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In Vitro Antioxidant Activity of *Sterculia Foetida* Seed Methanol Extract

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ABSTRACT

The objective of this study was to determine the phenolic content of the *Sterculia foetida* L. seeds methanol extract (SSME) and to evaluate the antioxidant activity of the extract. The Folin-ciocalteu procedure was used to assess the total phenolic content of the extracts as garlic acid equivalents. Antioxidant activity was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric reducing power and 2, 2-Azinobis-3-ethyl benzothiazoline-6-sulfonic acid (ABTS) diammonium salt methods. The seed methanol extract was yielded 9.5% crude material. The total polyphenol content was 14.32%. The antioxidant activity of extract was shown similar correlation between DPPH, ferric reducing power and ABTS.

Keywords: *Sterculia foetida,* Seed, Methanol extract, Polyphenols, Antioxidant

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INTRODUCTION

Natural antioxidants from fruits, vegetables, cereals, pulses, oil seeds and spices have gained increasing interest among consumers and the researchers because their clinical studies have proved that the natural antioxidants promote the health and wellness of human beaning [1-8] Canola, rapeseed seed hull and mushroom extracts are reported to be antioxidant potential, which due to the presence of tannins and polyphenols [9-10].

The natural antioxidants are classified in to three major groups such as vitamins, phenolics and carotenoids, in which ascorbic acid and phenolics are hydrophilic, while carotenoids are liphophilic nature[11]. A part from these, proteins, particularly smaller peptides are also responsible for antioxidant activity [12].

Sterculia foetida is belongs to the *Sterculiacae* family is widely distributed throughout tropics, and native to Asia and Sri Lanka. The tree yields edible seeds rich in fat (30 - 36%), and the seeds contain protein content to an extent of 11.4% [13]. Phospholipids of *Sterculia foetida* seed, in which phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol are the major components [14]. The de-oiled seed cake was reported to rich in protein 28.89% [15]. The seed protein solubility studies were also carried out by Narsing Rao and Rao [16] and they reported that the solubility of seed protein flour was higher (90%) at pH 12 and lower (25%) at pH 6.

The objective of the present study was to extract active components from *Sterculia foetida* seed and determination of total polyphenol content and in vitro antioxidant activity.

MATERIALS & METHODS

Plant Material & Chemicals

Sterculia foetida dry seeds (2 kg) were collected from the trees at Centre for Cellular and Molecular Biology (CCMB) campus, Hyderabad, India. Analytical grade chemicals and solvents used in the study were purchased from M/s. Sd Fine-Chem Ltd (Mumbai, India). Gallic acid, BHT, Potassium ferricynate, DPPH and ABTS reagents were purchased from M/s. SD Fine-Chem Ltd, Mumbai, India and M/s. Sigma Chemicals Co., St. Louis, USA.

Extraction of *Sterculia foetida* seed

Sterculia foetida whole seeds (250 g x 3) were course ground in a laboratory mixer (Sumeet, Nasik, India). The ground seed material was extracted with methanol (1:3 w/v) in a soxhlet extractor for 16 hours. The methanol extract was concentrated on rotavapor at 45 ± 2 °C and stored at 28 ± 2° C room temperature (RT) under nitrogen atmosphere in a glass container.

Estimation of whole seed moisture

The course ground whole seed moisture content was estimated in a hot air oven at 105 ± 2 °C for a period of 12 h [17].

Estimation of total polyphenol

The total polyphenol content in SSME was measured by dispersing 1 g in 50 ml of 80% ethanol at RT. The extract of 0.5 ml was taken in a test tube, 0.5 ml Folin–Ciocalteau reagent was added. The contents were mixed with 5 ml water and vortexed for 2 min, and allowed at RT for 5 min. Later 1 ml of sodium carbonate was added to the reaction mass, and the volume was made up to 10 ml with distilled water. The contents were vortexed for 2 min and allowed at RT for 60 min. The absorbance of the colour was recorded at 675 nm[18]. A standard calibration curve was drawn by using pure gallic acid 19-76 µg/ml, and was measured at 675 nm. The total polyphenol content was calculated and expressed as mg of gallic acid / 100 g SSME as follows:

$$\text{Polyphenols mg / 100 g} = \frac{\text{µg of polyphenols in the aliquot} \times \text{Total volume of solution (50 ml)}}{1000 \times \text{Weight of the sample}} \times 100$$

DPPH radical scavenging activity

Antioxidant activity of the methanol extract was determined on the basis of their scavenging acidity of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. Extract of 1-5 mg SSME was dispersed in 1 ml of methanol and methanolic solution, (4 ml) of DPPH (0.004% solution) was added. The contents were incubated at RT for 30 min and the colour absorbance was read at 517 nm [19]. The DPPH radical scavenging activity was compared with the activity of butylated hydroxytolulene (BHT) in the range 1-5 µg. The percent inhibition was calculated as follows:

$$\text{Inhibition, \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Ferric ions (Fe3+) reducing power

The ferric reducing power of the SSME was measured by dispersing 1-5 mg in a mixture of 1 ml methanol, 2.5 ml of phosphate buffer (pH 6.6) in different test tubes. Potassium ferricyanide (2.5 ml) 1% solution was added. The contents were incubated for 20 min at 50 °C. After incubation 2.5ml of 10% tri-chloroacetic acid was added and centrifuged at 8000 rpm for 10 min. The aliquot 2.5 ml was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The contents colour absorbance was read at 700 nm and expressed as absorbance per mg/µg sample or BHT. The reducing power activity was compared with BHT (1-5 µg) according to a reported method in the literature [19].

