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Antimicrobial evaluation of water and methanolic extracts of *Syzygium cumini* stem bark

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Abstract
*Syzygium cumini*stem bark was collected from near by area in Bijapur district, Karnataka in the month of January 2014. It was processed and subjected for soxhlet extraction using methanol followed by distilled water. Phytochemical screening of crude methanolic extract has showed the positive tests for Phenols, Glycosides, Saponins and Steroids. The water extract contains Phenols, Glycosides, Saponins and Steroids. Both the extracts were tested for their antibacterial activity using two gram positive and two gram negative organisms and two fungal species at different concentrations. The results were compared with a standard drug. Both the extracts have exhibited encouraging results.

Keywords: antimicrobial, *Syzygium cumini*, phytochemical

Introduction

Since the dawn of civilization, Man utilized plants for their medicinal and edible value. By trial and error, Man distinguished between the beneficial and poisonous plants. Man also observed that in large quantities medicinal and edible plants may be poisonous, and learned about the usefulness of plants by observing animals. Sick animals utilize certain plants that they usually ignore. Today, this method is used by scientists to isolate active compounds from medicinal plants.

Herbalism is thought to have started some 60,000 years ago, where pollen grains of several medicinal plants such as marshmallow (*Althaea*), yarrow (*Achillea*), ephedra and muscari were documented at burial sites at Shanidar in Iraq. This confirms the use of medicinal plants by the Neanderthal Man.

The earliest written historical information dates back to 2500 B.C. when Sumarian ideograms described the use of medicinal plants such as the poppy as the "the plant of joy" 1728 to 1686 B.C. in the Code of Hammurabi, the King of Babylon. Plants mentioned include mint, henbane, senna and licorice. It is impossible to determine at what point in time mankind first discovered the medicinal use of specific plants.

With time, more documents were written or drawn and by the sixteenth century B.C. the earliest written records of practices were produced by the Egyptians, who were greatly esteemed in the ancient Mediterranean world. Medicinal plants such as fennel, castor oil, opium, thyme, linseed, aloe and myrrh, were mentioned. Some of the early uses of medicinal plants are still valid today [1, 2]. The first documented healer by name was Imhotep. He was so famous that after his death his stature was elevated to that of a god.

Many plants with medicinal virtues are termed *officinalis*. The Latin name denotes that the plant is medicinally useful. This term dates back to the early Christian period, when monasteries were utilised as centres for the gathering and writing of information and usage of medicinal herbs.

After the first millennium after Christ, several botanists and herbalists wrote on the usage of medicinal plants. Authors include Hildegarde, Albertus Magnus, Valerius Cordus, Theophrastus, Pier Andrea Mattioli, William Turner Carolus Clusius, Nicholas Culpeper and Friedrich Hoffmann extending from 1098 to 1791. Later the isolation of chemical substances from plants was commenced by Caventou and Pelletier who isolated alkaloids such as caffeine, while Geiger and Hess isolated atropine and other alkaloids dating up to 1850. Later scientists from the mid-nineteenth century to date isolated most of the chemical constituents that we know of. Some of them are still in use in their natural form, while others are produced more efficiently by chemical synthesis in industry.

Introduction to present plant: (*Syzygium cumini*)

Scientific classification

Kingdom	:	Plantae
Order	:	Myrtales
Family	:	Myrtaceae
Genus	:	syzygium
Species	:	*S. cumini*

The genus *Syzygium* is one of the genera of the myrtle family Myrtaceae which is native to the tropics particularly to tropical America and Australia. It has a worldwide, although highly uneven, distribution in tropical and subtropical regions [4]. The genus comprises about 1 100 species, and has a native range that extends from Africa and Madagascar through southern Asia east through the Pacific. Its highest levels of diversity occur from Malaysia to northeastern Australia, where many species are very poorly known and many more have not been described taxonomically. Plants of this family are known to be rich in volatile oils which are reported for their uses in medicine and many fruits of the family have a rich history of uses both as edibles and as traditional

medicines in divergent ethno botanical practices throughout the tropical and subtropical world. Some of the edible species of *Syzygium*are planted throughout the tropics worldwide.

