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Research Article

Surveillance of *Invitro* Antioxidant and Anthelmintic Activity of Methanolic Extract of *Syzygium Cumini* Bark (*Myrtaceae*)

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Abstract

Introduction: Herbal medicines have been used for treating various diseases from ancient times. Even in the era of advanced modern medicine, natural sources of antioxidants and pharmaceutical compounds are of great importance, many of such rich sources are still unearthed.
Materials and methods: Methanolic extract of *Jamun* (*Syzygium cumini*) tree bark was screened for *in vitro*-antioxidant activity. Antioxidant studies includes DPPH scavenging activity, reducing capacity assessment, reducing effect, scavenging of hydrogen peroxide and nitrous oxide scavenging activity against ascorbic acid (ASA) standard.
Results: Preliminary phytochemical screening results showed *S. cumini* was positive for flavonoids, saponins, tannins and terpenoids. The phenolic content was 480 mg % of gallic acid equivalents, tannin 1.9 mg % of gallic acid equivalents, tannin was 250 mg % of catechine equivalents on ferric chloride estimation and flavon content 66.17 µg/ml was considerable. Anthelmintic property of methanolic and aqueous extract of *S. cumini* bark showed promising activity against control.
Discussion: *S. cumini* could be a promising agent in antioxidant and anthelmintic research and could lead in development of new drug molecule. To the best of our knowledge this is the first report on the antioxidant and anthelmintic activity of *S. cumini* bark.
Key Words: Antioxidant, anthelmintic, methanolic extract, *Syzygium cumini*.

1.Introduction

Plants have provided mankind with herbal remedies for several diseases for many centuries. In India herbal medicines have been the bases of treatment and cure for various diseases in traditional methods such as *Ayurveda*, *Unani* and *Sidha*. *Syzygium cumini* Linn (syn. *Eugenia jambolana*) commonly known as a "*Jamun*" (family *Myrtaceae*), is fast growing ever green tropical tree, native to India, Pakistan and Indonesia. In India, *S. cumini* bark extract is used for treatment of anaemia, bark and seeds are used for diabetes which reduces blood sugar level quickly[1,2]. Fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea, and ring worm infections. Leaves have been extensively used to treat many diseases like diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy, strangury and dermopathy[3] and to inhibit hematochezia[4]. It possesses acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin, quercetin, kaempferol and myricetin have been reported to possess antioxidant and free radical scavenging activities[5] and α- amylase inhibitor activity of seeds[6], in addition, pharmacological evaluation of this plant concerning their antidiabetic, hypolipidemic, antioxidant, anti HIV, anti-bacterial, anti-inflammatory, antipyretic and radioprotective and neuropsychological activity[7]. It has been proved that *S. cumini* bark extract has a potent anti-

inflammatory action against different phases of inflammation without any side effect on gastric mucosa[8]. Number of research is being carried out worldwide directed towards finding natural antioxidants of natural origin. Modern synthetic medicines are very effective in curing diseases but also cause a number of side effects. Crude drugs are less efficient with respect to cure of diseases but are relatively free from side effects[9].

2. Materials and Methods

The stem bark was collected, identified and authenticated by Dr. Madhavachetty; Assistant professor, Department of Botany, Sri Venkateswara University, Tirupathi, and voucher specimen (No: JCP/2010/153) was deposited in the herbarium of the same department.

Fully mature stem bark of *S. cumini* was collected from *Mellacheruvu* village, *Chittoor* district, *Andhra Pradesh*, India, was air dried at room temperature (RT- 27±2 °C) for 30 days and powdered using auto mix blender; the powder was stored in deep freezer at -40 °C. For further analysis 500 g of dry fine powder was suspended in 1.5 litres of methanol and stirred magnetically for 24 hrs at RT. Extract was double filtered using musline cloth and Watmann no. 1 filter paper. Filtrate was concentrated to dryness under reduced pressure at 40 °C using rotary vacuum evaporator (Buchi labortech AG, Switzerland). Percentage of yield was 17.9 and the dried methanol extract of *S. cumini* bark (MESCB) was stored in vacuum desiccators under controlled conditions for further analysis.

