



Proximate analysis, phytochemical screening and total phenolic and flavonoid content of the ethanolic extract of molave *Vitex parviflora* Juss. Leaves

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ABSTRACT

Air-dried leaves of molave (*Vitex parviflora* Juss.) were subjected to proximate chemical analysis and the results were compared with literature values of its close relative, lagundi (*Vitex negundo* Linn.), a clinically-proven, commercial cough medicine in the Philippines. Following AOAC standard method of analysis, the air-dried molave leaves were shown to contain 16.95 ± 0.07 % moisture, 9.37 ± 0.09 % ash, 1.80 ± 0.14 % crude fat, 33.18 ± 0.49 % crude fiber, 16.94 ± 2.69 % crude protein and 21.76 ± 2.60 % total carbohydrates (by difference). Phytochemical screening of the ethanolic extract of the molave leaves yielded secondary metabolites that include alkaloids, saponins, diterpenes, phenols, phytosterols and flavonoids. By Folin-Ciocalteu assay of the ethanolic extract, total phenolic content of 356 ± 19.4 mg gallic acid equivalent (GAE) per 100g air-dried sample and total flavonoid content of 321.16 ± 16.71 mg quercetin equivalent (QE) per 100g air-dried sample were obtained. The results suggest the high anti-oxidant potential of extractable components from the leaves of molave.

Keywords: molave, *Vitex parviflora* Juss., proximate analysis, phytochemical screening, phenolics

INTRODUCTION

Plants contain pharmacologically active compounds that enable their use in a wide variety of medicinal and other health-related applications. These phytochemicals possess therapeutic properties that help reduce oxidative damage and protect humans from cancer and coronary heart diseases [1]. The chemical characterization of indigenous flora in a given locality is a logical prerequisite to the isolation of bioactive chemical components for drug development [2]. Discovery of the presence of alkaloids, tannins, flavonoids and phenolic compounds provides important clues for the possible utilization of the plant as medicine or food supplement [3]. Current interest in phenolic compounds has been spurred by the many potential and beneficial effects in human health of these natural products [4].

The molave tree (*Vitex parviflora* Juss.) belongs to the family *Lamiaceae* and is a close relative of the five-foliolate small tree or shrub also known in the Philippines as lagundi (*Vitex negundo* Linn.). Lagundi is the source of a clinically proven, commercially available anti-cough medicine with the brand Ascof[®], which is sold in the pharmacy in tablet or syrup form. Molave, on the other hand, is a hardwood tree indigenous to the Philippines and other Asian countries that produces very durable timber[5]. The other known Philippine names for *Vitex parviflora* Juss. are "molawin," "tugas," "sagat," and "amugauan." Because of its close taxonomic relationship with Lagundi, molave is considered as an undiscovered medicinal plant in Philippine tropical forests. The characterization and evaluation of the chemical components of the molave tree will lead to further understanding and possible use as a source of active pharmaceutical ingredients.

The present study evaluated the chemical composition of Molave leaves. Screening of the ethanolic extract was likewise done to determine the presence of pharmacologically active compounds. The results would serve as basis for the commercial development of the plant for as a pharmaceutical or nutraceutical herbal product.

EXPERIMENTAL SECTION

Sample Collection and Preparation:

Molave (*Vitex parviflora* Juss.) leaves were collected from the Mt. Makiling Forest Reserve located in Los Baños, Laguna, in the island of Luzon in the Philippines. The forest reserve is under the management and control of the Makiling Center for Mountain Ecosystems (MCME), which provided the experts for the proper taxonomic identification of the tree. Extraction of the leaves and chemical analyses were conducted from August 2013 to October 2013 at the Institute of Chemistry, College of Arts and Sciences, University of the Philippines Los Baños (UPLB) and at the Wood Chemistry Laboratory, Department of Forest Products and Paper Science, College of Forestry and Natural Resources, also of UPLB, College, Laguna, Philippines. The molave leaves were thoroughly washed with tap water and air-dried at room temperature for at least five days. The air-dried leaves were powdered using Waring blender.

