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# Chemical Constituents, Antioxidant and Antibacterial Activity of *Syzygium cumini* (L.) Skeels (Myrtaceae)

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**ABSTRACT**

*This work investigated the phytochemical constituents, antioxidant and antibacterial activities of the leaves, fruit-pericarp, and stem-bark of Syzygium cumini, which is known for its uses in traditional folk medicine in the Philippines. The three plant parts contained alkaloids, terpenoids, glycosides, saponins, alkaloids, and tannins. All samples exhibited antioxidant activity. The fruit-pericarp had the highest radical scavenging activity of 56.73%, followed by the stem-bark and leaves having 51.97%, 47.51%, respectively. Leaves had the highest phenolic content with 382.75 mg AAE/ g sample while stem-bark had 369.17 mg AAE/ g sample. All samples showed inhibitory activity against Staphylococcus aureus and Escherichia coli with diameter zone of inhibitions range from 8.12 to 13.28 mm, except fruit-pericarp against E. coli. The three plant parts of S. cumini exhibits antioxidant and antibacterial activities which are mainly attributed to the various phytochemical constituents.*

**Keywords**: *Syzygium cumini*, phytochemicals, antioxidant, antibacterial.

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## INTRODUCTION

Medicinal plants are valuable resource of bioactive phytochemicals that play an important role in traditional folk medicine as therapeutic alternatives. In the Philippines, the use of medicinal or herbal plants as remedy for different illnesses was introduced and practiced by the different tribes or ethnic groups. Each ethnic group has their unique knowledge and skills on the utilization of medicinal plants. *Albularyos* (herbal doctors) were considered medicinal plant specialist that uses plants in curing different illnesses in many *barrios* (small community) in Philippines. Thus, in order to confirm the effectiveness of these medicinal plants utilized by the ethnic groups and *albularyos*, a number of investigations on the elucidation of active components and evaluation of functional activities have been conducted and reported. For instance, the secondary metabolites found and isolated from plants have shown to have anticancer, antibacterial, analgesic, anti-inflammatory, antitumor, antiviral and many other activities to a greater or lesser extent [1, 2].

*Syzygium cumini* (L.) Skeels, belong to Myrtaceae, is commonly known by Filipinos as *duhat*. This evergreen tree could reach up to 25 m high with coarse and discolored bark and grayish white young stems. The simple leaves are opposite, elliptic to broadly oblong, smooth, glossy, somewhat leathery, and short pointed at tips. Its fruit is ovoid, 1-seeded berry to 2 cm long, shiny dark purplish red, with white to lavender flesh. This plant also exhibits various

biological activities. The ethanolic extract of *S. cumini* bark has a potent anti-inflammatory action against different phases of inflammation without any side effect on gastric mucosa [3]. In a review conducted by Srivastava and Chandra [4], the fruit *S. cumini* is reported to have antidiabetic, antihyperlipidaemic, antioxidant, antiulcer, hepatoprotective, antiallergic, antiarthritic, antimicrobial, anti-inflammatory, antifertility, antipyretic, antiplaque, radioprotective, neuropsychopharmacological, nephroprotective and antidiarrhoeal activities. Herein, the phytochemical composition, antioxidant and antibacterial activity of the extracts of Philippine *S. cumuni* leaves, fruit pericarp and stem-bark were investigated.

## MATERIALS AND METHODS

### *Source of Plant Samples*

The leaves, fruit pericarp, and stem-bark of *S. cumini* were collected from Bambanaba, Cuyapo, Nueva Ecija, Philippines, and separately placed in a plastic bag with proper label. Samples were washed three times and air-dried in a shaded condition for 10 days. These were pulverized and processed for extraction.

### *Phytochemical Analyses*

The chemical screening of the aqueous extracts of the plants were carried out following the procedures described by Sofowora [5]. Among the phytochemicals considered include alkaloids, cardiac glycoside, flavonoids, saponins, tannins, and terpenoid. Distilled water was used a control and was used as a gauge in the changes of color/intensity of the reaction. Three replicates were laid out for each test parameter.