***2.1. Preliminary phytochemical screening*:** MESCB was dissolved in solvent to obtain a stock of concentration 1 % (w/v). The standard methodology of Harborne and Kokate[10,11] were adopted for screening. Alakloid was assessed using dragondroff's reagent; an orange/red precipitate produced immediately indicated the presence of alkaloids. To confirm the presence of amino acids, sample was treated with few drops of Ninhydrin reagent, boiled; appearance of purple colour indicates presence of amino acids. Presence of carbohydrate was confirmed by addition of molisch's reagent and H_2SO_4. Appearance of purple colour ring between the junctions of two liquids indicates the presence of carbohydrates.

For tannin extract and 1 % lead acetate were mixed, yellow precipitate confirms the presence of tannins. Appearance of yellow colour on addition of NaOH indicates presence of flavonoids. For saponin, extract was diluted and agitated in a graduated cylinder. Formation of layer of foam showed the presence of saponins. Presence of terpenoids was confirmed by the reddish violet colour in presence of chloroform, acetic anhydride and conc. H_2SO_4. For phytosterols, extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place, mixture was diluted and extracted with ether and following evaporation residue was tested for presence of phytosterol using acetic anhydride and conc. H_2SO_4. Appearance of bluish green colour shows positivity. For testing anthraquinones extract was hydrolyzed with conc. H_2SO_4 extracted with benzene, dilute ammonia was added, rose pink coloration suggested the positive response. For steroids, extract was dissolved in chloroform and conc. H_2SO_4, yellow with green fluorescence indicates presence of steroids.

***2.2. DPPH radical scavenging activity*:** 0.004 % of DPPH solution was added to varying concentrations of MESCB (100-1000 µg), read the OD absorbance at 517 nm after incubation (95 % methanol as blank and ASA as standard). Percentage inhibition activity was calculated by using following formula, absorbance of control-absorbance of extract/ absorbance of control ×100 .[12]

2.3. Determination of reducing capacity assessment (FRAP): FRAP reagent was added to 150 µl MESCB and OD reading at 593 nm has taken after incubation against ferrous sulphate standard[13].

2.4. Determination of reducing effect: Effect of reducing activity has done by the method of Oyaizu[14]. Absorbance was read at 700 nm (DW and phosphate buffer as blank). Higher absorbance indicates greater reducing capacity which is calculated using formula, OD of reaction mixture/ OD of blank - 1 × 100.

***2.5. Determination of scavenging of H_2O_2*:** H_2O_2 scavenging activity was carried out according to Ruch *et al*[15]; ASA was used as standard.

***2.6. Evaluation of NO scavenging activity*:** Done as prescribed previously by Green *et al*[16] and Marcocci *et al*[17]. Absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dichloride was read at 546 nm (methanol and sodium nitroprusside in phosphate saline as blank, and ASA as standard.

2.7. Quantitative determination of total phenolic content (TPC): TPC was estimated as per the method of Singleton and Rossi[18]. TPC was determined by the formula OD of test/OD of standard × concentration of standard × 100 against catechol standard.

2.8. Estimation of tannins: Tannin was estimated as per Oyaizu *et al*[14]. Tannin content of the sample was calculated by

formula OD of test/ OD of standard × 100 × 100 and expressed as tannic acid equivalent against tannic acid standard.

2.9. Tannin analysis with ferric chloride: Analysis was carried out as per Hagerman *et al*[19]. Catechin was used as standard; tannin content was expressed in mg/ g (catechin equivalent).

2.10. Estimation of flavones and flavonols: Flavones and flavonols estimated as per Woisky *et al*[20]. Total content of flavones and flavonols in plant extract was calculated in quercitine equivalent against quercitine standard.

