Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(1), 462-469 | ISSN:2251-6727

Comparative Phytochemical Profiling and Antibacterial Efficacy of *Syzygium Cumini (L.) Skeels* Extracts Against Extended-Spectrum Beta-Lactamase Producing Uropathogens

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(Received: 25 November 2025 Revised: 27 December 2025 Accepted: 11 January 2026)

KEYWORDS

Syzygium cumini
ESBL
uropathogens
phytochemical screening
antibacterial activity

ABSTRACT:

Introduction: Urinary tract infections are the one of the most common bacterial infections in the world. However, their multidrug resistance, due to the ESBL-producing uropathogens, is growing at an alarming rate. *Syzygium cumini (L.) Skeels* long used in traditional medicine, exhibits notable antibacterial activity against ESBL-producing uropathogens, highlighting its potential as a natural therapeutic option for managing multidrug-resistant urinary tract infections.

Objectives: In the present study was aimed to investigate the phytochemical composition and antibacterial activity of *Syzygium cumini L. Skeels* seed and fruit pulp extracts against clinical ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates.

Methods: The plant materials were extracted using the cold maceration method with increasing solvent polarity which included n-hexane, petroleum ether, ethyl acetate, acetone, and ethanol. The qualitative phytochemical evaluation was done using standard procedures. The antibacterial assay was done using agar well diffusion method. The bacterial strains were urinary tract pathogens which were identified as ESBL producers.

Results: The results of antibacterial activity by well-diffusion method revealed that the ethanolic and acetone extracts showed higher inhibition zones for all extracts with a maximum diameter of 29 mm from seed ethanolic extracts against *Escherichia coli* and *Klebsiella pneumonia*e. Also, low or no zones of inhibition were observed in nonpolar solvents like hexane and petroleum ether, not eliminated with bioactive polar constituents in the plant. Phytochemical analysis showed the presence of various bioactive compounds that contributes to the antibacterial activity.

Conclusions: The antibacterial activity of *Syzygium cumini L. Skeels* is a result of the presence of secondary metabolites such as flavonoids and tannins known to exhibit antimicrobial and antioxidant activity. The study concluded that the seeds of *Syzygium cumini L. Skeels* are more promisingly a source of natural anti-antibacterial compounds. Hence, our finding scientifically supports the traditional medicinal use of *Syzygium cumini L. Skeels* seed extracts, especially ethanol extracts, which could be potential alternatives against infections caused by multi drug resistant uropathogens in clinical conditions.

1. Introduction

Urinary tract infection (UTI) is the utmost common and serious ill-health condition both in the public and hospital surroundings all over the globe. It is the second most occurring infection after respiratory infection and a significant cause of morbidity worldwide affecting all

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JCHR (2026) 16(1), 462-469 | ISSN:2251-6727

age groups which necessitates the need for rapid medication (1,2). Urinary Tract infections being bacterial infections in nature have been a leading cause of life-threatening outcomes affecting 150 million patients each year with increasing morbidity and mortality among others (3).

Uropathogens are group of organisms associated with urinary tract infection, which have specific virulence factors that facilitate their invasion of the urinary tract. Bacteria, which are involved in urinary tract infections are caused by both Gram-positive and gram-negative pathogens, but most commonly by *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus* and yeast such as *Candida albicans*. *Escherichia coli* is the key etiological agent in causing urinary tract infections, which accounts for up to 90% of cases (4) and *Klebsiella pneumonia* is also another important bacterial agent in UTI infection *(*5). Finding ESBL producers in samples like urine may be crucial since it serves as an epidemiologic indicator of infection and could potentially spread the organisms to more people (6). Beta-lactamase antibiotics, the primary cause of gram-negative bacterial resistance, are the most widely used medication in the world for treating bacterial infections.