### *Ethanol Extraction*

Twenty grams of each air-dried milled plant sample were soaked in 500 ml of 95% ethanol for 48 hours. After which, these were filtered using Whatman No. 2 filter paper to separate the plant material. Each filtrate was evaporated in a rotary evaporator to remove the solvent used. Extracts were labeled and prepared for the different assays.

### *DPPH Radical Scavenging Activity Assay*

The stable 2,2'-diphenyl-1-1picrylhydrazyl (DPPH) radical was used to estimate the free radical scavenging activity of the extracts, following the standard method of Shimada et al. [6]. A 100 µl of test sample in ethanol was added with 5 µl DPPH solution (5 mg DPPH powder in 2 ml of ethanol) in 96-well microtitter plates. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The inhibition of DPPH free radicals was calculated.

### *Estimation of Total Phenolic Content*

The total phenolic content was estimated using Folin-Ciocalteu method of Slinkard and Singleton [7] with modifications. Sample solution (50 µl) was mixed 500 µl of 10% Folin-Ciocalteu reagent (Folin:Methanol, 1:1, v/v). After 2 min, 50 µl of 7.5% saturated was added and kept in the dark for 1h before absorbance was taken at 765 nm. A calibration curve was obtained using various concentrations of ascorbic acid. The total phenolic content of the sample was expressed as mg of ascorbic acid equivalents (AAEs) per gram of sample.

### *Antibacterial Screening*

The antibacterial activities of the ethanol extracts of the three parts of *S. cumini* were determined following the paper disc diffusion method of Bauer et al. [8]. Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* were cultured in 9 ml of nutrient broth (NB) medium and incubated at 37 °C. After 24 hours, the turbidity of each bacterial culture was adjusted to equal that of 0.5 McFarland standard, which approximated $1.5 \times 10^8$ $ml^{-1}$. The bacterial suspension was spread using a sterile cotton swab on nutrient agar plate. Six millimetre diameter paper discs impregnated with crude extract (20 µL) and ethanol extract (20 µL), and streptomycin as standard were placed equidistantly on the medium. Plates were incubated at 37 °C, and the zones of inhibition were measured using vernier calliper after 24 hours. Each test was done in triplicate.

### *Statistical Analysis*

Data were analyzed using Analysis of Variance (ANOVA). Means were compared using Duncan Multiple Range Test (DMRT) at 5% level of significance.

## RESULTS AND DISCUSSION

### *Phytochemical Constituents*

Phytochemicals are the primary and secondary chemical constituents of the plants. They can detoxify substances that cause various diseases. They neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogen [9]. In this study, the secondary metabolites such alkaloids, terpenoids, glycosides, saponins, alkaloids, and tannins were screened in the three plant parts of *S. cumini*. The results are presented in Table 1. Apparently, all chemicals were detected in all the three plant parts of *S. cumini*. These phytochemicals confirmed the antioxidant and antibacterial activity of the three plant parts of *S. cumini*. Moreover, the result strongly indicates that the three plant parts of *S. cumuni* hold valuable compounds with tremendous biological activities.

Flavonoids have anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [10]. Terpenoids are good source of antioxidant, can modify hormones, cholesterol absorption blocker, and protect cellular differentiation [11]. Tannins exhibit antiviral, antibacterial, anti-tumor, and diuretic activities [12]. Alkaloids are powerful chemicals known to treat some types of cancer, reduce spasms, and relieve pains and inflammation [13]. Saponin demonstrates hypercholestrolaemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, and antifungal properties [14].

**Table 1. Phytochemical composition of the three plant parts of *S. cumini.***

| Phytochemicals | *S. cumini* | | |
|---|---|---|---|
| | Leaves | Fruit-pericarp | Stem-bark |
| Flavonoids | + | + | + |
| Terpenoids | + | + | + |
| Cardiac Glycosides | + | + | + |
| Saponins | + | + | + |
| Alkaloids | + | + | + |
| Tannins | + | + | + |

*+ positive*

### *Antioxidant Property*

Antioxidants are substances that neutralize free radicals or their actions [15]. Several studies have showed the important functions of antioxidant to reduce effect of cancers, cardiovascular diseases, neurodegenerative diseases, diabetes, and many more diseases [16, 17, 18]. Thus, various medicinal plants have been investigated for their potential as source of antioxidant. In this present work, the DPPH radical scavenging activity of the three plant parts of *S. cumini* was evaluated. Table 2 shows the percentage radical scavenging activity of *S. cumini*. Among the plant parts, the fruit-pericarp had the highest radical scavenging activity of 56.73%, followed by the stem-bark having 51.97%. The leaves had 47.51% scavenging activity. The antioxidant property of the fruit skin may come in part from the antioxidant vitamins, phenolics or tannins and anthocyanins present in the fruit [19]. This result clearly dictate that the three plant parts of *S. cumini* hold promising free radical neutralizers that protect the human body against various diseases including cancer and cardiovascular diseases.