***2.11. Anthelmintic activity*:** Anthelmintic activity was evaluated as per Patel *et al*[21] on Indian earth worm (*Pheretima posthuma*), procured from Veterinary Sciences and Animal Sciences University, Tamilnadu (TANUVAS). Sample was prepared by two solvent extracts, i) Methanol extract of *S. cumini* bark– MESCB, ii) Aqueous extract of *S. cumini* bark– AESCB, the stock concentration of 100 mg/ ml was the stock. Different working dilutions were prepared to 4 final concentrations of 25, 50, 75 and 100 mg/ ml. 20 mg/ ml Albendazole (Glaxo Smith Kline) was used as standard. Four group of earth worms consisting of six earth worms (I- Control group in 1 % gum acacia in saline; II- Albendazole; III- a) 25, b) 50 c) 75 and d) 100 mg/ml of MESCB; IV- a) 25, b) 50 c) 75 and d) 100 mg/ml of AESCB) into each group were released in 50 ml of sample with desired concentration in petri plates. Time taken for paralysis (min) and death of the individual worms were noted when no movement could be observed, except when the worm was shaken vigorously. Worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). Paralysis assumed to occur as they do not revive even in saline solution. Potency was inversely proportional to time taken for paralysis and/or death of parasite.

3. Results

On preliminary phytochemical screening, it was observed that flavanoids, saponins, tannins and terpenoids were present in the MESCB whereas, alkaloids, amino acids, anthraquinones, glycosides, phytosterols, steroids were absent**.** In DPPH radical scavenging activity, concentration of sample at which the inhibition percentage reaches 50 % is its IC50 value. IC50 values are negatively related to the antioxidant activity, as its express the amount of antioxidant needed to decrease its radical concentration by 50 %. Lower IC50 value represents the higher antioxidant activity of the test sample (**Table 1**). **Fig. 1** shows the dose response curve of DPPH radical scavenging activity of the MESCB compared with ASA standard. The reducing ability of extract was in the range of 810 µg Fe^{2+}/ gm. **Fig. 2** illustrates a significant decrease in the NO radical due to the scavenging ability of extracts and ASA. MESCB showed maximum activity of 50 % respectively at 1000 µg/ ml whereas ASA was 54.08 % at the same concentration of the IC 50 values were found to be 1000 µg/ ml and 900 µg/ ml for ASA. The given results were diverted to MESCB is having no scavenging activity but less in ASA. The reducing power of extract was found to be remarkable which increasing gradually with a rise in the concentration (**Fig. 3**). Best hydroxyl radical scavenging activity was shown by MESCB*,* at 2 mg/ ml in the range of 65.22 % (ASA showed 94.2 % of H_2O_2 inhibition). IC50 value MESCB was 42.03 % at concentration of 1.2 mg/ ml, at same concentration of the ASA was achieved 52.17 % **(Fig. 4).**

TPC present in the sample was 480 mg % of gallic acid equivalents and tannin content in MESCB was 1.9 mg/ g of gallic acid equivalent against standard. When tannins analysed with ferric chloride indicates 250 mg % of catechin equivalents. Estimation of flavones and flavonoids shows the dose response curve of MESCB compared with standard quercetin. It was observed that MESCB is having 66.17 µg/ ml of the flavones and total content of flavones was found to be 33.08 µg/ mg of quercitine equivalents.

MESCB and AESCB produced dose dependant paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death. MESCB and its different concentrations exhibited anthelminitc activity in dose depend manner giving a paralysis time 36.58 min and death time 70.58 min respectively with the concentration of 100 mg/ ml and AESCB gives 76.25 min as paralysis time and 80.33 min for death at same concentration (**Table 2**).

Table 1: **Percentage of scavenging of H_2O_2 and NO**

H_2O_2				NO			
Test	**MESCB (IC %)**	**Control**	**ASA (IC %)**	**Test**	**MESCB (IC %)**	**Control**	**ASA (IC %)**
T1	13.04	C1	20.29	T1	6.66	C1	9.09
T2	23.19	C2	31.88	T2	15.78	C2	17.39
T3	42.03	C3	52.17	T3	40.32	C3	48.39
T4	65.22	C4	73.19	T4	46.83	T4	48.39
T5	86.96	C5	94.20	T5	50.00	C5	54.08

