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Der Pharmacia Lettre, 2017, 9 (2):74-78
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Phytochemical Screening of *Syzygium Cumini* (Myrtaceae) Leaf Extracts Using Different Solvents of Extraction

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ABSTRACT

Objectives: The study aimed to identify the phytochemical constituents present in the leaves of *Syzygium cumini* (Myrtaceae) using the following solvents with various polarity: distilled water (aqueous), ethanol, methanol, ethyl acetate, and hexane.

Methodology: The leaves were cleaned, air-dried, and ground to a coarse powder. The powders were divided into five and were soaked separately using distilled water, ethanol, methanol, ethyl acetate, and hexane for around one (1) week prior to phytochemical screening.

Results: The study showed that both the ethanolic and methanolic extracts contained most of the phytochemical constituents, followed by the ethyl acetate, hexane, and aqueous extracts, respectively. These phytochemicals in the leaves include alkaloids; flavonoids; saponins; tannins; glycosides; phenols; proteins; triterpenoids; steroids; and fixed oils and fats, having proteins as the highest amount in all the five solvents.

Conclusion: *S. cumini* leaves contain significant bioactive compounds that make the plant a potential antioxidant, anti-diabetic, and among other therapeutic uses.

KEYWORDS: *Syzygium*, *cumini*, Duhat, Phytochemical Screening

INTRODUCTION

Syzygium cumini commonly known as duhat, black plum, or Java plum, is found throughout the Philippines and is one of the most popular fruits. It is planted, and in many regions spontaneous. It is also a native of India, Myanmar,

Sri Lanka, Thailand, Australia, Colombia, Cuba, Mexico, Nepal, Kenya, United States of America, Zambia, and Zimbabwe.

Scientific Classification: *Syzygium cumini*

Kingdom: Plantae

Subkingdom: Viridaeplantae

Infrakingdom: Streptophyta

Division: Tracheophyta

Subdivision: Spermatophytina

Infradivision: Angiospermae

Class: Magnliopsida

Superorder: Rosanae

Order: Myrtales

Family: Myrtaceae

Genus: *Syzygium*

Specie: *Cumini*

Duhat is an evergreen tree that grows up to 25 meters (80 feet) tall, with grayish white stems and coarse and discolored lower bark. The leaves are simple, opposite, elliptic to oblong, smooth, glossy, and somewhat leathery. Also, the leaves are 5 to 10 centimeters (1.2 inches) long. The midrib of the leaves is prominent and yellowish. The leaf blades have many lateral veins closely parallel. The flowers are white to pinkish, about 1 centimeter (0.5 inch) across with four petals and many stamens. The calyx is cup-like. The fruits are ovoid, 1-seeded berry, with a length of 2 centimeters (0.8 inch), dark purple red, shiny, with white to lavender Flesh [2-9].

In leaves, ethanolic extract of *S cumini* showed the presence of tannins, alkaloids, flavonoids, sterols, glycosides, and carbohydrates while methanolic extract demonstrated the presence of flavonoid [8]. In seeds, saponins and flavonoids were in more quantity than alkaloids, glycosides, triterpenoids, steroids, and tannins in the ethyl acetate and methanol extracts [4]. In the bark, tannins were also present (13.4%) [6].

MATERIALS AND METHODS

Plant Materials

The leaves of *Syzygium cumini* were collected from a single tree during the third week of December 2016 at Novaliches, Quezon City, Philippines. The leaves were cleaned, air-dried at room temperature on a cool dry place away from direct sunlight, and finally ground to a coarse powder (250 g). The 250-gram-powder was then divided into five for the extraction using different solvents [1].

Preparation of Various Extracts

Dried leaf powder (50 g for each solvent) was soaked separately in 250 mL of each solvent namely: distilled water, ethanol, methanol, ethyl acetate, and hexane in a flask. The five flasks were covered and then kept at room temperature for around 1 week. After that, the separate solutions were filtered by Whatman filter paper (no. 1). Each filtrate was collected in a round bottom flask and was subjected to evaporation to achieve a gummy appearance. Then the gummy substance of each solvent was dried at room temperature. The powdered extracts were weighed and stored at 40°C for further work. From 50 g dried leaf powder, 7 g (14%) of extract was finally obtained from aqueous extract; 7.8 g (15.6%) of extract from ethanolic extract; 7.3 g (14.6%) from methanolic extract; 6.9 g (13.8%) from ethyl acetate extract; and lastly, 6.3 g (12.6%) from hexane extract. Then, the extracts were re-dissolved in their respective solvents before they were subjected to phytochemical screening [1].

Phytochemical Screening [3,10]

o Alkaloids

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Mayer's Test: To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids [4].

Valser's Test: A few drops of Valser's reagent is added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive.

Wagner's Test: A few drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive.

Flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Saponins

Froth Test. The extract (50 mg) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A two cm layer of foam indicates the presence of saponins

Tannins

Ferric Chloride Test: The extract (50 mg) is dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution are added. A dark green colour indicates the presence of phenolic compound.

Glycosides

For 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests.

Legal's Test: 50 mg of extract is dissolved in pyridine; sodium nitroprusside solution is added and made alkaline using 10% NaOH. Presence of glycoside is indicated by pink color.

Phenols

Lead Acetate Test: The extract (50 mg) is dissolved in of distilled water and to this 3 ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

o Proteins

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for proteins.

Millon's Test: To 2 ml of filtrate few drops of Millon's reagent are added. A white precipitate indicates the presence of proteins.

Carbohydrates

Molish's Test. To 2 ml of plant sample extract, two drops of alcoholic solution of α - naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

Triterpenoids

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Phyto sterols

Liebermann-Burchard's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of Phyto sterols.

Fixed Oils and Fats

Spot Test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

RESULTS AND DISCUSSION

Alkaloids	Aqueous	Ethanol	Methanol	Ethyl Acetate	Hexane
Dragendroff's Reagent	+	+	+	+	+
Mayer's Reagent	+	+	+	+	+
Valser's Reagent	-	+	+	-	-
Wagner's Reagent	-	+	+	-	-
Flavonoids	+	+	+	+	++
Saponins	++	++	+++	++	++
Tannins	-	++	++	++	+
Glycosides					
Phenols					
Proteins					
Carbohydrates					
Triterpenoids					
Steroids					
Fixed Oils and Fats					

Results in the table above showed that both the ethanolic and methanolic extracts contained most of the phytochemical constituents, followed by the ethyl acetate, hexane, and aqueous extracts, respectively. Among these phytochemicals present in the leaves of *S. cumini* are alkaloids; flavonoids; saponins; tannins; glycosides; phenols;

proteins; triterpenoids; steroids; and fixed oils and fats. Proteins were the highest constituents in all types of solvent. However, carbohydrates were absent in all extracts of the leaves.

CONCLUSION

It was observed that *S. cumini* leaves contained significant bioactive compounds that make the plant a potential antioxidant, anti-diabetic, anti-microbial, and among other therapeutic properties.

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