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Research Article

In vitro evaluation of phytochemical and antioxidant properties of *Syzygium cumini* leaves and their synergistic effect on its antimicrobial property

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ABSTRACT

One of the major causes of ageing and stress- induced damage to cellular metabolism is release of Reactive Oxygen Species produced during normal metabolic processes. Plants have been reported to show antioxidant property attributed to their polyphenolic content. Thus, plant derieved supplements should be incorporated in our daily diet as nutraceuticals. The methanolic extract of *Syzygium cumini* (L.) Skeels was evaluated for its phytochemical, antioxidant and antimicrobial properties. The extract showed the presence of tannins, alkaloids, flavonoids, saponins, terpenoids and glycosides. Total phenolic content, determined using gallic acid, was 88.4 ± 5.25 mg/ml while total flavonoid content was found to be 54.52±9.64 mg/ml. Quercetin was used as standard. Its antioxidant property was determined by DPPH method, using ascorbic acid as standard. The methanolic extract was taken at concentrations ranging from 100-500µg/ml. The percentage scavenging activity of the extract was found to be higher than the standard for the whole range. The antimicrobial property of the extract was determined by well-diffusion method. The extract showed potent antimicrobial activity against yeast and mold and Gram positive bacteria while the Gram negative bacteria were more resistant to the extract. The efficient antioxidant activity and antimicrobial potential suggests its use as dietary supplement to boost our immunity.

Keywords: DPPH; nutraceutical; reactive oxygen species; total flavonoid content; total phenolic content; well-diffusion method

INTRODUCTION

There are different free radical scavenging molecules like superoxide dismutase, catalase, peroxidase and glutathione present naturally in our body. Reactive Oxygen Species (ROS) viz., singlet oxygen (O^{l}), superoxide ion (O_2^-), hydroxide ion (OH^-) and hydrogen peroxide (H_2O_2) are produced during normal metabolic processes and cause oxidative damage to biomolecules like DNA and protein (Halliwell, 1997). These ROS are reportedly responsible for many grave human diseases eg cancer (Parmar et al., 2010), Alzheimer's, diabetes, Parkinson's etc (Ozgen et al., 2006). To counter the effects of ageing and stress-induced damage to cellular metabolism, it is imperative to add plant derived dietary supplements that boost our mechanism of scavenging ROS (Battacharya et al., 1997; Ilavarasan et al., 2001; Manonmani et al., 2002; Li et al., 2008).

Ancient Indian medicinal literature like *Sushrut Samhita* and *Charak Samhita* have emphasized on the therapeutic use of plant extracts. The active components of different plants possess high antioxidant properties which make them effective scavengers of ROS and potent antimicrobial agents. Different plant parts contain polyphenolic compounds like flavonoids, tannins and tocopherol which are responsible for their antioxidant properties (McCord, 2000). *Syzygium cumini* (L.) Skeels, a plant of Myrtaceae family, is a tropical evergreen tree, with its origins in India. The earlier reported work on this plant has been majorly on its fruits and seeds (Banerjee and Narendhirakannan, 2011; Borhade, 2012; Murti et al., 2012; Saha et al., 2013). These parts have been reported for their antioxidant, antiinflammatory (Muruganandan et al., 2001), neuro-psychopharmacological, antimicrobial (Bhuiyan et al., 1996; Shafi et al., 2002), anti-HIV, antileishmanial (Chandrasekaran et al., 2004; Ratnam et al., 2008), nitric oxide scavenging (Jagetia et al., 2002), free radical scavenging (Silva et al., 2006), antidiarrhoeal (Mukherjee et al., 1998), antifertility (Rajasekaran et al., 1988), gastroprotective, antiulcerogenic and radio-protective activities (Sagrawat et al., 2006). There is a wide scope to study the medicinal properties of its leaves like antimicrobial and antioxidant activity and correlate it with its secondary metabolites, which is the focus of this study.

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MATERIALS AND METHODS

Plant source

Fresh mature leaves of *S. cumini* were collected locally from Indore City, Madhya Pradesh in the month of July.

They were cleaned, air dried, deveined and pulverized into fine powder and stored in an air tight container at room temperature.

Preparation of extract

For extract preparation, 10gms of leaf powder was defatted using 250 ml petroleum ether (Boiling Point 40-60°C) followed by methanolic extraction using Soxhlet apparatus. The extract was then vacuum dried and stored at 4°C in air tight containers for further use.

