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Invitro Analysis of Antibacterial and Antioxidant Activity of Extract of Syzygium Cumini

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Abstract

Black plum (*Syzygium cumini*) is a prominent tropical tree cultivated extensively for its fruit. Black plum has a long history of medicinal use across various countries. Black plum is classified under ethnomedicine. It holds significant importance in the field of medicine. The presence of caryophyllene oxide indicates that the plant exhibits cerebral effects, as evidenced by its depressant impact on CN3. Examples of antioxidants include carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and endogenous metabolites. Various phytochemical analysis methods were employed to evaluate the composition of alkaloids, proteins, phenolic compounds, flavonoids, and saccharides, along with their antioxidant potential. The DPPH test, a free radical scavenging assay, was employed to measure antioxidant activity.

Keywords: antibacterial, antioxidant, phytochemical

Introduction

Natural dietary sources are rich in antioxidants, and the intake of these compounds may provide numerous potential health benefits. Natural dietary sources such as vegetables, fruits, and beverages are rich in antioxidants. Dietary antioxidants, including flavonoids, may play a role in reducing the risk of mortality from coronary heart disease and myocardial infarction.

The ethno-medicinal history of Syzygium cumini is comprehensive. Numerous parts of the plant are utilized in diverse traditional medical practices, especially for addressing gastrointestinal issues. The plant's antioxidant and free radical defense has been demonstrated to have long-term cardioprotective effects, and the fruit is rich in vitamin C [1]. The fruit is also rich in antioxidant dietary fiber. The decoction of Black plum tree leaves is utilized for the treatment of spasms, epilepsy, and various cerebral diseases. The presence of caryophyllene-oxide indicates that the plant exhibits cerebral features, as evidenced by its CN3 depressive activity [2].

The extract that prolonged phenobarbitone-induced sleep in mice demonstrated central nervous system depressant activity [3]. Epilepsy and chorea, which encompass various degenerative nervous disorders marked by involuntary spasmodic movements of the body and limbs, are addressed through the use of an extract [4]. The leaves serve therapeutic purposes for coughs, ulcers, boils, wounds, and antiallergic conditions [5]. The composition included anthraquinones, flavonoids, seccoirridoids, and was associated with malaria treatment and/or prophylaxis [6].

Black plum possesses significant levels of antioxidants and exhibits antibacterial properties [7]. Examples of antioxidants include carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and endogenous

metabolites. Even at low concentrations, antioxidants inhibit the oxidation process and play various physiological roles in the body. Antioxidants function as radical scavengers, facilitating the transformation of reactive radicals into less reactive forms. While oxygen is crucial for the survival of aerobic organisms, elevated levels can lead to toxicity.

A variety of diseases, including arthritis, cancer, and atherosclerosis, are causally associated with free oxygen radicals. Medicinal plants abundant in antioxidants are utilized to address disorders resulting from oxidative stress, serving as an alternative therapeutic option. In a chemical reaction referred to as oxidation, electrons are transferred from a material to an oxidizing agent. Intracellular oxygen might inadvertently trigger a series of processes at the cellular level, leading to damage to essential cell biomolecules. Free radicals are atoms or molecules characterized by an unpaired electron, which makes them highly reactive. They have the potential to disrupt other molecules and generate a significant quantity of additional free radicals. Spectrophotometric methods utilizing hydrogen atom transfer (HAT) and single electron transfer (SET) processes are currently employed to assess antioxidant activity. The oxygen radical absorbance capacity (ORAC) assay, the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, the 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) radical scavenging activity assay, the ferric reducing antioxidant potential (FRAP) assay, and the cupric reducing antioxidant potential (CUPRAC) assay.

The DPPH assay stands out as one of the most prevalent and uncomplicated procedures in this field. The process involves the reduction of the violet DPPH radical through a hydrogen atom transfer mechanism, leading to the formation of stable light yellow DPPH molecules. The measurement of antioxidant activity is conducted using a UV-Vis spectrophotometer at a wavelength of 515–520 nm, which assesses the residual activity of the violet DPPH radical.

Materials And Methods

Preparation of water extracts

A 100 g sample of Black plum fruit was cooked in 1.5 L of distilled water for 4 hours. Following that, the sample was filtered using Whatman filter paper No. 4. The filtrate was concentrated at 60°C using a rotary evaporator and subsequently dried in a freeze dryer. The extracts were maintained at 18°C until analysis.

Preparation of ethanol, methanol, and hydroethanolic extracts.