Members of the uropathogens belong to the family Enterobacteriaceae, which generate enzymes such as carbapenemase and extended-spectrum β-lactamases (ESBL), exhibit a greater degree of resistance (7). The ability of plants to biosynthesize a wide variety of secondary metabolites enhances their value as functional foods or nutraceuticals and provides a source of lead compounds for drug discovery, in addition to helping them maintain their secondary functions (8). Long ago, our ancestors also recognized this possible advantage, as demonstrated by highly developed herbal medicinal systems (9). Even though contemporary medicine already controls the majority of pharmaceuticals, side effects and antibiotic resistance are significant issues that call for more research to provide safe and efficient pharmaceuticals. Because of this, plants could be used as sources of lead compounds and alternative medications (10,11).

The tropical evergreen tree *Syzygium cumini* (L.) Skeels, sometimes called the Jamun or Indian blackberry, is indigenous to the Indian subcontinent and is a member of the Myrtaceae family. This plant's fruit is a popular dish in Southeast Asia, and it goes by a number of local names, including Jambul, Jamun, Jambolao, Java plum, and black plum (12). *Syzgium cumini L., Skeels* is a plant that has long been utilized for therapeutic purposes. The bark, leaves, seeds, and fruit of the plant, for instance, have all been used to cure different illnesses (13). The known antibacterial properties of medicinal plant extracts have gained interest because of their known antimicrobial nature. It has been demonstrated that the seeds of *Syzygium cumini L.Skeels* have antibacterial properties in addition to their antimicrobial characteristics. The extracts have the potential to yield natural antibacterial medicines because they have inhibitory actions against a variety of diseases (14). *Syzygium cumini* fruits are known to have high levels of vitamins, minerals, and fiber. The purple fruits are as a result of the presence of anthocyanins and the tannins that confer the astringent taste in the fruit (15). Although these seeds have not been fully utilized, there is currently growing interest in them being used as they are believed to have healing properties. The seeds also have numerous bioactive components such as tannins, flavonoids, and phenolics which are generally anti-bacterial (16). The bioactive elements could potentially inhibit the development of some bacteria, fungi, and viruses and hence, be an efficient anti-microbial agent. *Syzygium cumini* seeds have enormous medicinal applications because of the synergistic action of phytochemicals present in them (17). A major public health concern is the emergence of antibiotic resistance in the treatment of UTIs, especially in developing nations. while the highest rates of lack of education, poverty, and unsanitary behaviour are typical for developing states, which, more so, use counterfeit and falsified drugs in high prevalence (18). The aim of this study is therefore to evaluate the antimicrobial effects of *Syzygium cumini* (L.) Skeels extracts on some multidrug resistant extended spectrum beta lactamase producing uropathogenic bacteria isolated from clinical specimens.

2. Objectives

The objective of the study was to evaluate the antibacterial efficacy of the solvent extracts of *Syzygium cumini L Skeels* fruit pulp and seed against ESBL producing bacterial strains isolated from urinary tract infections. The most active solvent extract was subjected to qualitative phytochemical analysis to determine the presence of bioactive compounds.

3. Methods

Collection and Preparation of Plant Powder

The medicinal plant parts included in this study were the seed and fruit pulp of the *Syzygium cumini* (L.) Skeels. The fruits of the medicinal tree were collected and were washed twice with double distilled water and then surface sterilized using 70% ethanol. The seeds were separated from the fruits and the fruit pulp was obtained after removing the seeds. The medicinal plant parts were shade dried for 1-2 weeks and then after complete dryness was ground into a coarse powder using a mixer grinder.

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Preparation of plant extraction

Cold maceration method was used to obtain extracts from plant samples (19). Plum and seed of *Syzygium cumini* were dried and powdered. About 30 grams of each powdered samples were weighed and extracted using 90mL of Ethanol, Hexane, Acetone, Petroleum ether and Ethyl acetate. The mixed samples were stored in an air tight container and incubated for about 48 hours at room temperature. After incubation, the suspension present in the samples was filtered into sterile conical flasks.

