International Journal of Scientific Engineering and Research (IJSER)
ISSN (Online): 2347-3878
Index Copernicus Value (2015): 62.86 | Impact Factor (2015): 3.791

Phytochemical Screening and Anti-Bacterial Activity of Methanol and Hexane Extract of *Syzygium Cumini* Leaves

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Abstract: ***Phytochemical screening of syzygium cumini leaves was carried out and showed the presence of tannins, steroids, alkaloids, flavonoids and terpenes in both the methanol and hexane extract while saponins and glycosides were detected only in the methanol extract. The percentage yield of the methanol and hexane extract of S.cumini leaves were found to be 16.2% and 9.2% respectively. Invitro anti-bacterial activity of the methanol and hexane extract of S.cumini leaves were carried out using agar well diffusion method with clinical isolates of Escherichia coli and staphylococcus aureus. The methanol extract was observed to be more effective against both the E.coli and staphylococcus aureus with mean zones of inhibition of 21mm and 20mm at 200mg/ml respectively and 17mm at 200mg/ml for E.coli and S.aureus respectively in the hexane extract. This indicates that methanol extract is more potent than the hexane extract. Therefore the leaf extract of syzygium cumini can be used against enteric bacterial infections.***

Keywords: *syzygium cumini*, *staphylococcus aureus Escherichia coli*, zone of inhibition, agar well diffusion method

1. Introduction

The plant *syzygium cumini* is an evergreen tropical plant. It belongs to the family Myrtaceae. The synonyms of *syzygium cumini* are *syzygium jambolanum* and *Eugenia cumini* (Timbola et al 2002). It is native to West Africa, India, Burma ,Ceylon, and Indonesia. Leaves are elliptic-oblong, flowers are greenish white, fruits as 1.5-4cm long, violent black on ripened. Most of the parts of plant like bark leave seeds and fruits are used as alternative medicines to treat a variety of diverse diseases (Teixeira et al, 2006).

Figure 1: Flower buds of *S. cumini*

Figure 2: Leaves of *S. cumini*

It is a therapeutic plant and it contains a number of antibacterial, anti-inflammatory, antioxidant activities which are pharmacologically proved. (Mohamed et al 2013). In earlier days, all parts of the plant viz. fruits, leaves, dried seeds and bark were used in ayurvedic medicines for the treatment of several disorders (Subramanian and Nair, 2002). The fruits have been used for a broad variety of ailments, including cough, diabetes, dysentery, inflammation etc (Kirtikar and Basu, 2005).

The fruits of *Syzygium cumini* are not only used for medicinal purposes but also used in numerous food products (Natarajan and Paulsen, 2000). These fruits are fit for human consumption and they contain various components like gallic acid, tannins, vitamin C, anthocyanins etc. (Shyamala and Vasantha, 2010).. The pulp of the fruit is also used in making jams, jellies and puddings.. The leaves of the *Syzygium cumini* yield essential oils which are used in soaps and perfumes

(Sharma and Seshadri, 2003). The leaves which have an aroma similar to turpentine, are pinkish when young, changing to a leathery, glossy dark green with a yellow midrib as they mature. The leaves are used as food for livestock, as they have good nutritional value (Muruganandan *et al.,* 2001). Various extracts of fruit and seeds of *Syzygium cumini* have antidiabetic, anti-inflammatory, hepato-protective, antihyperlipidemic, diuretic and antibacterial activities. The seed extract was found to be promoting endogenous release of insulin (Goyal *et al.,* 2010). These properties of *Syzygium cumini* seed have been accredited to its saponins, tannins and Flavonoids (Chetri *et al.,* 2005). In another study, *Syzygium cumini* extract was found tohave anti-tumor and anti-oxidative potential against chemical induced stomach carcinogenesis (Teixeira *et al.,* 2006).

Despite the existence of potent antibiotic and antifungal agents, resistant or multi- resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Fahey, 2003).It is therefore very necessary that the search for newer antibiotic sources be a continuous process. Plants are the cheapest and safest alternative sources of anti-microbial agents (Pretorius, 2001).

2. Materials and Method

Fresh leaves of *sygygium cumini* were collected from kinkinau by fadama close Kaduna. It was identified at the herbarium of the biological sciences department of Ahmadu Bello University, Zaria with voucher number 1953.

Sample Preparations

The leaves were air dried for two weeks it pounded into a coarse powder using clean mortar and pestle. The coarse powder was weighed, labeled and kept for further analysis

Extract preparation

The extracts (methanol and n-hexane) were prepared using cold maceration. In this process 150g coarsely powdered leaves was dissolved in 100ml of solvents (methanol or n hexane). The mixture was allowed to stand at room temperature for the period of at least 3 days with frequents agitation. The mixture was then filtered using whatman filter paper. The filtrates as then heated under a water bath to evaporate the solvents so as to obtain the crude oil extracts (Naibe et al 2008).

Phytochemical Screening

Phytochemical analysis for qualitative detection of alkaloids, tannins, saponins, flavonoids, glycosides, steroids and terpenes was performed on the *Syzygium cumini* leaf (Trease and Evans, 1978).

Agar Preparation

Nutrients agar (28g) based medium was dissolved in 1000 $cm^3$of distilled water, brought to boiling and sterilized. Allowed to cool to 45°c before pouring.