ABTS radical scavenging activity

ABTS (2, 2-Azinobis-3-ethyl Benzothiazoline-6-Sulfonic acid) diammonium salt inhibition assay of SSME was carried out according to the reported method with minor modifications [20]. The ABTS solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulphate and incubated in the dark at RT for 16 h. The mixture was diluted with 80% (v/v) water to obtain an absorbance of 0.700 at 734 nm. ABTS solution (3 ml) was mixed with 1-5 mg/ml SSME vigorously. The control was prepared using water instead of SSME and its absorbance was recorded at 734 nm after 10 mn. The ABTS inhibition was compared with BHT in the range 1-5 µg/ml. The percent inhibition of the samples was calculated using following expression.

$$\text{Inhibition, \%} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The experiments were carried out in three replications and the mean values with standard deviation (mean ± SD) computed using MS Excel, 2007 were reported.

RESULTS & DISCUSSION

Yield and total polyphenol content

The seed of *Sterculia foetida* is presented as photograph in Figure 1.

Figure 1: Seed of *Sterculia foetida*

The whole seed was yielded 9.81% crude material after methanol extraction. The extract (SSME) total polyphenol content was found to be 14.61% (Table 1). The Chloroform/methanol (2:1, v/v) extract of *Sterculia urens* seed cotyledons was yielded 39.20% crude material [21]. The total poly phenol content 316mg/100g in gum karaya defatted seed flour was reported by Narsing Rao and Rao[22]. The polyphenol content of 1.1 and 0.8% of ethanolic and ethylacetate extracts from melon seed was reported [20]. Roasted sesame, pumpkins, sunflower seeds, soy beans and wheat germs polyphenol content 1.6 1.0, 15.8, 2.0, and 1.7% methanol extract was reported by Agnieszka and

Magdalena [23]. Stevia leaf extract also reported to contain 2.4-4.2 % total polyphenols, and which showed higher DPPH antioxidant activity was reported [24-25].

Table 1: Whole seed moisture content, yield and total polyphenol content[a] of SSME

Parameters, %	SSPF
Moisture	5.35 ± 0.252
Yield	9.81 ± 0.669
Total polyphenol,	14.32 ± 1.597

[a]Values are means of triplicate analyses with standard deviation.

SSME: *Sterculia foetida* seed methanol extract., %, Yield: Yield of extract based on whole seed

DPPH radical scavenging activity

The DPPH radical scavenging activity of SSME and BHT were presented in Figure 2. DPPH radical scavenging activity in terms of percent inhibition was found to be increased with enhancing the concentration of SSME. The inhibition was 30.65% (1 mg) and 71.07% (5 mg) for SSME, whereas the values were 23.01% (1 µg) and 32.56% (5 µg) for BHT respectively. The DPPH radical scavenging activity of free polyphenols extracted in 80% chilled ethanol of mushroom was in the range from 21.1% - 82.9% in different cultivars at Southern Vietnam was reported by Hung and Nhi [26]. Inhibition of 49.3% for 100 µg of *Cucumis melon* seed methanol extract was also reported in the literature [27]. Ethanol and water extracts of fennel seed was 77.5 and 99% inhibition for 100 µg was reported by Munir, Gulcin, Ilhami, and Irfan [28].

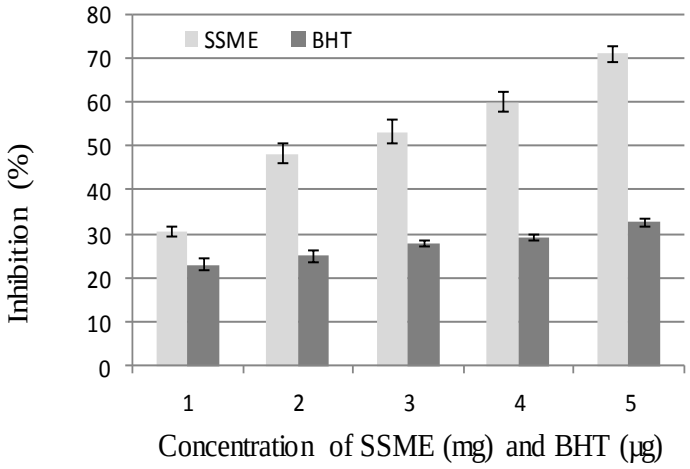

Figure 2: DPPH radical scavenging activity of SSME (mg) and BHT (µg)