Part used - Stem bark

The stem bark is rich in betulinic acid, friedelin, epi-friedelanol, β-sitosterol, eugenin and fatty acid ester of epi-friedelanol, β-sitosterol, quercetin kaempferol, myricetin and, gallic acid and ellagic acid, bergenins, flavonoids and tannins. The presence of gallo- and ellagi-tannins may be responsible for the astringent property of stem bark. Stem bark contains pentacyclic triterpenoid betulinic acid (m.p. 306-310ºC). Betulinic acid is a naturally occurring triterpenoid, which has demonstrated selective cytotoxicity against a number of specific tumor and active against a verity of infectious agent like HIV malaria immunomodulatory and the inflammatory. A plant sterol B-sitosterol is found in almost all part of plant [4, 5]. It has same chemical structure with cholesterol. It has much beneficial pharmacological activity like anti-inflammatory and lowering blood cholesterol. Friedelin ($C_{30}H_{50}O$, m.p. 256-260ºC) is also a pentacyclic triterpenoid found in plant. Plant bark also contains substance which is an ester of epi-friedelanol ($C_{30}H_{51}OH$) with a fatty acid ($C_{27}H_{55}COOH$) [6]. It also contains tannins (10-12%) gallic acid, ellagic acid and resin myricetin are also reported

Materials and Method

Collection of plant materials

Fresh bark of selected herbs, *Syzgium Cumini*, were collected from campus of Karnataka State Women's University, Bijapur in the month of February, 2014 and the specimen was authenticated by several literature surveys. The bark were washed, cleaned and chopped into pieces and dried at 40^0 C in thermostatically controlled oven until they attained a constant weight. The samples were then crushed into powder, using mechanical grinding machine, so as to enhance effective contact of solvent with sites on the plant materials.

Hot continuous extraction (Soxhlet)

Finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber Esiphon into flask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated.

Phytochemical Screening

The phytochemical screening of crude extract of the present plant has been done using chemical methods and it has revealed the presence of various class of chemical compounds. The details are summarized in the table. No.1

Antimicrobial Activity

The antimicrobial activity of these extracts was studied comparatively using Streptomycin as standard drug following cup-plate method [7]. The microorganisms used were *Escherichia coli and S. aureus*

The medium used for the test was prepared by dissolving bacteriological peptone (6 grams), pancreatic digest of casein (4 grams), yeast extract (3 grams), beef extract (1.5 grams), dextrose (1 gram) and agar (15 grams) in distilled water to produce 1 liter of medium. The pH was adjusted to 6.5-6.6 using 1M sodium hydroxide and 1M hydrochloric acid. It was sterilized for 30 minutes at 15 lbs o pressure.

Nutrient broth was prepared by dissolving bacteriological peptone (6 grams), pancreatic digest of casein (4 grams), yeast extract (3 grams), beef extract (1.5 grams) and dextrose (1 gram) in distilled water to produce one liter of nutrient broth [8]. The pH was adjusted to 6.2 and sterilized by autoclaving. The test solutions both standard and extracts were prepared as follow.

Streptomycin: 10 mg of streptomycin was dissolved in 100 ml of water to get a final concentration of 10 μg/0.1ml

Test samples: 10 gram of extract was dissolved in 10 ml of DMSO to get a final concentration of 1000 μg/0.1 ml.

The organisms used in the present study were obtained from the laboratory stock on the day of testing. The organisms were subcultured into sterile nutrient broth. After incubating the same for three hours it was used as inoculum for the test.

Previously liquefied medium was inoculated with the appropriate quantity of broth of microorganisms between 40-45ºC and inoculated medium was poured into sterile Petri-dishes to give a depth of 3-4 mm. ensured that the layers of medium were uniform in thickness by placing them on a leveled surface. With the help of a suitable sterile cork borer a cup of 6 mm diameter was scooped out off the set agar in each Petri-dish. Using sterile pipettes the standard and test solution (0.1 ml) were fed into the bored cups. The fed dishes were incubated for 24 hours at 37ºC. The zone of inhibition developed was measured and recorded. Each zone of inhibition recorded is an average of six measurements. And are given in the table No. 2

Results and Analysis

In the present study, the phytochemical screening and antibacterial activities were performed with methanol and aqueous extracts of the stem bark of *S. cumini*. The study was made against bacteria using the standard cup plate method[6]. The stem bark of *S. cumini* were rich in flavonoids, glycosides, steroids, phenols, tannins and saponins. These Phytochemical confer antimicrobial activity on the stem bark extracts. The antimicrobial activity results of the plant material under investigation are encouraging. The activity increases with increase in the concentration of test solution.

Table 1: Phytochemical screening results

Phytochemical Constituent	Methanol	Aqueous
Phenols	++	+++
Saponins	+	+
Flavonoids	+++	+++
Steroids	+++	+
Alkaloids	++	+++
Glycosides	++	++

+ = present, ++ = moderately present, +++ =Appreciable amount

Table 2: Results of antibacterial activity.

Test Sample	Concentration (µg/ml)	Zone of inhibition (in mm)	
		E. Coli	***S. aureus***
Sample-1	500	09	09
Sample-2	1000	11	10
Sample-3	1500	13	13
Sample-4	2000	15	14
Streptomycin	100	15	15
D. Water	--	06	06

Cup diameter= 6 mm Quantity of drug solution and control fed into each cup = 5µl

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