**Full Length Research Paper**

**Preliminary Phytochemical, Antimicrobial and Acute Toxicity Studies of the Stem, bark and the Leaves of a cultivated *Syzygium cumini* Linn. (Family: Myrtaceae) in Nigeria.**

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The phytochemical, antimicrobial and acute toxicity studies of the leaf and stem bark extracts of *Syzygium cumini* Linn. grown in Abuja, Nigeria was carried out. The phytochemical screening revealed the presence of carbohydrates, saponins, tannins, terpenes, volatile oil, sterols, resins balsam, phlobatanins and flavonoids in the leaves and stem bark of the species studied. Alkaloids and anthraquinones were absent in all the plant parts studied. 70% methanol extracts were used for antimicrobial and acute toxicity studies. The six test organisms used for antimicrobial studies were: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Bacillus subtilis* and *Salmonella typhi*. The oil from the leaves showed activity on all the test organisms; the leaf extract showed activity on all the test organisms except *P. aeruginosa* while the stem bark extract showed no activity on any of the test organisms used. The oil had a saponification value of 363 and an acid value of 4.21. The lethal dose 50% (LD₅₀) in mice was found to be >5000 mg/kg for the stem bark and at 3,873 mg/kg for the leaf extracts.

**Key words:** Phytochemical, antimicrobial, acute toxicity, *Syzygium cumini*, Nigeria.

**INTRODUCTION**

Drug chemotherapy still remains one of the major curative options worldwide. Currently, attention is being given to the use of herbal medicinal products and therapy for the treatment of most ailments whether physiological disorder or bacterial, viral or of parasitic origin. Screening of plants extracts for antibacterial, viral or parasitic properties are one of the basic steps in identifying target drugs after extraction.

The World Health Organization (WHO) reported that about 80% of the world’s population depends mainly on traditional medicine. Traditional treatment involves mainly the use of plant extracts. Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases, this practice being an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (Sofowora, 1989). Unfortunately, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. The rationale for the utilization of these remedies rested largely on long term clinical experience. But now, with the upsurge in the use of herbal medicine, a thorough scientific investigation of the plants will go a long way in validating their folkloric usage (Sofowora, 1989).

*Syzygium cumini* Linn. belongs to the family, *Myrtaceae*. The species is an evergreen tropical tree, native to India, Pakistan and Indonesia. The tree was introduced to Florida and USA in 1911 by the United States Department
of Agriculture (USDA) and is also now commonly planted in the tropics including Nigeria. Although, a tropical tree, it grows easily in subtropical climates. *S. cumini* Linn is a fast growing species; it can reach a height of 30 m and can live for 100 years. Its dense foliage provides shade and is grown just for ornamental value. The flowers are fragrant and small, about 5 mm in diameter, the fruits develop in May/June and are oblong, ovoid, starts green and turns pink to shining crimson black as it matures. The fruit has a combination of sweet, mildly sour and astringent flavour and tend to colour the tongue purple. The fruit juice is said to be carminative and a soothing stomachic. The sap from the leaves may be similarly used. The plant has analgesic uses in Madagascar for neuralgias, inflammations and sprain (Burkill, 1997) (Figure 1).

All parts of plant can be used medicinally and it has a long tradition in alternative medicine. In India, the bark extract is used for anaemia, the bark and seeds are used for diabetics which reduce blood sugar level quickly, the fruits are used for dysentery and the leaves extract for gingivitis (bleeding gum) (Burkill, 1997). In the Philippines and Suriname, wine is made from fermented ripe fruits (www.tropilab.com). A decoction of the chopped bark and an infusion of the powdered leaves are also used for diabetes and for high blood pressure. The leaves are used for diarrhea and fever; the fruits are used for diarrhea and abdominal pains and the seeds for diabetes and high blood pressure. Bark, leaves and flowers are used to treat diabetes (www.tropilab.com).

The leaves are said to be antibacterial and are used for strengthening the teeth and gums. The bark is astringent, sweet sour, diuretic, digestive and anthelmintic (www.search.yahoo.com). The bark and leaves are used for diarrhea.

Acute toxicity is the toxicity produced by a substance when administered in one or more doses during a period not exceeding 24 h. Acute toxicity studies in animals are usually necessary for any medicinal substance intended for human use. The information that is obtained from this study will be useful in choosing doses for repeat-dose studies. Acute toxicity may also aid in the selection of starting doses for phase1 studies and provide information relevant to acute overdosing in humans (Guide for industry, 1996). Synonyms of the plant include *Syzygium jambolanum* (Lam) DC, *Eugenia cumini* (L) Druce and *Eugenia jambolana* Lam. Common names include java plum, jambal, jaman, jamun, black plum and Indian black berry (Burkill, 1997).

The aim of the present study is to carry out a preliminary phytochemical, antimicrobial and acute toxicity studies of the stem, bark and the leaves of *S. cumini* Linn. (Family: Myrtaceae) found in Nigeria.

**MATERIALS AND METHODS**

**Preparation of plant material**

The plant materials (stem bark and leaves) were collected from Kado Estate, Abuja and air dried for 30 days. A voucher specimen was deposited and given the herbarium number (NIPRDH/P/6175). The plant parts were then pounded using a wooden mortar and pestle to increase the surface area for better extraction.

