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RESEARCH ARTICLE

In-vitro Antioxidant activity of Methanolic Extract of *Syzygium cumini* Linn. Bark

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ABSTRACT

The present research was subjected to screen *invitro* antioxidant activity of methanolic extract of *Syzygium cumini* bark. Preliminary Phytochemical investigation was carried out on the methanolic extract of *Syzygium cumini* bark. It indices presence of Carbohydrates, Amino acids, Tannins, Saponins, Phytosterols, Terpenoids, phenols and flavones. The antioxidant activity was determined by *invitro* methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, Hydrogen peroxide scavenging assay, and Ferric reducing antioxidant power (FRAP) assay. The IC_{50} value of methanolic extract of *Syzygium cumini* for DPPH and hydrogen peroxide scavenging activity were found to be 53.3% at concentration of 600mg and 42.03% at 1.2mg/ml respectively. FRAP value found to be 810µg Fe^{2+}/gm. The extract showed significant antioxidant activity in all antioxidant assays when compared to ascorbic acid. The results of this research work are promising thus indicating the utilisation of the bark of *Syzygium cumini* as a significant source of natural antioxidants.

Keywords: Antioxidant activity, DPPH, FRAP, H_2O_2 scavenging assay, *Syzygium cumini*.

1. INTRODUCTION

Investigation of traditional medicine is very important for the welfare of rural and tribal communities for the conventional illness. Antioxidants are the agents which are used to prevent oxidation and provide protection to living organisms from damage caused by uncontrolled production of ROS and concomitant lipid peroxidation, protein damage and DNA strand breaking. Oxidation is the basic part of aerobic life and our metabolism. During oxidation, many free radicals are produced which have one or more unpaired, nascent electron. Atoms of oxygen or nitrogen having central unpaired electron called as reactive oxygen species (ROS). ROS react easily with free radicals to become radicals themselves. This may be harmful to body and may cause peroxidation. Lipid peroxidation is a destructive process, alters the structure and function of cellular membrane [1, 2].

Typical ROS are superoxide, hydroxyl, peroxyl and alkoxy radicals [3]. These free radicals may oxidise nucleic acids and proteins which can inhibit a chain of events resulting in the onset of disease [4]. Now a days, the role of free radicals in many ailments and diseases including inflammation, rheumatoid arthritis, cancer and cardiovascular diseases has been widely established [5].

Antioxidant agents like tannins, flavonoids, phenols, polyphenols, and nitric acid, scavenges free radicals such as peroxidase, hydrogenperoxidase or lipid peroxyl thus inhibits the oxidative mechanism that lead to degenerative diseases [6,7,8, 9]. Antioxidants are found in all parts of plants such as bark, stalks, leaves, fruits, roots, flowers, pods and seeds [10]. The most effective components seem to be flavonoids and phenolic compound of many plant raw materials, particularly in herbs, seeds and fruits [11]. Increasing the antioxidants intake can prevent diseases and lower the health problems. Research is increasingly showing that antioxidant rich foods, herbs reap health benefits [12].

Syzygium cumini Linn. belongs to the family myrtaceae [13]. The synonym of *Syzygium cumini* are *Eugenia jambolana*

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lam.,Myrtue cumini linn.,Syzygium jambolana D.C.,Eugenia cumini (Linn). Druce., Eugenia caryophyllifolia lam., calyptranthes jambolana wild., etc [14]. It is commonly called as Jamun in Hindi, Indian blackberry in English, Navva pazham in Tamil. It is a large tree found in all forest over the greatest part of India from the sub Himalayan tract to extreme south and also found in Thailand & Philippines [15].

The *syzygium cumini* bark is acrid, sweet, digestive, astringent to the bowels, and used for the treatment of sore throat, bronchitis, asthma, thirst, dysentery, ulcers and it is also a good blood purifier [16]. Different parts of *Syzygium cumini* were pharmacologically proved to posses neuropsychopharmacological, antimicrobial, anti HIV, antileishmonial and antifungal, gastro protective & radio protective activities [17], hypoglycaemic [18], antibacterial [19], antidiarhoeal effect[20] , nitric oxide scavenging activity[21] and antihelmintic[22], anti-inflammatory activity of leaf and barks[23,24]. In unani system of medicine the ash of leaves is used for strengthening the teeth & gums, the seed are astringent, diuretic, stops urinary discharge & the bark showed good wound healing property [16].

