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Free radical scavenging potential of root extracts of *Syzygium caryophyllatum* (L.) Alston

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ABSTRACT

Medicinal plants are commonly used in treating or preventing specific ailments or diseases are considered to play a beneficial role in health care. Therefore, the study of plants as a resource of medicine has become more important in the present world for preventing the human beings from oxidative stress diseases and deaths. Objective of the present study is to evaluate the effects of acetone, methanol and aqueous extracts of *Syzygium caryophyllatum* roots for its antioxidant activity. Using different *in vitro* models like DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) assay in a concentration dependent manner (500, 400, 300, 200 and 100 mg/ml) and ABTS radical scavenging activity. The results showed that the methanol root extract had high DPPH radical scavenging activity (62.33%) and ferric reducing capacity than the other extracts. But the results were less when compared to the standard used. The results of this research work indicate the utilization of the root extract of *S. caryophyllatum* as a significant source of natural antioxidants.

Keywords: Oxidative stress, Antioxidants, *Syzygium caryophyllatum*, DPPH, FRAP and ABTS.

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INTRODUCTION

It is increasingly being realized that many of today's diseases are due to "oxidative stress" that results from an imbalance between formation and neutralization of pro-oxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases.[1]

All human cells protect themselves against free radical damage by enzymes such as superoxide dismutase (SOD) and catalase, or compounds such as ascorbic acid, tocopherol and glutathione.[2-3] Sometimes these protective mechanisms are disrupted by various pathological processes and antioxidant supplements are vital to combat oxidative damage. Recently, much attention has been directed towards the development of ethno medicines with strong antioxidant properties but low cytotoxicities. Myrtaceae family includes numerous popular and also less known largest trees with pronounced therapeutic properties.

It consists of 130 genera and 3,000 species.[4-5] Members of this family occur in temperate, sub-tropical, and tropical regions however; the family is centered in Australia and tropical America.[5] *Syzygium* is considered as one of the most important genera of Myrtaceae family and are traditionally used for various purposes.

Traditionally *S. cumini* seeds, fruits, leaves, flowers and bark have been used in folk medicine for various diseases. Charaka prescribed seeds, leaves and fruits as decoctions for diarrhoea and the bark as an astringent. Sushruta prescribed the fruit for obesity, vaginal discharges, menstrual disorders and cold infusion in intrinsic haemorrhage.[6] *Syzygium caryophyllatum* is one of the very important species of the genus *Syzygium* and are enlisted in the Red List of Threatened Species.[7] The preliminary phytochemical analysis of leaves, bark and roots showed the presence of alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides, triterpenoids and fats and fixed oils and the leaves of *S. caryophyllatum* showed a very good hypoglycaemic activity on alloxan induced diabetic mice.[8-9]

Despite the various bioactive phytochemical constituents and medicinal properties attributed to the *S. caryophyllatum* no detailed antioxidant studies have been carried out in the root extracts so far. So the present study aimed to evaluate the antioxidant potential of the root extracts of *S. caryophyllatum*.

MATERIALS AND METHODS

Collection of the plant material

Syzygium caryophyllatum(L.) Alston (Myrtaceae) is collected during the month of October 2009 from Palani Hills, Tamil Nadu, India. It is identified and authenticated by Dr. S. Padmavathy, Associate Professor, Department of Botany, Nirmala College for Women (Autonomous), Coimbatore, Tamil Nadu.

Preparation of extracts

Solvent extraction

Freshly collected samples of *S. caryophyllatum* roots were washed 2-3 times with water followed by distilled water and shade dried. All the dried parts were pulverized by mechanical grinder (Willy mill) to get the powder through 100-mess sieve and then stored in a refrigerator. The shade dried powdered plant material (250g) was extracted first with acetone, methanol using a soxhlet apparatus. Then the extract was concentrated in a rotary evaporator.

Aqueous Extraction

Air-dried ground roots (250 g) of *Syzygium caryophyllatum* was extracted with double distilled water for 24 hrs. The extract was stored in a vial and wrapped with aluminum foil and kept in the refrigerator for further processing.