**Phytochemical screening**

The standard method of Odebiyi and Sofowora (1990) and Fadeyi et al. (1989) were adopted for the phytochemical screening. 70% Methanol extracts of the leaves and stem bark were used for the antimicrobial screening and the acute toxicity studies.

**Antimicrobial studies**

The freeze dried 70% methanol extract was screened for antimicrobial activity against clinical isolates (Table 2) from NIPRD clinic using Agar Dilution streak technique (Mitscher et al, 1972). The test organisms were prepared by incubating them in freshly prepared nutrient broth at 37°C for 3hrs. (having approximately 1.25 x 106 – 1.25 x 107). 64mg of the crude extracts were dissolved in 1ml Dimethyl Sulphur Oxide (DMSO) and made up to 2ml with sterile distilled water to give a concentration of 32mg/ml of extract. 1ml of the prepared extract was introduced into 15ml of molten nutrient agar placed in water at 56oC. This was mixed properly and poured into sterile petri dishes to give a final concentration of 2mg/ml. The dish was then allowed to gel and thereafter, the test organisms were inoculated by streaking onto the nutrient agar using a wire loop meant to deliver 0.002ul containing approximately 2.5x103-2.5x104 colony forming units (cfu). The organisms were also streaked on dishes containing only agar (Organism Viability Control) and dishes containing nutrient agar and DMSO, which also served as controls. The petri dishes were then incubated over night at 37oC. They were then observed for microbial growth inhibition. Both water and DMSO showed no inhibiting effect on the organisms. The two controls were used in all the tests Viz. control 1 had only the agar and control 2 had agar and DMSO. The controls were set up to show that the agar supports growth and also that the DMSO does not inhibit the growth of the test organisms.

**Acute toxicity studies**

Methanol extract (70%) was used for the acute toxicity studies. The model of Lorke, (1983) and Dietrich (1983) were used to estimate...
Table 1. Phytochemical analysis of the leaves and stem bark of the Nigerian S. cumini.

<table>
<thead>
<tr>
<th>Test</th>
<th>Leaves</th>
<th>Stem bark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatanins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Balsams</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Volatile oil</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Present, - = absent.

Table 2. Antimicrobial screening of the oil, leaf and stem bark of S. cumini.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Ps</th>
<th>Ca</th>
<th>Ec</th>
<th>Sa</th>
<th>St</th>
<th>Bs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Stem bark</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oil</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = Activity/no growth, - = no activity/growth, PS = Pseudomonas aeruginosa, Ca = Candida albicans, Ec = Escherichia coli, Sa = Staphylococcus aureus, St = Salmonella typhi and Bs = Bacillus subtilis.

The acute toxicity profile. Nine mice were divided into three groups of three each for the phase I study. Three dose levels of 10 mg/kg, 100 mg/kg and 1000 mg/kg were administered orally to group 1, 2, and 3 respectively. Saline water was used as control for both the phase I and phase II studies. In the phase II study, doses of 4 sets of doses corresponding to the outcome of phase I study was adopted from the table provided by Lorke (1983) and given to 4 groups of one mouse and the LD$_{50}$ was estimated according to Lorke's model:

LD$_{50} = \sqrt{a} \times \sqrt{b}$

Where, a = Lowest dose that is lethal; b = highest dose that is safe.

Acute toxicity studies

70% methanol extract was used for the acute toxicity study and the leaf extract produced a lower LD$_{50}$ (3,873 mg/kg) compared to the stem bark (> 5000 mg/kg). This observation may infer the presence of more potent compounds in the leaf as compared to the stem bark (Table 3).

Physicochemical analyses of the oil from the leaves of S. cumini

The acid value of 4.21 is lower than that of olive oil (6.6). Acid values indicate the amount of fatty acid in the oil and is the number of milligrams of potassium hydroxide required to neutralize the free acid present. A saponification value of 363 was recorded (Table 4). The saponification value is the number of potassium hydroxide required to completely saponify 1 g of the oil.

DISCUSSION

The phytochemical investigations revealed the presence of carbohydrates, saponins, tannins, terpenes, volatile oil, sterols, resins, balsam, phlobatanins and flavonoids and absence of alkaloids and anthraquinone in the leaves and stem bark of the species studied. This shows that the plant parts contain many secondary metabolites. The presence of such metabolites suggests that the plant might be of great importance in phytomedicine. For instance, the presence of flavonoids and resins might be responsible for the use of this plant as analgesic in Madagascar for neuralgias, inflammations and sprain...
The methanol extracts of the leaf and stem bark were screened for antimicrobial activity. The leaf extract had activity on *C. albicans*, *E. coli*, *S. aureus*, *S. typhii* and *B. subtilis* while the oil from the leaves had activity on all the test organisms used. The stem bark had no activity on any of the organisms used. The acute toxicity study of the leaf extract produced a lower LD<sub>50</sub> (3.873 mg/kg) compared to the stem bark (> 5000 mg/kg). This observation may infer the presence of more potent compounds in the leaves as compared to the stem bark. The beneficial traditionally useful components however, can only be located through bio-guided assay of the two plant parts, using efficacy studies. The findings in this study show that the leaf extract is indeed more potent than the stem bark. This suggests that the lethal principle present in the leaf extract may not be present or may be present in small proportion in the stem bark.

In conclusion, the presence of a variety of secondary metabolites, moderate acute toxicity values and activity of the extracts on most of the micro organisms used suggests that the plant can be exploited for phytomedicine development.

**ACKNOWLEDGEMENTS**

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