African Journal of Biotechnology Vol. 9(41), pp. 6943-6747, 11 October, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.1205
ISSN 1684–5315 © 2010 Academic Journals

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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Accepted 13 April, 2010

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 auriginosa, Escherichia coli, Staphylococcus aureus, Candida albicans, Bacillus subtilis and Salmonella typhii.* The oil from the leaves showed activity on all the test organisms; the leaf extract showed activity on all test organisms except *P. auriginosa* 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% (LD_{50}) 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

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Figure 1. *Syzygium cumini* Linn. plant.

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 37oC 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

Table1. Phytochemical analysis of the leaves and stem bark of the Nigerian *S. cumini*.

Test	Leaves	Stem bark
Carbohydrates	+	+
Saponin	+	+
Alkaloids	-	-
Terpenes	+	+
Sterols	+	+
Phlobatanins	-	+
Resins	+	+
Balsams	+	+
Flavonoids	+	+
Phenols	+	+
Anthraquinone	-	-
Tannins	+	+
Volatile oil	+	-

+ = Present, - = absent.

the acute toxicity profile. Nine mice were divided into three groups of three each for the phase I study. Three dose levels of 10mg/kg, 100mg/kg and 1000mg/kg were administered orally to group 1, 2, and 3respectively. Saline water was used as control for both the phase I and the phase II studies. In the phase II study, doses of 4 sets of doses corresponding to the outcome of phase 1 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.

Oil extraction

The oil was extracted from dried leaves of *S. cumini* by hydro/steam distillation method that is, using water as the solvent. Acid and saponification values of the oil were determined using the standard methods of Rendle (1993).

RESULTS

Phytochemical screening

The phytochemical investigation revealed the presence of carbohydrates, saponins, tannins, terpenes, volatile oil, sterols, resins, balsam, phlobatanins and flavonoids and absence of alkaloids and anthraquinone in the leaf and stem bark of the species studied (Table 1).

Antimicrobial studies

The 70% methanol extracts of the leaf and stem bark were screened for antimicrobial activity. The leaf extracts were found to have activity on *C. albicans, E. coli, S. aureus, S. typhii* and *B. subtilis,* the oil from the leaves had activity on all the test organisms while the stem bark extract had no activity on any of the organisms used (Table 2). There were growths in all the controls used showing that the agar supports growth of the organisms and also that the DMSO does not inhibit growth of the organisms as well.

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

Plant part	Ps	Ca	Ec	Sa	St	Bs
Leaf	-	+	+	+	+	+
Stem bark	-	-	-	-	-	-
Oil	+	+	+	+	+	+

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

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 barks 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

Table 3. Acute toxicity studies of the leaves and stem bark of *S. cumini*.

Plant part	LD_{50} value
Leaf	3,873 mg/kg
Stem bark	> 5000 mg/kg

Table 4. Saponification and acid value of the oil from the leaves of *S. cumini*.

Acid value	Saponification value
4.21	363

(Burkill, 1997).

The presence of tannins and saponins in plant extracts suggest that it has anti-bacterial activity. Isaac and Chinwe (2001) reported that alkaloids, tannins and saponins are responsible for anti-bacterial activity in *Fetracarpidium coropheoum.* Onoruvioe and Olorunfemi (1998) also attributed the anti-bacterial effect of the root extract of *Dichrostachys cinerea* to alkaloids, saponins and flavoniods. The presence of tannins could also mean that it is an astringent, helping in wound healing and anti-parasitic which is in line with its folkloric use for gingivitis (bleeding gum). It is also reported that the presence of phenols shows anti-oxidative, antibacterial and anti-viral effects (Yihang et al., 2007; Shuyunshi, 2007).

The presence of terpenes shows that it is anti-tumor and has anti-viral activity. Presence of volatile oil in the leaves suggests it can be used in natural body cosmetic and scents. This is even more important since the oil had activity on all the test organisms. This shows that the oil can be used topically to cure skin infections. The presence of terpenes shows that it is anti-tumor and has anti-viral activity. The presence of saponin could also mean that the oil could be used in soap making.

The oil from the leaves had a saponification value of 363 and an acid value of 4.21. The acid value of 4.21 is lower than that of olive oil (6.6). This is the amount of fatty acid in the oil and is the number of milligrams of potassium hydroxide required to neutralize the free acid present. The value indicates that a lower amount of potassium hydroxide will be required to neutralize the free acid present in the species studied than that of olive oil. A saponification value of 363 was recorded. This is the amount of potassium hydroxide required to completely saponify 1 g of the oil. This value is on the high side (higher than that of coconut oil: 246 - 260) and the oil will be good for soap making. The saponification value gives information on the character of the fatty acid of the oil and in particular concerning the solubility of their soap in water. The higher the saponification value of an oil free from moisture and unsaponifiable matter, the more soluble the soap that can be made from it (Lewkowitsch, 1922; Carl et al., 2007). This oil also has activity on all test organisms which shows that the oil is highly anti-microbial and can be used to treat infections.

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_{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 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

We would like to acknowledge the DG NIPRD Dr. U.S. Inyang, for providing the enabling environment for this work, Prof. J.I. Okogun, Dr. (Mrs.) J. Giwa Amu for proof-reading the manuscript, Mr. and Mrs. Okaro, O for allowing us to collect the plant material from their house and all those who have contributed to the success of this work and above all, the Almighty God for keeping all of us.

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