Collection of Clinical Isolates of Bacteria

Clinical isolates of *Escherichia coli* and *Staphylococcus aureus* were collected from the Shehu Kangiwa Hospital Kaduna Polytechnic in agar slants. These were sub-cultured into nutrient agar media and incubated at 37^OC for 24 hours. The organisms were then stored until needed. Series of tests such as gram staining, indole test, methyl red test, citrate utilization test, motility test, oxidase test and coagulase tests were carried out on the basis of the bacteria biochemical properties and enzymatic reactions in the presence of some specific substrates.

Antibacterial Activity Test

The agar well diffusion method was used to determine the antibacterial activity of the methanol and hexane extracts of *Syzygium cumini*. 20ml of nutrient agar was pour in sterile plates after which it was allowed to solidify and then 1ml of broth culture of each microorganism isolates was spread all over the surface of the plates using a spreader. A standard cork-borer of 6mm in diameter was used to cut wells on the surface of the agar and then filled with 0.1ml of the methanol and hexane extracts of *Syzygium cumini* at various concentrations with the aid of a sterile syringe. All plates were allowed to stand for 1 hour at room temperature for proper diffusion of extract after which the plates were incubated at 37^OC for 24 hours and they were observed for zones of inhibition. A zone of clearance around each well signifies inhibition and the diameter of the clear zones were measured in millimeter using a ruler.

3. Result

The leaves of S.cumini in methanol extract appears dark green in colour with powdery texture while in Hexane extract it appears green with slightly gummy texture with percentage yield of 16.2% and 9.2% respectively (table 3.1).

3.1 Physical Characteristics and Percentage Yield of *Syzygium cumini* leaf extracts

Characteristics	Methanol extract	Hexane Extract
Colour	Dark green	Green
Texture	Powder	Slightly gummy
Initial weight	50g	50g
Final weight	8.1g	4.6g
Percentage yield	16.2%	9.2%

3.2 Phytochemical Screening of Methanol and Hexane Extract of *S. cumini.*

The Phytochemical constituents of methanol and hexane extracts of *Syzygium cumini* leaf. Is shown in table Tannins, steroids, alkaloids, flavonoids and terpenes were all present in both extracts but saponins and glycosides were only present in the methanol extract.

Table 3.2: Phytochemical constituents of the methanol and n-hexane extracts of *Syzygium cumini* leaf

Phytochemical constituents	Methanol extract	Hexane extract
Saponnins	+	-
Tannins	+	+
Steroids	+	+
Flavonoids	+	+
Cardiac glycosides	+	-
Alkaloids	+	+
Terpenes	+	+

Key: + =present, - = absent

Cultural and Biochemical identification of Clinical Isolates

Table 3.3 shows the Cultural and Biochemical Identification of the clinical isolates of *Escherichia coli* and *Staphylococcus aureus.* The test organisms showed positivity to methyl red and catalase and negativity to oxidase. In the coagulase test, *E. coli* was negative while *Staphylococcus aureus* was positive.

Table 3.3: Cultural and biochemical identification of clinical isolates *E. coli S.aureus.*

Bacteria	Colony morphology	Gram reaction	Cell morphology	Indole test	Methyl red	Motility test	Coagulase test	Citrate utilization	Catalase test	Oxidase test
E. coli	Greenish metallic sheen	Negative	Rod- shaped	+	+	+	-	+	+	-
S. aureus	Abundant opaque golden growth	Positive	Cocci	-	+	-	+	-	+	-

4. Discussion

The methanol extract of *S. cumini* (16.2%) had a higher yield than the hexane extract (9.2%). The difference in the yield may be due to the difference in polarity of the two solvents. Methanol is polar while n-hexane is non polar.

The results of Phytochemical screening of methanol and hexane extract of S. cumini leaves concur with the findings of Mahmud *et al.* (2014) where saponins and glycosides were present in polar solvents extracts such as water and ethanol but absent in non-polar solvents extracts such as hexane and chloroform The clinical isolates of bacteria were confirmed as *E. coli* and *Staphylococcus aureus* in (Table 3.3) with *E. coli* being gram negative while *S. aureus* was gram positive.

The antimicrobial activity of the methanol and hexane extracts of *S. cumini* leaves against *E. coli* and *Staphylococcus aureus* showed varying degrees of antimicrobial activity with the exception of the hexane extract at 25mg/ml where resistance was observed. The results in table 3.3 and showed that the most active extract was the methanol extract at 100mg/ml showed significant activity against *Staphylococcus aureus* with 20mm as mean zone of inhibition. These results are in agreement with the observations of Dorman and Deans (2000) where 25mm and 26mm were observed as mean zone of inhibition for the methanol extract of *S. cumini* leaves.

The result of the hexane extract, however, is in contrast to the report of Al-Bakri and Afifi (2007) where much higher activity against enteric bacteria such as *E. coli*, *S. typhi* and *S. aureus* were observed.

These results may be attributed to the actions of the phytochemicals that the extracts contained. Saponins show antibacterial activity by binding to adhesins produced by the bacteria to stick to target cells or tissue, form complexes with cell walls of bacteria or inactivate enzymes secreted by pathogens. Steroids exhibit antibacterial activity by enhancing intestinal absorption of sodium ion and water as observed by Cowan (1999). Tannins, like saponins, show antibacterial and antidiarrheal activity by binding to adhesins produce by the bacteria, enzyme inhibition, substrate deprivation, complex with cell membrane of the pathogen. In some cases, they cause membrane disruption of the bacteria thereby killing it (Cowan, 1999).

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