Even though more research works has been carried out on this plant but there is no enough scientific data available proving the antioxidant activity of methanolic extract of the bark of *Syzygium cumini.* Keeping above fact in the view, we have carried out the research work on antioxidant activity by using radical scavenging assays such as DPPH, hydrogen peroxide scavenging assay and FRAP assay. The effort was also made to estimate the total phenolic content, tannins and flavones by using standard methods.

2.1. MATERIALS AND METHODS:

2.1.1. Collection and identification of plant material:

The fully mature, fresh stem bark of *Syzygium cumini* was collected (during September) from Midhilanagaram, Mellacheruvu village, Chittoor district, Andhra Pradesh. The bark was air dried at room temperature (25^0C) for 30 days and converted into fine powder with an auto mix blender. The powder part was kept in a deep freezer until the time of use. The stem bark was identified and authenticated by Dr.Madhavachetty; Assistant Professor, Department of Botany, S.V.University, Tirupathi, and voucher specimen (No.JCP/2010/153) was deposited in the Herbarium of the same department.

2.1.2. Preparation of plant extract:

500 gm of dry fine powder was suspended in 1.5 litres of methanol and then stirred magnetically for 24 hours at room temperature. The extract was double filtered by using muslin cloth and whatmann No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40^0C using rotary vacuum evaporator (Buchi labortech AG, Switzerland). The percentage of yield was 17.9%. The dried MESC (Methanol Extract of *Syzygium cumini*) was stored in vacuum desiccators under controlled conditions till it used for experimental purpose.

2.2. Preliminary Phytochemical Screening:

1 gm of the methanol extract of *Syzygium cumini* Bark were dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (w/v). The standard methodology of Harborne (1998) [25] and Kokate (2001) [26] were adopted for the Phytochemical screening.

2.3.1. *Invitro* Antioxidant Studies:

2.3.2. DPPH Radical Scavenging Activity (DPPH):

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants [29]. The reaction mixture consisting of 3ml of 0.004% solution & different concentration (100-1000µg) of extract (0.1 to 1ml) & was incubated for 30minutes in dark, after which the absorbance was measured at 517nm. Ascorbic acid was used as positive control. The inhibition curve was prepared and IC50 values were calculated. The percentage inhibition activity was calculated by using following formula

% inhibition = Ao-A1 /Ao X100

Where, Ao= is the Absorbance of the control,

A1= is the Absorbance of the extract.

2.4.3. Determination of reducing capacity assessment (FRAP):

FRAP assay is a simple & reliable colorimetric method commonly used for measuring total antioxidant capacity [30]. 2850µl of freshly prepared FRAP solution were taken in all test tubes, except blank. In control tubes, 150µl of FeSo4 were taken, In the same way for sample test tubes,150µl of sample extracts ranges from 100-1000µg were taken. The absorbance of reaction mixture at 593nm was measured spectrophotometrically after incubation at room temperature for 30minutes in dark. The standard curve was linear between 200 & 1000µM FeSo4.results are expressed in µMFe^{2+}/g dry mass & compared with that of ascorbic acid.

2.4.4. Determination of Scavenging of Hydrogen Peroxide:

Different concentration of sample extract 0.2-1ml were taken in test tube, added 0.6ml of 2mM of hydrogen peroxide in phosphate buffer (pH=7.4). 1ml of colour reagent added to each test tube and incubated 10 mins. The absorbance of H2O2 at 230nm was determined against a blank solution containing phosphate buffer solution without H2O2. The scavenging of hydrogen peroxide was determined as follows: **% of scavenge= (Am/Ab) X100**

Where,Am=absorptionofractionmixture,

Ab= absorption of blank (in PBS without hydrogen peroxide)

3. RESULTS AND DISCUSSION:

3.1. Preliminary Phytochemical screening: The variety of plant and plant extracts contains different phytochemicals with biological activity that can be of valuable therapeutic Index. The results obtained in the present investigation is shown on the table (1)-phytochemical screening.