Proximate Analysis: Air-dried molave leaves in powder form were tested for the moisture content, ash content, crude fiber content, crude fat content, and crude protein content using standard analytical methods by Association of Official Analytical Chemist[6].

Sample Extraction: Powdered air-dried molave leaves were extracted by soaking with 70% ethanol for 24h with frequent agitation. The ethanolic extract was then concentrated by evaporating under reduced pressure using Büchi Rotavapor R-110 at temperature below 40°C.

Phytochemical Screening: The ethanolic extract was evaluated for the presence of specific phytochemicals. Positive reaction to the different tests taken to mean the presence of a specific class of phytochemicals[7, 8].

1. Alkaloids (Wagner's test): Two mL extract was treated with few drops of Wagner's reagent (iodine – potassium iodide solution). Formation of brown/reddish precipitate indicates the presence of alkaloids.

2. Carbohydrates -Reducing Sugars (Benedict's test): Two mL extract was treated with few drops of Benedict's reagent (CuSO₄, Na₂CO₃, sodium citrate). Formation of orange red precipitate indicates the presence of reducing sugars.

3. Cardiac glycosides (Legal's test): Two mL extract was treated with few drops of sodium nitroprusside in pyridine and NaOH. Formation of pink to blood red colored solution indicates the presence of cardiac glycosides.

4. Anthranol glycosides (Modified Borntrager's test): Two mL extract was treated with few drops of ferric chloride solution and then immersed in boiling water for 5 mins. The resulting solution was extracted with an equal volume of benzene. The benzene layer was separated using ammonia. Formation of rose-pink colored solution (ammoniacal solution) indicates the presence of anthranol glycosides.

5. Cyanogenic glycosides (Picrate paper test): Two mL extract was treated with few drops of 10mL water and 1mL dilute HCl. Picrate papers (paper strips dipped in saturated aqueous picric acid previously neutralized with NaHCO₃) were suspended above flask containing the solution. The solution was heated at 45°C for 1h. A picrate paper color change from yellow to red indicates the presence of cyanogenic glycosides.

6. Saponins (Froth test): Two mL extract was diluted with distilled water up to 20 mL then mixed. Formation of about 1 cm layer of foam indicates the presence of saponins.

7. Diterpenes (Copper acetate test): Two mL extract was treated with three drops of copper acetate solution. Formation of emerald green solution indicates the presence of diterpenes.

8. Triterpenes (Salkowski's test): Two mL extract was treated with few drops of chloroform and then filtered. The resulting solution was treated with few drops of concentrated H₂SO₄, shaken, and allowed to stand for 5 min. Formation of golden yellow colored solution indicates the presence of triterpenes.

9. Phenols (Ferric chloride test): Two mL extract was treated with three drops of ferric chloride solution. Formation of bluish black colored solution indicates the presence of phenols.

10. Phytosterols (Liebermann - Burchard's test): Two mL extract was treated with few drops of chloroform and then filtered. The resulting solution was treated with few drops acetic anhydride, boiled and then cooled. Concentrated H₂SO₄ was added after cooling. Formation of blue green colored solution indicates the presence of phytosterols.

11. Tannins (Gelatin test): Two mL extract was treated with few drops of 1% gelatin solution containing NaCl. Formation of white precipitate indicates the presence of tannins.

12. Flavonoids (Alkaline reagent test): Two mL extract was treated with few drops of 2M NaOH. Formation of an intense yellow colored solution, which turns colorless when dilute acid is added, signifies the presence of flavonoids.

13. Amino acids (Ninhydrin test): Two mL extract was treated with few drops of 0.25% w/v ninhydrin and then boiled for 5 min. Formation of blue colored solution indicates the presence of free amino acids.