A sample of 100 g of Black plum fruits was immersed in 1.5 L of pure ethanol (purity 94.0%) and 1.5 L of pure methanol (purity 99.8%) for a duration of 4 days at room temperature to obtain the ethanol and methanol extracts. Hydroethanolic extracts were obtained using hydroethanol solvents with water:ethanol ratios of 70:30, 50:50, 30:70, and 10:90 (v/v). Following a duration of four days, the extracts underwent filtration using Whatman filter paper No. 4 and were subsequently concentrated using a rotary evaporator at a temperature of 50°C. The filtrates were subsequently dried using a freeze dryer and stored at 18°C for future analysis.

Assay for phenolic compounds

A modified Folin–Ciocalteu method was used to assess the samples' phenolic component concentration [8]. 2 mL of distilled water and 0.5 mL of Folin–Ciocalteu reagent were employed to dilute one milliliter of each extract (Sigma Co.). Following a duration of 3 minutes, 0.5 mL of a 10% Na2CO3 solution was incorporated into the mixture, which was subsequently permitted to rest at room temperature for 1 hour in

a dark setting. A UV–visible spectrophotometer detected absorbance at 760 nm. A calibration curve was established utilizing a standard solution of caffeic acid (Sigma Co.) at concentrations ranging from 10 to 100 µg/mL. The findings were expressed in milligrams of caffeic acid per gram of extract. The tests were conducted in triplicate, and the results were averaged.

Assay of flavonoid content

Moreno's methodology was employed to assess flavonoid content [9]. Each extract (1 mL) was combined with 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 mol/L aqueous potassium acetate, and 4.3 mL of 80% ethanol in a test tube. Absorbance was recorded at 415 nm following a 40-minute incubation at room temperature in darkness. The total flavonoid content was quantified using standard quercetin (Sigma Co.) at concentrations ranging from 0 to 100 µg/mL.

TLC

TLC method was used for the identification of compound. The filtered extract was used for this study. The chromatographic sheet was set with 5cm width and 8cm length and spotted the sample 1cm above from the bottom using capillary tubes (about 50 µl). Sample placed and this was run in solvent system of methanol, acetic acid, formic acid and water (1: 1: 1: 2). After 1cm from the top of the plate this was taken out from the solvent and dried to visualise the compound, the same method was used for the TLC study. Rf value was calculated after visualisation of the compound as spot in plate. Rf value can be calculated by the following formula,

Rf value=(Distance moved by solute)/(Distance moved by solvent)

UV –VIS Spectrophotometric Analysis

The extract was examined under UV Visible spectral analysis. The extracts were scanned in the wavelength ranging from 200-800 nm using UV-Visible spectrophotometer and the characteristic peaks were detected.

DPPH Assay

The DPPH assay was employed to assess the free radical scavenging activity of the extracts. A DPPH solution (0.004% w/v) was prepared in methanol. Methanol served as the solvent for the preparation of the stock solution at a concentration of 1 mg/ml, alongside standard ascorbic acid at 0.05 g/ml. 0.5 ml of the sample solution and 1 ml of DPPH solution were combined in a tube, along with 0.4 ml of 50 mM Tris-HCl buffer. The absorbance was measured at 517 nm using a spectrophotometer. Methanol served as a blank, and ascorbic acid was employed as a reference to calculate the mg/g of DPPH.

Antibacterial Activity

Agar diffusion was employed to evaluate the antibacterial activity of the leaf extracts. The extracts' antibacterial activity was evaluated against *Bacillus coagulans* and *Staphylococcus aureus*. Each extract derived from the solvents was assessed at five concentrations: 10%, 20%, 30%, 40%, and 50%, with the solvent acting as a control. The experiment was conducted at 37ºC. The diameter of inhibition zones was measured after 24 hours, and the extract exhibiting the greatest diametrical inhibition at the lowest concentration was selected for subsequent analysis. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were assessed utilizing the Bloomfield technique (1991). The extracts were evaluated at five distinct pH levels: 4, 5, 6, 7, and 8, to assess the

impact of pH on their properties. The extract underwent testing across four sugar concentrations (10%, 20%, 30%, and 40%), four salt concentrations (1%, 2%, 3%, and 4%), and two temperatures (80°C and 100°C) for durations of 5, 10, and 15 minutes. The extract was evaluated against *Bacillus cereus* for 24 hours at 37 degrees Celsius.