1. Qualitative Phytochemical Analysis

Tannins, phenols, alkaloids, flavonoids, glycosides, saponins and steroids were analyzed qualitatively by using standard protocols (Harborne et al., 1973; Trease et al., 1989).

2. Quantitative Phytochemical Analysis

Total phenolic content was estimated spectrophotometrically using Gallic acid as standard, by Folin-Ciocalteau method modified by Singleton et al., 1999. Total flavonoid content was determined using the methodology of Jia et al., 1999 with slight modifications. Quercetin was used as standard. All the determinations were carried out in triplicates.

3. Antioxidant assay

To assay the antioxidant property, DPPH free radical scavenging activity (Chang et al., 2002) was performed. Ascorbic acid was used as standard. The stock solution of extract (1mg/ml) was diluted to final concentrations ranging from 100-500 µg/ml in methanol. To 2.5ml of each sample concentration, 1 ml of 0.3mM DPPH solution was added. Blank was made using methanol and negative control had DPPH (1ml) and methanol (2.5ml). After reaction for 30mins, the absorbance was measured at 518nm. Similar procedure was followed for the standard. All the procedures were carried out in triplicates. The percentage scavenging activity was calculated by,

% Scavenging activity = 100 --- [(Absorbance of sample --- Absorbance of blank) x 100/ Absorbance of control]

4. Antimicrobial activity

In vitro antimicrobial activity of methanolic extract of *S. cumini* leaves was determined against few Gram positive and Gram negative bacteria and fungal cultures. The standard cultures were procured from NCIM, Pune (Table No.2). They were sub-cultured on MRS medium and stored under refrigerated conditions till further use.

For testing, 24 hrs old cultures were inoculated on Nutrient Agar plates for bacterial cultures and on SDA plates for fungal cultures. The antimicrobial activity was determined by well diffusion method (Perez et al., 1990). The test cultures were lawn cultured and 100-200µl of extract was added to wells aseptically. The plates were incubated for 24 hrs at 37°C for bacterial cultures and for 48-72 hrs at 28°C for fungal cultures.

RESULTS AND DISCUSSION

1. Qualitative analysis

The phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, phenols, steroids and saponins (Table No.1). These compounds are known to have beneficial importance in medicine. Flavonoids have been reported by different workers as an antiallergic, antiinflammatory, antimicrobial and anticancerous agent (Aiyelaagbe and Osamudiamen, 2009). Yoshizawa et al., 1987 and Okuda et al., 1992 have earlier reported activities like antiviral, antibacterial and antitumor for tannins. Saponins are surface active agents and therefore allow antibody access in intracellular proteins. In medicine they have reportedly being used in hyperglycemia, hyper-cholestrolemia, weight loss etc (Gowri and Vasantha, 2010).

2. Quantitative analysis

The total phenolic content of methanolic extract of *S. cumini* leaves was 88.4±5.25 mg/gm. The results indicate that the total phenolic content was significantly high suggesting that methanolic extract of *S. cumini* leaves possess higher amounts of secondary metabolites. This further confirms that phenolic compounds are better extracted in polar solvents like methanol and ethanol as compared to aqueous extraction (Sultana et al., 2009).

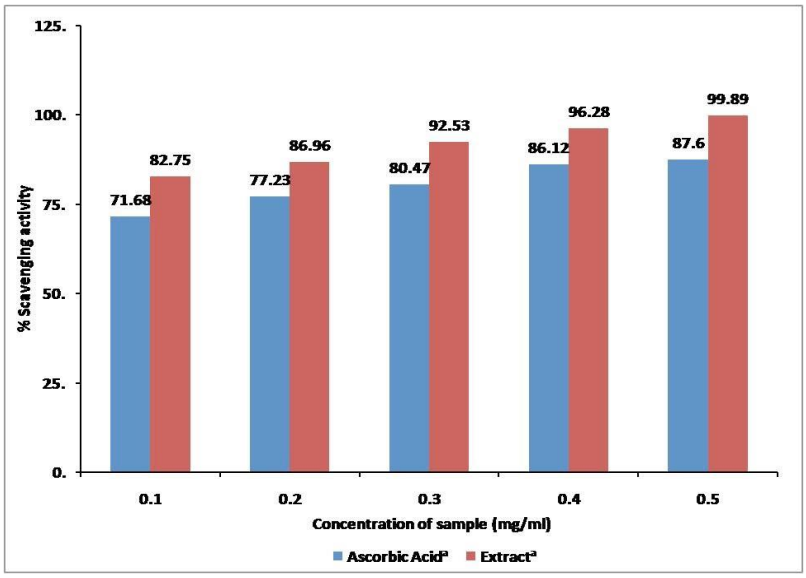
Reports obtained earlier have focused greatly on the free radical scavenging activity of the phenolics. They are produced as secondary metabolites in plants and chelate transitional metals and scavenge free radicals; thus they work as good antioxidants (Mohamed et al., 2010).