Chemicals

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma Chemical Co. (St., Louis, USA). Ascorbic acid, Folin Ciocalteu reagent, and methanol were purchased from Merck Co. (Germany).

In vitro antioxidant activity

DPPH radical scavenging activity[8]

Different concentrations (500, 400, 300, 200 and 100 µg/ml) of *S. caryophyllatum* extracts were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. Then 5 ml of 0.1m-methanol solution of DPPH (1, 1, Diphenyl-2- Picrylhydrazl) was added to each of the test tubes and were shaken vigorously. They were then allowed to stand at 37^0 C for 20 minutes. The control was prepared without any extracts. Methanol was used for base line corrections in absorbance (OD) of sample measured at 517nm. A radical scavenging activity was expressed as 1% scavenging activity and was calculated by the following formula.

$$\%\ \text{Radical scavenging activity} = \frac{\text{Control O.D - Sample O.D}}{\text{Control O.D}} \times 100$$

Reducing Power Assay [9]

Different concentrations (500, 400, 300, 200 and 100 mg/ml) of the extracts of *S. caryophyllatum* were dissolved in DMSO (dimethyl sulfoxide) and taken in test tubes in triplicates. To the test tubes 2.5 ml of sodium phosphate buffer and 2.5 ml of 1% Potassium ferric cyanide solution was added. These contents were mixed well and were incubated at 50^0 C for 20 min. After incubation, 2.5ml of 10% TCA was added and were kept for centrifugation at 3000 rpm for 10 minutes. After centrifugation, 5ml of supernatant was added with 5ml of distilled water. To this about 1ml of 1% ferric chloride was added and was incubated at 35^0C for 20 min. The absorbance (O.D) was taken at 700 nm and the blank was prepared by adding every other solution but without extract and ferric chloride (0.1%) and the control was prepared by adding all other solution but without extract. BHA was used as the standard.

ABTS [2, 2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)] radical cation assay[10]

The radical scavenging capacity of root extract of *S. caryophyllatum* was evaluated against ABTS radical cation. The assay is based on the ability of antioxidant molecules to decolourise the radical cation 2, 2-azino-bis (3-ethylbenzothiazoline- 6-sulphonate) [ABTS·+], a blue-green chromophore with characteristic absorption at 734 nm. The addition of antioxidants to the radical cation reduces it to ABTS. Briefly, ABTS radical cation (ABTS·+) was produced by reacting 7 mmol/L of ABTS stock solution with 2.45 mM potassium persulphate ($K_2S_2O_8$) in the dark at room temperature for 12–16 h before use. The next day, ABTS·+ solution (1 %, by volume) was diluted with 96 % ethanol to an absorbance at (0.73±0.02) 734 nm. After the addition of 0.16 mL of sample or 25–300 mM aqueous solution of Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) to 2 mL of diluted ABTS·+ solution, absorbance readings were taken after 1 min at 734 nm using the spectrophotometer Unicam Helios b (SpectronicUnicam, Cambridge, UK). Ethanol was used as a blank. The results were calculated according to the calibration curve for Trolox (y = 0.00219x+0.678662, y = absorbance at 734 nm, x = concentration of Trolox in mM, R2 = 0.9989) and the ABTS values, derived from triplicateanalyses, were expressed as mmol.

RESULTS AND DISCUSSION

The production of free radical can be controlled by the natural antioxidants, which increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke. The antioxidative properties of the phytochemicals in medicinal plants, vegetables, fruits, seeds and leaves have received an increasing attention for their potential role in preventing human diseases, especially on diabetes.[11-12]

DPPH Radical Scavenging Assay

The effects of acetone, methanol and aqueous extracts of *S. caryophyllatum* roots were evaluated for its antioxidant activity on different *in vitro* models like DPPH radical scavenging activity, ferric reducing assay in a concentration dependent manner (Table 1 & 2).