Phytochemical screening of *Syzygium cumini samples*

Phytochemical screening was carried out to identify the active components in the dried seed and fruit pulp extracts of *S. cumini* powder derived using various solvents. The qualitative analysis of secondary metabolites encompassed alkaloids, flavonoids, steroids, terpenoids, tannins, carbohydrates, glycosides, phenolic compounds, amino acids, proteins and saponins, employing standardised methodologies (20)

Test for Alkaloids

Approximately 2-3mL extract was taken, and few drops of Wagner's reagent was added and reddish-brown precipitate is formed to indicate the presence of alkaloids.

Test for Flavonoids

Add two millilitres extract was added to a pinch of magnesium turnings then 1–2drops of concentrated hydrochloric acid were added. Pink color is formed to indicate the presence of flavonoids.

Test for Steroids

Approximately 0.1mL of the crude plant extracts was taken in a test tube and dissolved with chloroform 1mL, then added by the sides equal volume of concentrated sulphuric acid to the test tubes. The upper layer in the test tube turns into reddish brown color, which is steroid ring positive.

Test for terpenoids

Then 2 ml of the chloroform was mixed in the extract of the selected plant sample 2mL, and 1 ml of sulfuric acid was added in the sample extract chose. The formation of reddish-brown color is used to indicate the presence of terpenoids in the selected plants.

Test for Carbohydrates

Five millilitres of Fehling's solution was added to 2mLof extract, and it was boiled in water bath. The yellow or red precipitate is formed indicates the presence of reducing power. Test for Phenolic compounds

Test for Phenolic compounds

One drop of neutral ferric chloride 5% was added to the 2 ml of test solution in alcohol. The development of intense blue colour indicates the presence of phenolic compounds. Test for tannins

Test for tannins

Ten milligram (2-3mL) of extract, 0.5 ml of 1% lead acetate solution, and a precipitate is formed. It indicates the presence of tannins and phenolic compounds in extract. Test for glycosides

Test for glycosides

Two millilitres of extract 2 ml glacial acetic acid. One drop of 5% FeCl3 from the side of the test tube. A reddish- brown ring appear at the junction of the two liquid layers indicated the presence of cardiac glycosides. Test for Amino acids and proteins

Test for Amino acids and proteins

One drop of 1 % ninhydrin in alcohol was added to 2 mL of test solution. Blue or violet colour development indicates the presence of amino acids in the extract. Test for Saponins

Test for Saponins

Two millilitres of test solution, 2 ml of water, shaken well, and the formation of foamy lather. The presence of saponins is indicated.

Test Strains used for the study

The test strains used for the study were extended spectrum beta lactamase producing uropathogenic bacteria *E.coli* and *Klebsiella* pneumoniae isolated from urine.

Anti-bacterial Activity of different solvent extracts of *Syzygium cumini* (Seed and Pulp) well diffusion method

A well diffusion assay was used to examine the antibacterial properties of seed solvent and fruit pulp solvent extracts from *Syzygium cumini L.* against the test organisms. The test organisms used in this study are *E.coli* 1, *E.coli* 2, *E.coli* 3, *E.coli* 4, *E.coli* 5 and *Klebsiella pneumoniae strain* 2. The strains were sub cultured and grown in nutrient broth. Log phase cultures of test organisms in nutrient broth were seeded by spread-plate method on Mueller-Hinton agar. Ampicillin was used as a positive control and Dimethyl sulfoxide (DMSO) was used as a negative control. Approximately wells of uniform size were made with a cork-borer onto the plates inoculated with test organisms. Crude plant extracts of 25µl, 50µl and 100µl were respectively added

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into the well aseptically and were incubated at 37°C for 24 hours.