**Table 2. Radical scavenging activity and total phenolics of the three plant parts of *S. cumini***

| *S. cumini* | Radical Scavenging Activity (%) | Total Phenolics (mg AAE / g sample) |
|---|---|---|
| Leaves | $47.51^{d}$ | $382.75^{a}$ |
| Fruit-pericarp | $56.73^{b}$ | $247.79^{c}$ |
| Stem-bark | $51.97^{c}$ | $369.17^{b}$ |
| Cathechin | $90.80^{a}$ | - |

*In the mean column, means having the same letter of superscripts are not significantly different from each other using DMRT at 5% level of significance.*

Phenolic compounds, ubiquitous in plants, are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. The antioxidant activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups and the nature of substitutions on the aromatic rings [20]. The present study also analyzed the total phenolic content of the three samples. The result of the analysis is presented in Table 2. Apparently, leaves had the highest phenolic content with 382.75 mg AAE/ g sample. The stem-bark and fruit-pericarp have 369.17 mg AAE/ g sample and 247.79 mg AAE/g sampe, respectively. Interestingly, the fruit-pericarp having the highest scavenging activity had the lowest phenolic content using

ascorbic acid as the standard. In the study reported by Singh et al [21], gallic acid and quercetin standard showed higher antioxidant activity using DPPH radical scavenging activity of *S. cumini* fruit than the other standards (caffeic acid, sinapic acid and delphinidin chloride). This clearly indicates that the antioxidant activity of *S. cumini* is dependent on the wide species of phenolic compounds.

***Antibacterial Activity***

The antibacterial activities of the three plant parts of *S. cumini* against reference pathogenic strains, *Staphylococcus aureus* and *Escherichia coli*, were *in-vitro* assayed. The diameter zone of inhibitions of the extracts of the three plant parts against the two pathogens is shown in Table 3. Interestingly, all the samples showed inhibitory activity against the two bacteria except fruit-pericarp against *E. coli*. Leaves ethanol extract had diameter zone of inhibitions of 9.51 mm against *E. coli* and 13.28 mm against *S. aureus*. On the other hand, stem-bark ethanol extract had 8.12 mm and 12.73 mm against *E. coli* and *S. aureus*, respectively. Therefore, *S. cumini* extracts could exhibit antibacterial activity against pathogenic microbes indicating its important role in wound healing and bacterial infections. Similarly, *S. cumini* fruit polyphenol extracts exhibited a broad spectrum antimicrobial activity against reference pathogenic strains, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli*, *Klebsiella pneumonia* and *Candida albicans* minimum inhibitory concentrations in the range from 0.5 to 2.5 mg/ml [21].

**Table 3. Diameter zone of inhibition of ethanol extract of the three plant parts of *S. cumini* against *E. coli* and *S. aureus in vitro*.**

| ***S. cumini*** | **Diameter zone of inhibition (mm)** | |
|---|---|---|
| | ***E. coli*** | ***S. aureus*** |
| Leaves | $9.51^{b}$ | $13.28^{b}$ |
| Fruit-pericarp | $0.00^{c}$ | $8.25^{c}$ |
| Stem-bark | $8.12^{b}$ | $12.73^{b}$ |
| Streptomycin | $27.89^{a}$ | $31.52^{a}$ |

*In the mean column, means having the same letter of superscripts are not significantly different from each other using DMRT at 5% level of significance.*

In conclusion, the three plant parts of *S. cumini* exhibits antioxidant and antibacterial activities which are mainly attributed to their several phytochemical constituents such as alkaloids, terpenoids, glycosides, saponins, alkaloids, tannins and phenolic compounds.

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