Total Phenolic and Flavonoid Content: The total phenolic and flavonoid content of the ethanolic extract of molave leaves were determined using the Folin-Ciocalteu assay[9]. The absorbance values of the sample were measured using Shimadzu UV Mini 1240 at 725 nm. The standards used for estimating the total phenolic and total flavonoid content were gallic acid and quercetin. The total phenolic content was expressed as mg gallic acid equivalent (GAE) per 100g air-dried sample and the total flavonoid content was expressed as mg quercetin equivalent (QE) per 100g air-dried sample.

RESULTS AND DISCUSSION

Proximate Analysis: The proximate analysis of the air-dried molave leaves was done in order to evaluate the nutritional content of the sample. The moisture content was 16.95 ± 0.07 %, ash content 9.37 ± 0.09 %, crude fat content 1.80 ± 0.14 %, crude fiber content 33.18 ± 0.49 %, crude protein content 16.94 ± 2.69 % and total carbohydrates 21.76 ± 2.60 %. In comparison, previously reported values for the proximate analysis of air-dried lagundi leaves showed 16.85 % moisture, 8 % ash, 7 % crude fat, 28 % crude fiber, 13.73 % crude protein and 26.43 % carbohydrates [10] .

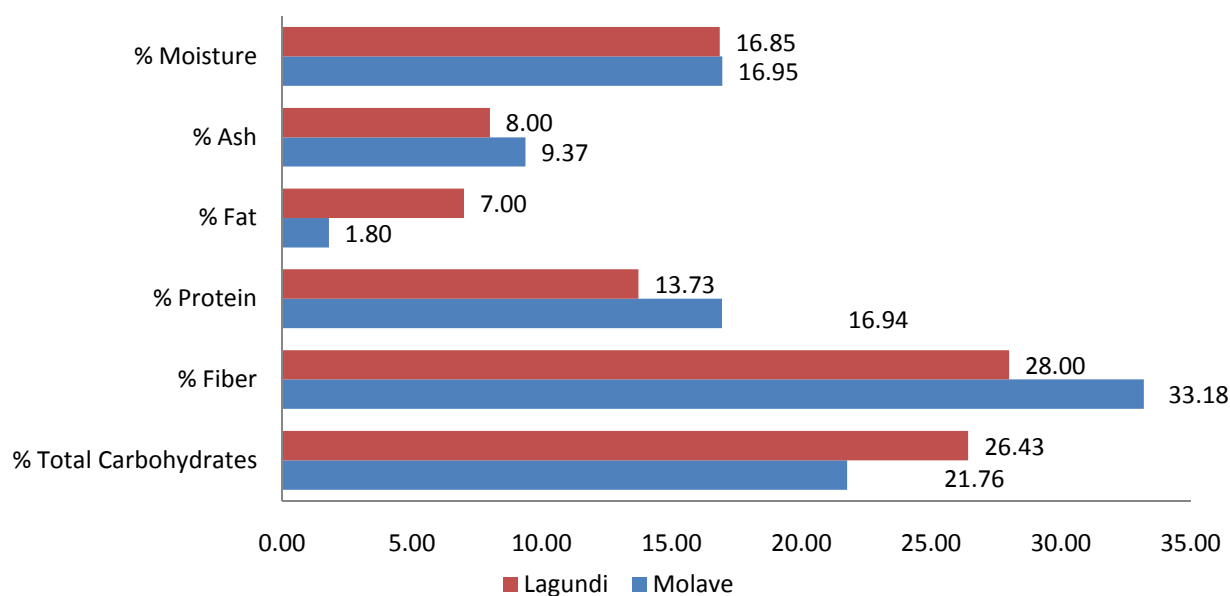


Figure 1. Comparison of the proximate analysis of air-dried molave leaves (*Vitex parviflora* Juss.) and air-dried lagundi leaves (*Vitex negundo* Linn.) [10]

The moisture content of the molave and lagundi leaves at the time of their analysis were almost the same. The ash content, crude protein content and crude fiber content of the molave leaves are higher compared to those of lagundi leaves as reported in the literature. In terms of inorganic compounds, structural carbohydrates, and protein content, molave leaves contained higher amounts of these components. For the crude fat content and total carbohydrates, lagundi leaves gave higher values compared to the molave leaves. The latter suggests that lagundi leaves are higher in energy-providing metabolites such as fat and carbohydrates than molave.