Table 1: DPPH Radical scavenging activity of *S. caryophyllatum* root extracts

Plant part	Solvents used	Concentrations µg/ml	% of inhibition *S. caryophyllatum*	StandardAscorbic acid
Root	Acetone	100	13.33±0.01	
		200	19.11±0.03	
		300	33.46±0.02	
		400	45.66±0.01	
		500	59.22±0.01	
	Methanol	100	20.22±0.10	
		200	33.25±0.20	79.8
		300	42.56±0.02	
		400	49.38±0.03	
		500	62.33±0.3	
	Aqueous	100	5.41±0.01	
		200	10.17±0.02	
		300	20.45±0.02	
		400	42.23±0.04	
		500	58.23±0.06	

Values are mean ± SD of three samples in each group

Table 2. Reducing power assay of *S. caryophyllatum* root extract

Sample Tested	Absorbance at 700nm Solvents	Concentrations of the extracts (mg/ml)	100	200	300	400	500
Root	Acetone	0.35 ± 0.001	0.70 ± 0.001	0.78 ± 0.01	0.95 ± 0.001		1.12 ± 0.02
	Methanol	0.65 ± 0.001	0.81 ± 0.01	1.07 ± 0.001	1.26 ± 0.001		1.58 ± 0.02
	Aqueous	0.25 ± 0.01	0.32 ± 0.01	0.41 ± 0.001	0.53 ± 0.03		0.69 ± 0.01
BHA (Standard)		0.486±0.014	1.11±0.06	1.57±0.06	2.05±0.072		2.69±0.041

Values are mean ± SD of three samples in each group

The methanol extract showed significant activity when compared to the other extracts. Among these solvent extracts, the methanol root extract had high DPPH radical scavenging activity (62.33%) followed by acetone extract (59.22%) when compared to the water extract but, it showed less inhibition when compared to the standard ascorbic acid (79.8%).The DPPH radical is widely used as the model system to investigate the free radical scavenging activities of several natural compounds such as phenolics or crude extracts of plants. In the present study the free radical scavenging activity was varying with the extracts. The highest percentage of inhibition was noticed in the methanolic extract of *S. caryophyllatum* and lowest in the aqueous extract. The degree of discolouration indicates the scavenging potential of antioxidants. In this investigation, DPPH radical is scavenged by antioxidants through the donation of proton from the reduced DPPH. According to the report of Ruan*et al.*[13] on *S. cumini* root, the DPPH radical scavenging activity might be due to the presence of phytochemicals such as, phenols, flavonoid, tannins etc. Antioxidants cause the reduction of Fe^{3+} ferric cyanide complex to the ferrous form and the activity is measured as the increase in absorbance at 700 nm. Table 2 shows the reducing power of root extracts of *S. caryophyllatum* in comparison with BHA as a reference antioxidant. The tested concentrations showed that all the samples possess the ability to reduce iron (III) ions. Although, the reducing power was significant in the methanolic extract (1.58 ± 0.02 mg/ml) which is also increased with increasing concentrations of the sample but the reducing capacity of methanolic root extract was less when compared to the standard used (2.69±0.041mg/ml). This suggests that the root extract is an electron donor and could neutralize the free radicals[14]. These findings supported the investigation of Ruan *et al.*[13] In the reducing power assay the presence of antioxidants in the extract reduced Fe^{3+}ferric cyanide complex to the ferrous form and the reducing ability of a compound depends on the presence of reductants[15] which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. The reducing capacity of compounds could serve as indicator of potential antioxidant properties and an increasing absorbance could indicate the increase in reducing power.[16] The ABTS radical cation scavenging activity of acetone, methanol and aqueous extracts of *S. caryophyllatum* are presented in the table 3. The activity of the tested samples was expressed as trolox equivalent-the micro molar trolox solution having an antioxidant capacity equivalent to 1 gm dry matter of the substance under investigation. In this assay, Total Antioxidant Activity (TAA) reflects the hydrogen donating ability to scavenge ABTS radical cation, compared with that of trolox. Thus, all the samples had exhibited the ABTS radical scavenging activity, but acetone root extract (20095.6 ± 60.2 µmol/g extract) exhibited more scavenging activity followed by methanol (20008.8 ± 16.7). From the antioxidant studies it has been noted that *S. caryophyllatum* root methanolic extract exhibits highest DPPH radical scavenging activity, ferric reducing capacity. The ABTS radical scavenging activity was not only a rapid and reliable test for total antioxidant capacity but also an advantageous assay applicable to both hydrophilic and hydrophobic antioxidant system. The scavenging activity was measured by the absorbance at 734 nm, which decreased as the ABTS radical is scavenged. The free radical scavenging activity of extracts measured along with the reference standard, trolox. In this study all of the extracts had strong antioxidant abilities and the order of total antioxidant activity of sample extract is acetone root followed by methanol extract.