Result

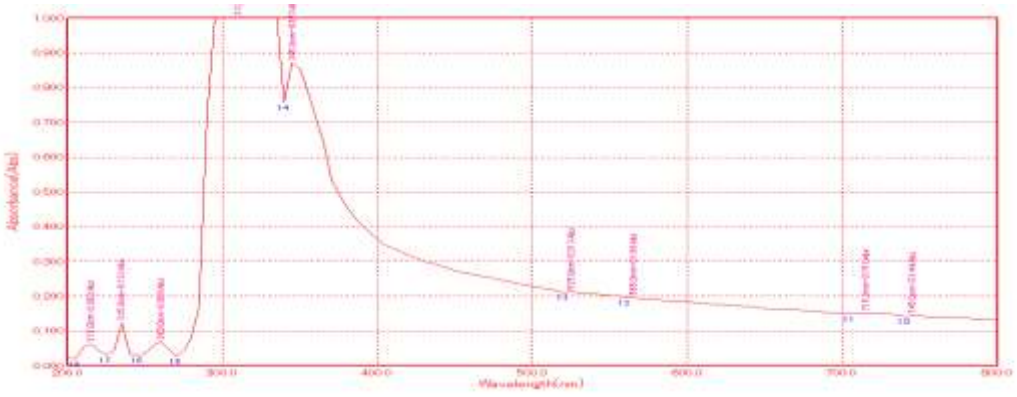
Results obtained for antioxidant compounds and antioxidant assays are expressed on a fresh fruit basis. With regard to visual colour, aqueous extraction of Black plum white colored extract, respectively. In this study the aqueous extract of the Black plum were evaluated for their antioxidant and antimicrobial properties.

Thin Layer Chromatography(TLC)

TLC is a technique for separating and studying the constituents of a mixture. TLC can be used to determine the number of components in a mixture, compound identity, and chemical purity. Various components in the original spot will migrate different distances from the original spot position and appear as separate spots as a result of the polarity difference. When the solvent has almost reached the top of the plate, the plate is removed and the solvent front is marked with a pencil before allowing the solvent to evaporate.
The Rf number is used to calculate how fast the materials are moving over the plate. Rf is calculated by dividing the distance travelled by the solvent by the distance travelled by the material. Its value is always in the range of zero to one. After seeing the compound as a spot in the plate, the Rf value was computed.
The formula for calculating the Rf value is as follows
Rf value=(Distance moved by solute)/(Distance moved by solvent)
Spct1 = (2.2)/(5.4)=0.407cm.
Spct2 =(4)/(5.4) = 0.740cm.

The Rf value of compound travel based on phenolic compounds present in Black plum fruit.
UV –VIS Spectrophotometric Analysis
The Black plum aqueous extract was analyzed by UV Visible spectral analysis. The extracts were scanned with a UV-Visible spectrophotometer at wavelengths ranging from 200 to 800 nm, and the typical peaks were identified (Figure 1). The primary function and essentially identical Black plum were discovered by spectral analysis.

Figure 1 The aqueous extract of Black plum observed the wavelength of UV from 200nm to 800nm.



Phenolic compound and flavonoid content

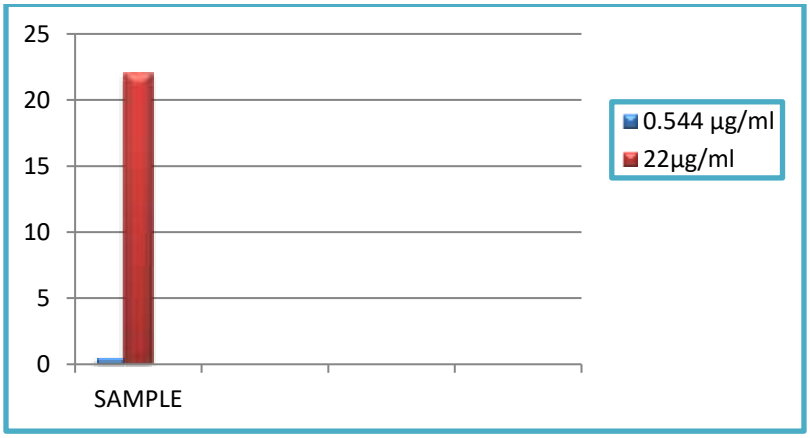
The total phenolic component and flavonoid concentration of Black plum leaf extracts are shown in Table 1. Water extract has a higher phenolic component concentration than pure ethanol and pure methanol extracts, according to previous research [10,11]. Several studies that looked into the relationship between phenolic compounds and antioxidant capacity support our findings. Antioxidant ability varies depending on the phenolic component profile, according to a recent study [12]. According to another study, there are positive relationships between phenolic component concentration and antioxidant capability [13].