They concluded that the antioxidant activity depends on type of solvent used for extraction and concentration of polyphenols. The guarana seed water, ethanol and acetone extracts showed antioxidant activity and further it was reported to have antibacterial, antimicrobial activity reported by Lucija and Zeljko [29]. They concluded that the seed extract further can be used as a natural additive in food, cosmetic and pharmaceutical industries

Reducing power

The iron reducing power of SSME and BHT were presented in Figure 3.The reducing power of iron was measured by noting colour absorbance. The absorbance was 0.326 (1 mg) and 0.004 (1 µg), and 1.33 (5 mg) and 0.037 (5 µg) for SSME and BHT respectively. The increasing trend in iron reducing power absorbance, 0.25 – 1.2 with increasing concentration from 0.2 – 1.0 mg was noticed in sun flower seed methanol extract [23]. Aqueous extract of *Cordyceps militaris* (1 – 8 mg), absorbance was 0.04 – 0.14 reported by Yu, Cai-Hong, and Yi-Jian [30].

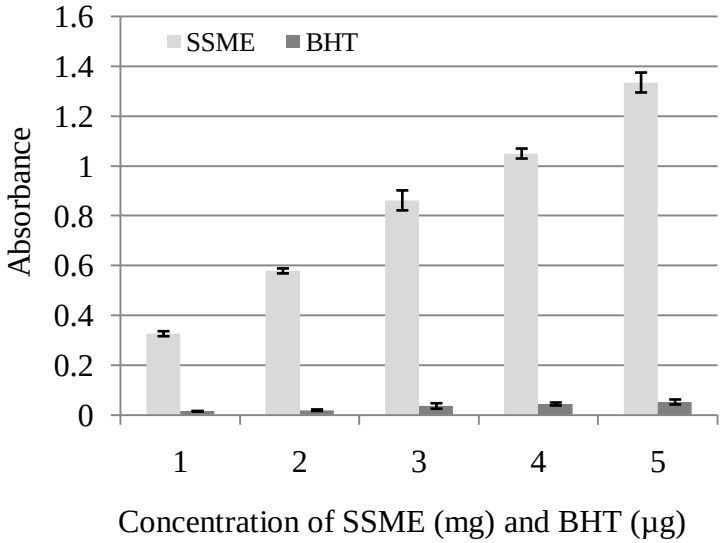

Figure 3: Ferric reducing power of SSME (mg) and BHT (µg)

ABTS antioxidant activity

The ABTS radical scavenging activity of SSME and compared with BHT were presented in Figure 4. The inhibition was 33.76% (1 mg) and 36.04% (1 µg) and 64.59% (5 mg) and 79.00% (5 µg) for SSME and BHT respectively. Ethanol extract of winter melon seeds was 27.0% (0.1 mg) radical inhibition was reported [20]. The inhibition of lipid oxidation could be by donation of a proton, stabilize and or termination of free radicals and by chelation of metal ions by phenols [12]. The increasing trend in antioxidant activity of extract was observed with increasing concentration of SSME. Generally, antioxidant activity was depends on several factors such as type of solvent used, class of bio active compounds, solubility, stability, storage, nature and its interaction with other compounds [12, 20, 31, 32]. Polyphenols (59-109 mg gallic acid/g) present in methanolic extract of certain fresh medicinal plants are responsible for antioxidant activity [33]. The free polyphenols shows higher antioxidant activity than bound polyphenols [34].

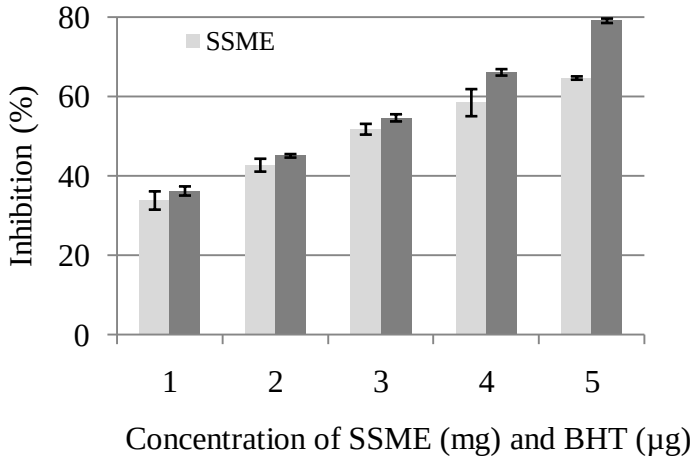

Figure 4: ABTS radical scavenging activity of SSME (mg) and BHT (µg)

CONCLUSIONS

In the present study, for the first time, methanol extraction of *Sterculia foetida* L. seed was carried out by soxhlet method and evaluated their antioxidant activity. The seed methanol extract was rich in polyphenol. It was found that DPPH radical scavenging activity, ferric reducing power and ABTS radical scavenging activity were comparable to oil seed extracts. This study revealed a positive linear correlation between the concentration of methanol extract and antioxidant activity. The knowledge derived would be helpful in the isolation of polyphenols and their characterization. These finding suggest that this seed is a potential source of natural antioxidant. This study encourage to work further on biological activity and health implications of methanol extract.

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