S. cumini methanolic leaf extract showed significantly high total flavonoid content of 54.52± 9.64 mg/gm. Earlier reports have emphasized the antioxidant properties of flavonoids (Zheng et al., 2011). These compounds possess certain hydroxyl functional groups which impart antioxidant effect through either chelating or scavenging mechanisms (Das and Pereria, 1990; Kessler et al., 2003). The present study confirms high flavonoid content in methanolic extract of *S. cumini* leaves, thus suggesting its high antioxidant effect. This further suggests its use in human nutrition for considerable health benefits.

The present work further corroborates with previous reports that the antioxidant potential of a plant is directly proportional to its total phenolic and flavonoid content (Mohamed et al., 2013).

3. Antioxidant activity

The different increasing concentrations of the methanolic extract of *S.cumini* leaves showed increment in the free radical scavenging activity with the increase in



*The value is mean of three determinants

Figure 1: DPPH scavenging activity of standard and methanolic extract of *S. cumini* leaves

Table 1: Phytochemical constituents of methanolic extract of *S. cumini* leaves

S.No.	Phytochemical constituents	Methanolic Extract of *S.cumini*
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponins	+
5.	Terpenoids	+
6.	Glycosides	+

Table 2: Antimicrobial activity of methanolic extract of *S. cumini* leaves

S.no.	Name of microorganisms	NCIM No.	Diameter of zone of inhibition (mm)
1.	*Staphylococcus aureus*	2079	19
2.	*Escherichia coli*	2065	17
3.	*Bacillus subtilis*	2063	16
4.	*Streptococcus faecalis*	5024	--
5.	*Candida albicans*	3471	23
6.	*Aspergillus niger*	1196	20

the extract concentration (Figure 1). There was significant decrease in the colour of DPPH free radical indicating scavenging activity of methanolic extract. The scavenging capability of the extract and ascorbic acid, used as standard, was found to be significant (P<= 0.05). These results are in sync with the high total phenolic content in the extract. The phenolics with their redox properties have the ability to chelate transitional metals, scavenge free radicals, inhibit lipooxygenase and show good antioxidant properties (Decker, 1997; Naik et al., 2003; Mohamed et al., 2013).

Apart from phenolics, the extract is rich in polar phytochemicals like flavonoids, tannins etc., which have been extracted efficiently by polar solvent, methanol in this study. Therefore the extract has shown good antioxidant activity which corroborates with the previous works of Sharma et al., 2003; Reynertson et al., 2008; Al-Reza et al., 2009; Mohamed et al., 2013.

4. Antimicrobial activity

The methanolic extract of *S. cumini* showed good antifungal and antibacterial activity *in vitro*. The results showed highest zone of inhibition against yeast and mould; *C. albicans* was comparatively more sensitive than *A. niger*. Good sensitivity was observed for *S. aureus* followed by *E. coli* and *B. subtilis*. *S. faecalis* was found to be resistant to the extract ((Table No.2). This suggests that the extract is significantly effective against Gram positive bacteria than Gram negative bacteria. This antimicrobial property can be attributed to the synergistic effect of high total phenolic and total flavonoid content of the extract, which are reportedly good antioxidants (Gowri and Vasantha 2010; Mohamed et al., 2013).

CONCLUSION

The current study has shown that the total phenolic content and total flavonoid content of the methanolic

extract of *S. cumini* leaves was very high suggesting it to be a good antioxidant. This high polyphenolic content is also responsible for its potent antifungal and antibacterial activity. Therefore it is recommended that this extract should be incorporated in daily diet as a nutraceutical supplement.

CONFLICT OF INTEREST

No conflict of interest lies between Authors

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