Fig. 1, 2, 3: Percentage of scavenging of H_2O_2 and NO

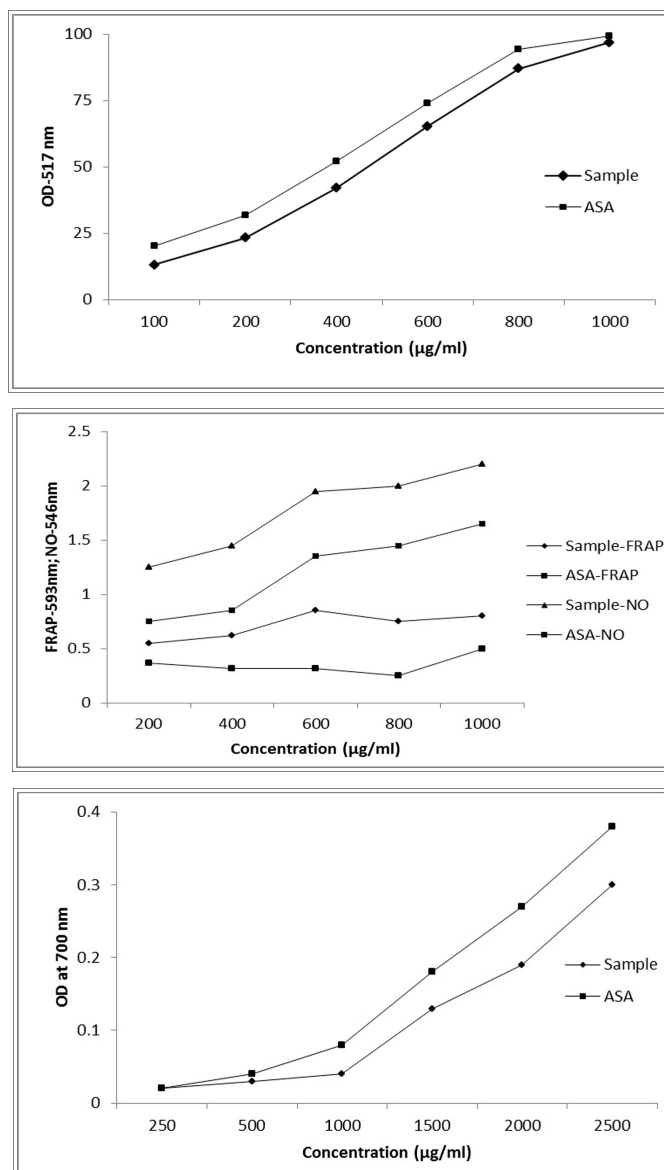
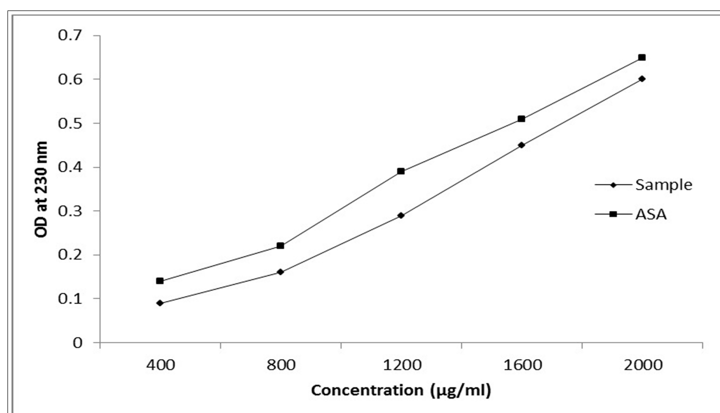


Table 2: **Anthelmintic activity**

Group	Sample	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
I	Gum acacia in saline	1 %	Nil	Nil
II	Albendazole	20	12.16±1.83	13.5±1.37
III	MESCB	25	72.75±0.81	72.75±0.81
		50	57.5±2.28	76.08±1.13
		75	44.58±5.73	70.33±5.63
		100	36.5±5.53	100.91±4.81
IV	AESCB	25	91.66±1.43	88.5±3.42
		50	88.5±1.84	88.5±3.42
		75	80.66±1.43	88.5±3.42
		100	76.25±0.34	80.33±3.69