Antioxidant analysis

DPPH

A DPPH forage test was used to know the antioxidant activity of the extracts. The DPPH test is frequently used to assess antioxidants' ability to forage free radicals, which are recognized to be a vital role in oxidative stress-induced cellular damage[14]. This assay is proven to provide accurate information on the antioxidant capacity of the substances being evaluated. The chemical (phenol) detected in Black plum fruit was 0.544g/ml and 22µg/ml.(Figure 2) In the result clearly illustrated that aqueous extracts of Black plum fruit exhibited rich scavenging effect on DPPH.

Figure 2 The aqueous extract of Black plum fruit increases the (phenol) compound level



Antimicrobial activity

Antimicrobial activity of aqueous extracts produced from Black plum fruit was tested against *Bacillus coagulans* and *Staphylococcus aureus* bacteria. Black plum fruit aqueous extracts at high concentrations showed antibacterial activity. Black plum aqueous extract was tested for antibacterial activity against *Bacillus coagulans* and *Staphylococcus aureus* in this study. The cup-plate method was used to determine antibacterial activity in terms of inhibition zone using four concentrations of aqueous Black plum extract, as shown in the table.

With increasing aqueous extract concentration, all bacteria show an increase in inhibitory zone diameter. The maximum zone inhibition was obtained for *Bacillus coagulans* (1mm) using Black plum aqueous extract at a concentration of 1mg/ml, followed by *Staphylococcus aureus* (0.6mm) using the same Black plum extract concentration (Table 1).

Table 1- Antimicrobial activity of aqueous extract of Black plum fruit against *Staphylococcus aureus* and *Bacillus coagulans* bacteria.

Bacteria	Inhibition zone (mm)			
	Sample 25ml	Sample 50ml	Distilled water	Disc
Staphylococcus aureus	-	0.6mm	0	0.7mm
Bacillus coagulans	-	1mm	0	0.5mm

The highest inhibitory zone was found in aqueous Black plum extract at a high concentration (1mm). The aqueous extract of Syzygium cumini has an antibacterial activity at high concentrations, according to the growth of *Bacillus coagulans* .

Based on the available reports, aqueous extract have been shown to result in high extraction yields with strong antimicrobial activities. The extract of Black plum controlled the growth of *Bacillus coagulans* bacteria and kills them. The bacteria are harmful in human body so the supplement of Black plum fruits intake our body to kill that bacteria.

Based on the outcome of the present study, the aqueous extract of Black plum fruit with moderate antimicrobial activity showed the strongest antioxidant activity. On the other hand, the extract of Black plum with the highest antimicrobial activity showed the lowest antioxidant activity.

The results of the phytochemical test show that Black plum has a variety of biologically active substances (phenol, vitamin, and ascorbic acid) that might be used as a source of herbal medicinal medications. At all concentrations, action against all test organisms was seen, with the largest inhibition zones of *S. aureus* 0.6 mm at 50 ml and *Bacillus coagulans* 1mm at 50 ml. Phytochemical components have antibacterial characteristics, according to Plant Database (2008), which were confirmed in this investigation.

Discussion

The growing recognition of free radicals as a fundamental component of the body's defense system against reactive oxygen species (ROS) highlights that oxygen-derived radicals are the most significant free radicals in biological reactive systems. Humans have developed a highly advanced and intricate antioxidant defense system to protect the body's cells and organ systems from reactive oxygen species. The system comprises various components, originating both internally and externally, that collaborate to neutralize free radicals through an interactive and synergistic approach.

The results of the current study have recorded the Antioxidant and Antimicrobial activity. The evaluation of Black plum's antioxidant and antimicrobial activities revealed that it exhibits significant antioxidant and antimicrobial properties. The findings indicate that Black plum may serve as a staple food for the prevention of certain diseases.

This study developed a novel approach for high throughput antioxidant testing utilizing a DPPH dry result. *Syzygium cumini*, commonly known as Black plum, is a prominent tropical tree cultivated for its fruit in various tropical regions. Research indicates its efficacy in addressing diarrhea, dysentery, gastroenteritis, hypertension, diabetes, dental caries, pain relief, cough, oral ulcers, and enhancing locomotor coordination and liver inflammation. The skin contains a significant amount of phytochemicals found in its fruit, which is rich in vitamins A and C, as well as iron, phosphorus, calcium, and various other minerals. The phenolic compounds found in Black plum contribute to the treatment of cancerous cells and help in delaying the

signs of skin aging. The leaves contain a variety of fungistatic and bacteriostatic substances, along with significant oxidants. The ethyl acetate extract includes quercetin, which plays a role in inhibiting thymus formation and germ infection. The medicinal Black plum fruit exhibits antimicrobial properties, especially antibacterial properties.