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PHYTOCHEMICALS	*METHANOL*
carbohydrate	+
Alkaloids	_
Amino acids	_
Anthraquinones	_
Flavonoids	+
Glycosides	_
Phytosterols	_
phenols	+
Saponins	+
Steroids	_
Tannins	+
sterols	+
Terpenoids	+
Volatile oil	_

Table 1: Preliminary Phytochemical screening of Methanolic extract of *Syzygium cumini* Bark *+ = Presence*

_ = Absence

3.2. Antioxidant Testing Assays

3.2.1. DPPH Radical Scavenging Activity:

In the present work, the methanolic extract of bark of *Syzygium cumini* was evaluated for their DPPH Radical scavenging activity. From the figure 1, it was observed that methanolic extract had lower activity when compared to ascorbic acid. At a concentration of 1mg/ml, the scavenging activity of standard ascorbic acid reached 98% while at the same concentration, methanolic extract of *Syzygium cumini* bark was 78.94%.the effect of antioxidant on DPPH radical scavenging abilities of the extract were less than those of ascorbic acid at 1mg/ml. This study showed that the extract are proton donating ability and could serve as free radical inhibitors or scavengers, activity possibly as primary antioxidants. When observing, the IC_{50} values of methanolic extract reached 53.3% at 600µg concentration. The effect of antioxidant on DPPH is thought to be due to their hydrogen donating ability.

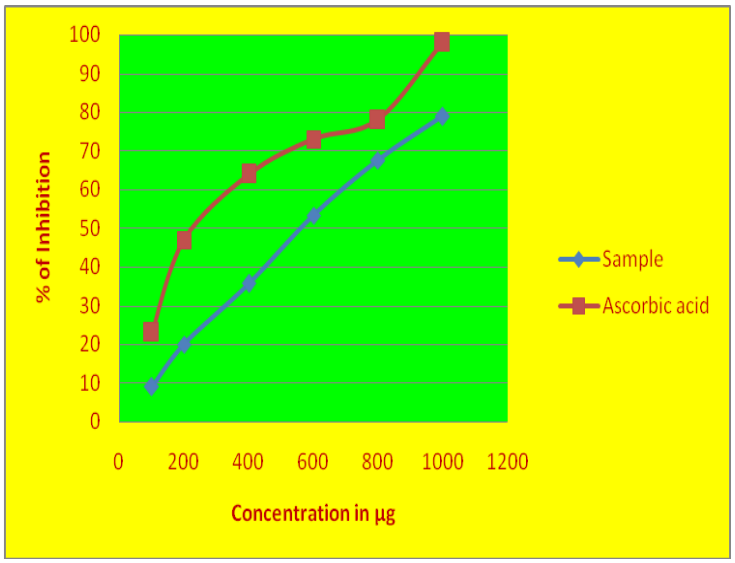

Figure 1: DPPH radical scavenging activity of the methanolic extract of *Syzygium cumini.*

3.2.2. Determination of Reducing Capacity Assessment:

The reducing ability of extract was in the range of 810µgFe^{2+}/gm. The Antioxidant potential of the methanolic extract of the *syzygium cumini* bark was estimated from the ability to reduce TPTZ- Fe^{3+} complex to TPTZ- Fe^{2+}. It has been evidenced from the figure 2, that the FRAP values of the methanolic extract of *Syzygium cumini* were significantly lower than that of ascorbic acid 980µgFe^{2+}/gm. Antioxidant activity is increased proportionally to the polyphenols content. According to the recent reports, a highly positive relationship between total phenols and Antioxidant activity appears to be the trend in many plant species [31].