Fig. 4: Anthelmintic activity



4. Discussions

Antioxidant properties of *S. cumini* leaves earlier studied by Ruan *et al*[4]. Phenolic and poly phenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages[22] and phenolic compounds are an antioxidant agent which acts as free radical terminators[23]. DPPH free radical is a stable one, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants[24]. The degree of discoloration indicates the scavenging potential of antioxidant compound of extracts in terms of H_2 donating ability. Experimental observations showed that MESCB had lower activity than that of ASA. The scavenging activity of standard ASA reached 98 % at a concentration of 1 mg/ ml, while MESCB was showing 78.94 % at same concentration. Though DPPH assay radical scavenging abilities of the extracts were less than those of ASA, this study shows the extracts have proton donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary anti-oxidants. Antioxidant potential of the MESCB estimated from the ability to reduce TPTZ- Fe^{3+} complex to TPTZ-Fe^{2+}. FRAP values for the MESCB were significantly lower than that of ASA 980 µg Fe^{2+}/ g. Antioxidant activity is increased proportionally to the polyphenol content. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species[25].

As in **Fig. 3**, Fe^{3+} transformed to Fe^{2+} in presence of extract and the reference compound ASA to measure the reducing capability at 0.25mg/ml and the absorbance of the plant extract and ASA was same (0.02). While at 2.5 mg/ ml, absorbance of both sample and ASA was 0.35 and 0.38, indicates that MESCB also acting as potent reducing agent. Hydroxyl radical is extremely reactive free radical formed in biological systems and had been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecules found in living cells[26]. The most reactive free radical is the hydroxyl radical, known to initiate lipid peroxidation and cause fragmentation of DNA and to mutation[27]. NO is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is diffusible free radical, plays many roles as an effector molecules diverse biological system including neuronal messenger, vasodilatation, antimicrobial and anti-tumour activities[28] suppression of released NO may be partially attributed to direct NO scavenging, as MESCB decreased the amount of nitrite generated from the decomposition of SNP *in vitro*. Scavenging of NO by the extracts was increased in dose dependant manner. Results obtained in the present study revealed that the level of tannin in the MESCB were considerable. The tannins have antioxidant activity and it is likely that the activity of extract is due to these compounds. This activity may be mainly due to their redox properties, which play an important role in absorbing and neutralizing free radical.

Parasitic diseases cause severe morbidity of population in the endemic areas in worldwide[29]. Majority of infections are due to helminthics are generally restricted to tropical regions and cause enormous hazards to the health and contribute to the prevalence of under nourishment, anaemia, eosinophilia and pneumonia. Parasitic disease cause ruthless, morbidity affecting population in endemic areas, caused by round worm, hook worm, thread worm, tape worm and filarial, guinea worm in intestine results in complication like blood loss in stool, injury to organs, intestinal, lymphatic obstruction[30]. Tannins possess antiparasitic activity and anthelmintic activity of tannin can bind to free protein in GIT of host animal or glycoprotein on the cuticle of the parasites and can cause death[31]. Intestinal infections with worms can be more easily treated than those infections that occur in other locations in the body because the worms need to be killed by the drug and the drug need not be absorbed when given by oral route. However, increasing problems of development of resistance in helminths against anthelminthics have led to the proposal of screening medicinal plants for their anthelmintic activity[32]. A large number of medicinal plants are utilized by ethnic groups worldwide. To the best of

our knowledge, there is no scientific data available on the MESCB has employed for the study of antioxidant and anthelmintic effect.

In the present study we found that MESCB is showing significant antioxidant and anthelmintic activity. To the best of our knowledge this is the first research report on antioxidant and anthelmintic activity of MESCB. Results of indicates that antioxidant activity of the extract as a source for natural antioxidants and MESCB and AESCB has beneficial anthelmintic effect, indicates that *S. cumini* could be promising agent for anthelmintic effect and scavenging of free radical.

In vitro methods provide a means to rapid screening for potential anthelmintic activity and the result obtained could not be extrapolated for *in vivo* activity, therefore the results should be ascertained by *in vivo* evaluation. Exact component of *S. cumini* bark could be identified and quantified by HPLC and LCMS. We strongly believe that the outcomes of the study will trigger exciting research opportunities on addressing antioxidants and anthelmintic drug in a cost effective manner.

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