4 Results

Phytochemical screening of seed and fruit pulp of *Syzygium cumini* samples

Syzyzium cumini seed and fruit pulp extracts displayed remarkably different compound profiles during phytochemical screening due to the solvent-dependent variation and underscored the paramount role of solvent polarity in extraction efficiency. The solvent polarity dictates the phytochemicals' class solubility whereby it affects the yield and diversity of the extracted constituents. In the present study, polar solvents such as ethanol extracted a broad range of phytochemicals whereas non-polar solvents like hexane and petroleum ether demonstrated selectivity behaviour to specific types of compounds.

Hexane was notably more efficient in flavonoids extraction more than ethanol and petroleum ether (Table 1a, 1b). Although flavonoids are more soluble in solvents of medium polarity, the high extraction in hexane could be attributed to non-polar substituents or conjugated systems in some flavonoid derivatives in *S. cumini (*21). Flavonoids are commonly known for their antioxidant activity because they can scavenge reactive oxygen radicals and prevent both infection and degeneration-related diseases. The high flavonoids content in *S. cumini* extracts is, therefore, evidence that the plant could be utilized for its natural antioxidant capability (22). Terpenoids were consistently found in the seed extracts in all of the solvents, which also indicates that they are highly distributed and extracted regardless of their polarity. Terpenoids are pharmacologically active phytochemicals that exhibit multiple roles, including antioxidants, anti-inflammatory, antimicrobial, anticancer, and antiallergic effects. Their presence correlates with the various reports on *S. cumini* which identifies it as the valuable medicinal plant with diverse pharmacological uses (23).

Anti-bacterial Activity of *Syzygium cumini* Extract

The antimicrobial activity of *Syzygium cumini* seed and fruit pulp extracts against different *Escherichia coli* strains and bacterial isolate *Klebsiella pneumoniae* strain 2 demonstrated notable variation depending on both the solvent used for extraction and the concentration of the extract applied. The differences observed in the zone of inhibition indicate that solvent polarity plays a major role in determining the nature and quantity of bioactive compounds extracted from *S. cumini* (Table 2). In the antibacterial efficacy of the seed solvent extracts against the uropathogens, the ethanolic solvent extract of the seed showed maximum activity of around 29 mm for both the *E.coli* 3 and *E.coli* 5 at 100 µL, followed by the acetone solvent extract the highest inhibition was at 23 mm. And the moderate zone of inhibition was observed in ethyl acetate solvent extract

Similarly, acetone extracts showed strong inhibition, particularly against *E. coli* 4 and *E. coli* 5, further supporting that medium-polar solvents are efficient in extracting both polar and moderately non-polar phytochemicals such as tannins, saponins, and alkaloids. Petroleum ether solvent extract of the seed showed less activity and hexane seed solvent extract showed no antibacterial activity. The least activity was observed at a concentration of 25µl/ml and the maximum activity was observed at a concentration of 100 µl/ml of the extract. Among the bacterial isolates, *E. coli* 3 and *E. coli* 5 were the most susceptible, while *E. coli* 2 and *E. coli* 4 showed comparatively moderate sensitivity. The variability among strains may be related to differences in cell wall composition, efflux mechanisms, or inherent resistance profiles.

Anti-bacterial Activity of *Syzygium cumini* fruit pulp extract

In the antibacterial efficacy of the pulp solvent extracts against the uropathogens, the ethanolic solvent extract of the pulp showed maximum activity for *E.coli* 1, *E.coli* 3 and *Klebsiella pneumoniae strain* 2 at a range of 24 mm, followed by the acetone solvent extract and ethyl acetate solvent extract. Petroleum ether and hexane solvent extract of the pulp showed no antibacterial activity (Table 3). On comparing to, solvent seed extract the antibacterial activity was less in fruit pulp. The least activity was observed at a concentration of 25µl/ml and the maximum activity was observed at a concentration of 100 µl/ml of the extract. Overall, the results confirm that *S. cumini* seeds and fruit pulps possess potent antibacterial compounds, particularly in ethanol and acetone extracts. These findings align with previous studies reporting that polyphenols, flavonoids, and tannins present in *S. cumini* exhibit strong antimicrobial activity through mechanisms such as disruption of bacterial membranes, enzyme inhibition, and interference with nucleic acid synthesis (24).