Extraction of Phenolic Compounds: The phenolic compounds in molave leaves (*Vitex parviflora* Juss.) were extracted using 70% ethanol. In general, 70 to 80% ethanol and methanol are the solvents used for the extraction of phenolic compounds and the most useful for all types of living tissues. Phenolic compounds tend to be water-soluble because they frequently occur in combination with sugar as glycosides and are usually located in the cell vacuole [11].

Phytochemical Screening: Phytochemicals are chemical compounds found in plants which are responsible for the pigment and the plant's bioactive properties, thereby enabling its use as nutritional or dietary fiber for protection against various diseases. Phytochemical screening consists of preliminary tests that are important for knowing major molecules or drug entities present in biological materials. Table 1 summarizes the results of the phytochemical screening of the ethanolic extract of molave leaves. Based on the results, the ethanolic extract of molave leaves was shown to contain secondary metabolites like alkaloids, saponins, diterpenes, phenols, phytosterols and flavonoids.

Reported phytochemical screening results for *Vitex negundo* Linn. methanolic extract revealed the presence of flavonoids, flavones glycosides, diterpenes, triterpenes, and sesquiterpenes [12]. The presence of major phenolic compounds in the leaves of molave suggests that it can be a promising medicinal plant.

Table 1. Phytochemical screening of the ethanolic extract of molave leaves

Phytochemical Test	Result	Observation
Alkaloids (Wagner's test)	+	Turbid/Brown precipitate
Carbohydrates (Benedict's test)	-	Dark green solution without formation of orange precipitate
Cardiac glycosides (Legal's test)	-	Dark brown solution
Anthranol glycosides (Modified Borntrager's test)	-	Colorless solution
Cyanogenic glycosides (Picrate paper test)	-	Yellow (Picrate paper)
Saponins (Froth test)	+	Formation of foam
Diterpenes (Copper acetate test)	+	Emerald green solution
Triterpenes (Salkowski's test)	-	Yellow solution
Phenols (Ferric chloride test)	+	Dark brown to black
Phytosterols (Liebermann-Burchard's test)	+	Brown ring formation
Tannins (Gelatin test)	-	No white precipitate
Flavonoids (Alkaline reagent test)	+	Intense yellow color, then colorless upon addition of dilute acid
Amino acids (Ninhydrin test)	-	Dark brown solution

+ - present

- - absent

Total Phenolic and Flavonoid Content: The total phenolic content and total flavonoid content were quantified by using the Folin-Ciocalteu assay. Table 2 shows the comparison of the total phenolic content and total flavonoid content of the ethanolic extract of molave and lagundi leaves. The total phenolic content in terms of gallic acid equivalent (GAE) of the molave ethanolic extract is higher which shows that molave contains more ethanol-soluble phenolic compounds.

Table 2. Total phenolic content and total flavonoid content of the ethanolic extract of molave and lagundi leaves

Ethanolic Extract	Total Phenolic Content	Total Flavonoid Content	
	Gallic Acid Equivalent (mg/100g dried sample)	Quercetin Equivalent (mg/100g dried sample)	Catechin Equivalent (mg/100g dried sample)
Molave	356.00 ± 19.4	321.16 ± 16.7	Not Tested
Lagundi	249 ± 8.34*	NA	166.67 ± 9.14*

* - Literature values [13]

CONCLUSION

Proximate chemical analysis of the air-dried *Vitex parviflora* Juss. leaves was done to evaluate its nutritional content. Phytochemical screening detected several phytochemicals in the ethanolic extract that can be used as basis for developing dietary products with potential health benefits. Analysis of the total phenolic content showed comparable to or even slightly higher amounts of phenolic compounds in *Vitex parviflora* Juss. leaf extract (356 mg GAE/100g) than *Vitex negundo* Linn. leaf extract (249 mg GAE/100g).

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