Plant species and their derivatives serve as the primary sources of antioxidant potential. White Black plums exhibited notable hydrophilic antioxidant activity, with phenolic compounds and vitamin C suggesting that the consumption of Black plums may be beneficial to health. The strong associations observed between hydrophilic antioxidant activity, total phenol, and vitamin C indicate that the content of total phenol or vitamin C could serve as a reliable estimate for antioxidant activity in Black plum fruit. The antioxidant activity of Black plum fruits primarily stems from phenol and vitamin C, while carotenoid contributes to a lesser extent. The investigation into the antioxidant and antibacterial properties of Black plum has yielded findings that demonstrate its significant antioxidant and antimicrobial activity. The hydrophilic antioxidant activity, identified as the most significant, exhibited strong correlations with both phenol and vitamin C. This suggests that assessing antioxidant activity levels in Black plum fruit could be effectively achieved through the measurement of total phenols or vitamin C content. Black plums featuring white flesh exhibited notable hydrophilic antioxidant activity, with phenolic compounds and vitamin C suggesting that the consumption of Black plum may be beneficial for health. The antibacterial activity of Black plum extract was found to be more pronounced against gram-positive bacteria. Black plum extract possesses antibacterial properties that may be useful in managing food-borne infections, especially those leading to diarrhea. Black plum serves as a natural antibacterial agent that may be utilized in addressing various detrimental bacterial infections.

REFERENCES

1. Jimenez-Escriq A, Rincon M, Pulido R, Saura-Calixto F. 2001.Black plum fruit (*Syzygium cumini* L.) as a new source of antioxidant dietary fiber. J Agric Food Chem. 49:5489–5493.
2. Zakaria, M. and M.A. Mohd, 1994. Traditional Malay Medicinal Plants. Fajar Bakti Sdn. Bhd., Kuala Lumpur.
3. Kolawole O.T, Makinde J.M and Olajide O.A 2007.Central nervous system depressant activity of russelia Equisetiformis Nigerian Journal of Physiological Sciences 22 (1-2): 59-63.
4. Quisumbing, E., 1978. Medicinal Plants of the Philippines. Katha Publishing, Quezon, Philippines, pp: 640-642.
5. Suzuki, I., M. Kishida, M. Okada, M. Izawa and F. Kurita et al., 2000. A clinical evaluation of the effectiveness of *Syzygium cumini* in atopic dermatitis. In The International Congress of Allergy and Clinical Immunology.
6. Nundkumar, N. and J.A. Ojewole, 2002. Studies on the antiplasmodial properties of some South African medicinal plants used as antimalarial remedies in Zulu folk medicine. Methods Find Exp. Clin. Pharmacol., 24: 397-401.
7. Masuda, T., S. Yonemori, Y. Oyama, Y. Takeda and T. Tanaka et al., 1999. Evaluation of the antioxidant activity of the environmental plants: Activity of the leaf extracts from the sea shore plants. J. Agric. Food Chem., 47: 1749-1754.
8. Ainsworth, E. A., and K. M. Gillespie. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat. Protoc.2:875–877.

9. Moreno, M. I. N., M. I. Isla, A. R. Sampietro, and M. A. Vattuone. 2000. Comparison of the free radical scavenging activity of propolis from several regions of Argentina. J. Ethnopharmacol. 71:109–114.
10. Reddy, N. S., S. Navanesan, S. K. Sinniah, N. A. Wahab, and K. S. Sim. 2012. Phenolic content, anti-oxidant effect and cytotoxic activity of Leea indica leaves. BMC Complement Altern. Med. 12:128–134.
11. Aktumsek, A., G. Zengin, G. O. Guler, Y. S. Cakmak, and A. Duran. 2013. Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic Centauea L. species. Food Chem. Toxicol. 55:290–296.
12. Kosinska, A., K. Magdalena, I. Estrella, T. Hernandez, B. Bartolome, and G. A. Dykes. 2012. Phenolic compound profiles and antioxidant capacity of Persea americana Mill. Peels and seeds of two varieties. J. Agric. Food Chem. 60:4613–4619.
13. Kim, K. M., P. Seguin, J. K. Ahn, J. J. Kim, S. C. Chun, E. H. Kim, et al. 2008. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. J. Agric. Food Chem. 56:7265–7270.
14. Krings, U., & Berger, R. G. (2001). Antioxidant activity of some roasted foods. Food Chemistry.