Thus, the ethanol and acetone extracts of *S. cumini* may help to recognize specific bioactive compounds responsible for antibacterial activity, thereby having the potential to develop natural sources of antimicrobial agents. The various phytochemical compounds identified have beneficial significance in medicinal sciences. The flavonoids have been called nature's biological response modifier, because of their ability to modify the body's reaction to allergies and virus and they exhibited their

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anti-allergic, anti-inflammatory, antimicrobial, and anti-cancer activities. The antibacterial effectivity of jamun or *Syzygium cumini L. Skeels* is because of bioactive phytoconstituents such as tannins and flavonoids (25).

Phytochemical compound	Ethanol	Acetone	Hexane	Ethyl Acetate	Petroleum Ether
Alkaloids	++	++	++	++	++
Flavanoids	+	_	++	_	+
Steroids	++	++	++	_	++
Terpenoids	++	++	++	++	++
Carbohydrates	+	++	_	+	+
Phenols	++	++	_	_	_
Tannins	++	++	++	+	++
Cardiac glycosides	++	++	++	+	+
Amino acids	_	_	_	_	_
Saponins	+	++	++	++	++

Table (1a): Phytochemical screening test of *Syzygium cumini seed* extracts

Phytochemical compound	Ethanol	Acetone	Hexane	Ethyl acetate	Petroleum ether
Alkaloids	++	++	++	++	++
Flavanoids	++	++	_	_	++
Steroids	++	++	++	++	+
Terpenoids	++	++	++	++	++
Carbohydrates	++	++	_	+	_
Phenols	++	_	_	_	_
Tannins	++	++	++	++	++
Cardiac glycosides	+	++	++	++	++
Amino acids	_	_	_	_	_
Saponins	++	++	++	++	++

Table 1(b): Phytochemical screening test of *Syzygium cumini fruit* pulp extracts

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Table 2: Anti-bacterial Activity of *Syzygium cumini L.* Seed Solvent Extracts SSolvextract

Test organisms	Zone of inhibition (in mm)											
	Ethanol			Ethyl acetate			Acetone			Hexane & Petroleum Ether		
	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl	25 µl	50 µl	100 µl
***E.coli* 1**	16.5 ± 0.408	21.5 ± 1.08	**24 ± 0.816**	-	11 ± 0.408	13.3 ± 0.235	11.5 ± 0.408	13.5 ± 0.408	15.16 ± 0.623	-	-	-
***E.coli* 2**	15 ± 0.667	19.6 ± 0.471	22.3 ± 0.623	-	10.1 ± 0.235	10.8 ± 0.235	11.1 ± 0.235	12.83±0.235	15.5 ± 0.408	-	-	-
***E.coli* 3**	17.6 ± 0.840	20.5 ± 1.08	**24.5 ± 0.408**	-	11 ± 0.408	13 ± 0.408	10.5 ± 0.408	12.6 ± 0.623	15.83 ± 0.623	-	-	-
***E.coli* 4**	17 ± 0.408	19.3 ±0.471	20.5 ± 0.408	-	10.5 ± 0.408	12.1 ± 0.235	10.8 ± 0.849	12.5 ± 0.408	14.3 ± 0.623	-	-	-
***E.coli* 5**	17.8 ± 0.235	21.3 ± 0.471	23.5 ± 0.408	-	11 ± 0.408	12 ± 0.408	11± 0.408	13 ±0.408	14.5 ± 0.408	-	-	-
***Klebsiella pneumoniae strain* (K2)**	14.8 ± 0.623	19 ± 0.861	**24.1 ± 0.235**	-	11.1 ± 0.623	13 ± 0408	11.5 ± 0.408	14 ± 0.816	16.5 ± 0.408	-	-	-

Table 3: Anti-bacterial Activity of *Syzygium cumini L.* fruit pulp Solvent Extracts