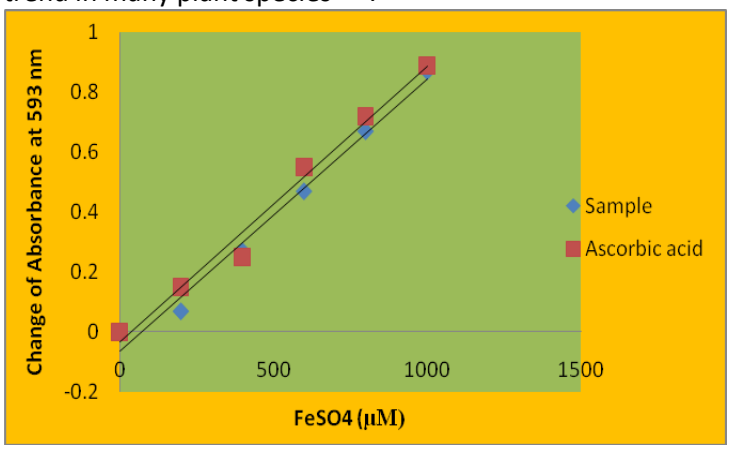

Figure - 2: FRAP activities of the methanolic extract of *Syzygium cumini* bark

3.2.3. Determination of Scavenging of Hydrogen Peroxide:

Although H_2O_2 itself is not very reactive, it can sometimes, cause cytotoxicity by giving rise to Hydroxyl radicals in the cell. Thus removing H_2O_2 is very important throughout the food system. Scavenging of H_2O_2 by antioxidants may be

due to donation of electrons to H_2O_2, thus neutralising to water [32]. Figures-3, illustrate H_2O_2 scavenging activity of methanolic extract of *Syzygium cumini* are compared with standard ascorbic acid. According to the results shown in figure, the hydroxyl radical scavenging activity was shown by Methanolic extract of *Syzygium cumini*, at 2mg/ml in the range of 86.96% at the same way standard ascorbic acid show 94.20% of H_2O_2 inhibition. The IC_{50} of Methanolic extract of *Syzygium cumini* was 42.03% at concentration of 1.2mg/ml and at the same concentration of the ascorbic acid was achieved 52.17%.

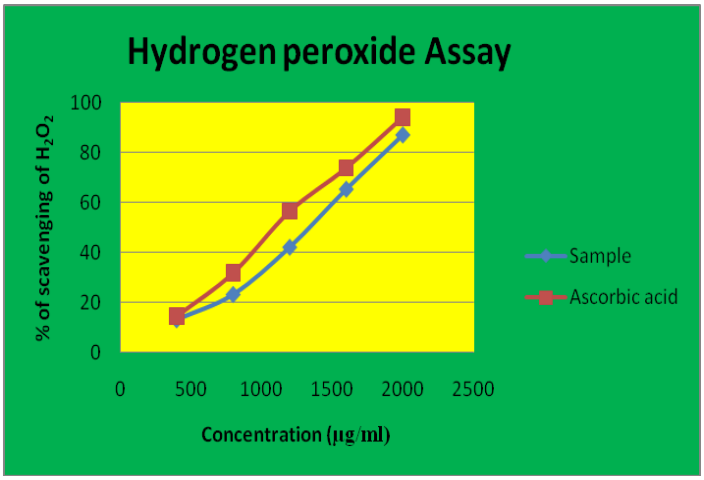

Figure 3: Hydrogen peroxide scavenging of the methanolic extract of *Syzygium cumini*

4. CONCLUSION:

The results of the present study lead us to inference that the bark extract posses antioxidant properties. The preliminary Phytochemical analysis evidenced that the methanolic extract of *Syzygium cumini* bark exhibited antioxidant activity might be possible due to the presence of phenolic compound, tannins and flavones. The *in vitro* assays indicate that this bark extract is significant source of natural antioxidants, which might be helpful in preventing the diseases associated with oxidative stresses.

Furthermore, detailed studies on isolation, characterisation of phytochemicals and pharmacological and biochemical investigation is needed to elucidate the exact mechanism of action and will be helpful in projecting this *Syzygium cumini* bark as a therapeutic target in Antioxidant research.

We strongly believe that the outcomes of the study will trigger exciting research on addressing Antioxidants in a cost effective manner.

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Conflict of Interest: None Declared

