed as mg AE/ g sample, in the ethanolic leaf extracts of *L. camara, C. citratus, C. alata, T. catappa, and C. cainito*. Error bars are standard deviations (*n*=3).

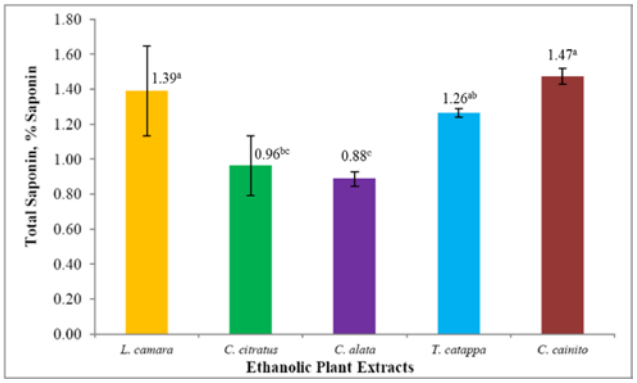

Figure 2: Total saponin content, expressed as % saponin, in the ethanolic leaf extracts of *L. camara*, *C. citratus*, *C. alata*, *T. catappa*, and *C. cainito*. Error bars are standard deviations (*n*=3).

(Figure 1). The *L. camara* extract gave the highest alkaloid content among the ethanolic leaf extracts.

Total Saponin Content

The presence of saponins in the ethanolic leaf extracts, although at a low level, may provide additional scientific basis on the medicinal value of *L. camara*, *C. citratus*, *C. alata*, *T. catappa*, and *C. cainito*. Among the plant samples, the ethanolic leaf extracts of *C. cainito* gave the highest total saponin content, followed by *L. camara*, *T. catappa*, *C. citratus*, and *C. alata* (Figure 2).

DISCUSSION

Total Alkaloid Content

As reported, alkaloids and saponins were detected in the leaves of *L. camara,*[10] *C. alata,*[11] *T. catappa,*[12] and *C. cainito* stem barks.[13] Although *C. citratus* are known for their essential oils, there rhizomes are found to contain alkaloids.[14] The results of the Analysis of Variance (ANOVA) indicate that there are significant differences in the total alkaloid content among the ethanolic leaf extracts of *L. camara, C. citratus, C. alata, T. catappa*, and *C. cainito*. Subsequent Post hoc Tukey's Test show that the total alkaloid content among the ethanolic leaf extracts are statistically different in the order of *L. camara* > *C. alata* > *C. citratus* > *C. cainito* ~ *T. catappa*. The results suggest that the total alkaloid content in the ethanolic leaf extracts of *L. camara, C. citratus, C. alata, T. catappa*, and *C. cainito* may vary depending on their taxonomic classification. The pharmacological potential of any plant is dependent on the composition of secondary metabolites, which is unique for the individual taxa.[15] Accordingly, different taxa of plants differ in genomic sequence.[16] Transcription of these genes which encode biosynthetic enzymes lead to the production of phytochemicals which are usually restricted to a few families or genera.[17]

Total Saponin Content

ANOVA shows that there are significant differences in the total saponin content among the ethanolic leaf extracts of *L. camara*, *C. citratus*, *C. alata*, *T. catappa*, and *C. cainito*. Subsequent Post hoc Tukey's Test at 0.05 level of significance revealed that the total saponin content in the ethanolic leaf extracts of *C. cainito*, *L. camara*, and *T. catappa* are statistically comparable but is significantly higher than that of *C. citratus* and *C. alata*. These findings imply that the total saponin content in the ethanolic leaf extracts of *L. camara, C. citratus, C. alata, T. catappa*, and *C. cainito* is dependent on their taxonomic classification. The detection of both alkaloids and saponins in the ethanolic leaf extracts of *L. camara*, *C. citratus*, *C. alata*, *T. catappa*, and *C. cainito* are consistent with the results of the studies showing their various pharmacological properties which are usually associated to the presence of alkaloids and saponins which are found to be significant for protection and survival of the plants.[18] Alkaloids have been reported to exhibit antimicrobial, anti-parasitic, anti-diarrhea, cytotoxic, and insecticidal activities.[19] On the other hand, saponins show antihepatotoxic, antidiarrheal,[20] antimicrobial,[21] and antifungal.[22]

CONCLUSION

The results suggest that the total alkaloid and saponin content in the ethanolic extracts vary depending on the taxonomic classification of the plant sample. The detection of alkaloids and saponins in the leaves of *L. camara, C. citratus, C. alata, T. catappa*, and *C. cainito* provide scientific basis to support the medicinal value

of the above-mentioned plants as potential sources of therapeutic bioactive components.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CNS: Central nervous system; **AE:** Atropine equivalents.

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