Table 3: ABTS radical scavenging activity of *S. caryophyllatum*(L.) Alston

Plants	***TAA (µmol/g extract)**		
	Extracts Used		
	Acetone	**Methanol**	**Aqueous**
S. caryophyllatum	20095.6 ± 60.2	20008.8 ± 16.7	19912.4 ± 60.2

Total antioxidant activity (µmol equivalent Trolox performed by using ABTS radial cation), Values are means of three independent analyses ± standard deviation (n=3)

CONCLUSION

The present result indicated that the methonolic root extract of *S. caryophyllatum* possesses potent antioxidant activity when compared to the acetone and aqueous extracts. It may be due to the presence of secondary metabolites in the *S. caryophyllatum*.

REFERENCES

1. Braca A, Sortino C, Politi M, Morelli I, Mendez J. Anti Oxidant activity of flavanoids from *Licanialicaniaeflora*. J Ethnopharmacol 2002; 79: 379-81.
2. Maxwell SR. Prospects for the use of Antioxidant therapies. Drugs 1995; 49(3):345-61.
3. Niki E, Shimaski H, Mino M. Antioxidantism - free radical and biological defense. Gakkai Syuppn CenterTokyo: 1994; pp. 3-16.
4. Stebbins GL, Flowering Plants:evolution above the species level. Balknap Press of Haward University. Cambridge. Massachussets; 1974; pp. 399.
5. Watson L, Dallwitz MJ. The families of flowering plants: descriptions, illustrations, identification, and information retrieval. Version: 2000 December 14.
6. Kumar AR, Jayachandran T, Deecaraman M, Aravindan P, Padmanabhan N, Krishnan MRV. Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. J Med Res 2008; 2(9):246-49.
7. *IUCN, 2006, en.wikipedia.org/wiki/syzygium_caryophyllatum.
8. Savitha RC, Padmavathy S. Comparative phytochemical analysis of *S.caryophyllatum* (L.) Alston and *S. densiflorum* Wall. Scientia 2011; 7(1): 125-31.
9. Savitha RC, Padmavathy S hypoglycaemic effect of *S.caryophyllatum* (L.) Alston on alloxan induced diabetic albino mice. Asian J Pharm Clin Res 2013; 6(4): 203-5.

10. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature* 1958; 181:1199 -200.
11. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr 1986; 44:307- 15.
12. Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. J Nut 2003; 133:2812-19.
13. Naziroglu M, Butterworth PJ. Protective effects of moderate exercise with dietary vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin-induced diabetes. Can J ApplPhysiol 2005; 30:172-85.
14. Pallauf K, Rivas-Gonzalo JC, Castillo MD, Cano MP, Pascual-Teresa S. Characterization of the antioxidant composition of strawberry tree (*Arbutus unedo*L.) fruits. J Food Compos Anal 2008; 21:273-81.
15. Ruan Z, Zhang PLL, Lin YM. Evaluation of the antioxidant activity of *Syzygium cumini* leaves. Molecules 2008; 13:2545-56.
16. Zhu N, Wang M, Wei GJ, Lin JK, Yang CS, Ho CT. Identification of reaction products of (-)-epigallocatechin, (-)-epigallocatechingallate and pyrogallol with 1, 1-diphenyl-2-picrylhydrazyl radical. Food Chem 2001; 73:345-49.
17. Wong C, Li H, Cheng K, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem 2006; 97:705-11.
18. Meir S, Kanner J, Akiri B, Hada SP. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaves. J Agric Food Chem 1995; 43:1813-19.