Test organisms	Zone of inhibition (in mm)												
	Ethanol			Ethyl acetate			Acetone			Petroleum Ether			Hexane
	25µl	50 µl	100 µl	25µl	50 µl	100 µl	25µl	50 µl	100 µl	25µl	50 µl	100 µl	25-100 µl
***E.coli* 1**	11.5 ± 0.408	17.1 ± 0.623	20.6 ± 0.471	-	13.5 ± 0.408	15 ± 0.408	16.5 ± 0.408	21 ± 0.408	**23.1 ± 0.471**	-	10.2 ± 0.235	11.3 ± 0.235	-
***E.coli* 2**	10.6 ± 0.235	13.5 ± 0.408	21 ± 0.408	10.5 ± 0.408	13.1 ± 0.235	14.5 ± 0.408	15.3 ± 0.471	18.5 ± 0.408	21 ± 0.408	-	10.2 ± 0.235	11.3 ± 0.235	-

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***E.coli* 3**	16 ± 0.816	20.5 ± 1.08	**29.1 ± 0.849**	11.3 ± 0.471	13.6 ± 0.471	15.8 ± 0.623	16 ± 0.816	18.8 ± 0.623	21 ± 0.408	10.3 ± 0.25	11.5 ± 0.408	13.5 ± 0.408	-
***E.coli* 4**	12.5 ± 0.408	16.5 ± 0.408	19.5 ± 0.408	12.3 ± 0.235	14.5 ± 0.408	16.5 ± 0.408	16.3 ± 0.623	19.8 ± 0.235	22 ± 0.408	-	-	-	-
***E.coli* 5**	15.1 ± 0.623	19 ± 0.816	**29.1 ± 0.849**	12 ± 0.816	14.5 ± 0.408	17.1 6 ± 0.255	16± 0.408	19 ± 0.408	22 ± 0.408	-	-	11.5 ± 0.408	-
Klebsiella pneumonaie strain 2	12.1 ± 0.623	15 ± 0.816	22 ± 0.408	12.5 ± 0.408	14.8 ± 0.235	17.1 ± 0.235	18± 0.408	20.5 ± 0.408	**23.5 ± 0.408**	-	11.1 ± 0.235	12.1 ± 0.849	-

5 Discussion

Urinary tract bacterial infection is the major issue to a community, which seeks medical attention (26). The rapid increase of resistance to broad spectrum β-lactams among uropathogens has recently a threat to the society globally (27). The serious increase in the prevalence of ESBL's worldwide creates a need for effective and easy to perform screening methods for detection. In the development health care system, the exploration of new drugs sharing better and expanded therapeutic actions from medicinal plants exhibiting ethnobotanical use is progressively valuable.

The current investigation explained the wide variety of phytochemicals and antibacterial activities against ESBL-producing uropathogens in *Syzygium cumini* seeds. This revealed that the extracts of seeds and fruit pulp differential solvent extracts represent their active biological properties. The extractions of ethanol and acetone are universal solvents expliant diverse phytocompounds such as flavonoids, tannins, terpenoids, phenols known their antimicrobial and antioxidant properties. Ethanolic seed extract showed significant antibacterial activity, especially against *E.coli* and *Klebsiella pneumoniae*, which explained the edible therapeutic efficacy for urological corruption. In contrast, all non-polar solvents like Petroleum Ether and hexane revealed moderate antibacterial activities while they underlined polarity playing that imparts on the extraction of biological components. The complete absence of amino acids in all explants indicates the antibacterial effects are primarily accounting for secondary metabolites and not primary metabolism products. The study concluded that the seeds of *S.cumini* are more promisingly as a source of natural anti-antibacterial extracts. However, more studies need to isolate, evaluated their pharmacological mechanisms for active principles and phytochemicals or other investigations and continue with their physiological regulator; thus, it indicated a promising solution for ESBLs -resistant.

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