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TOTAL PHENOLIC, FLAVONOID CONTENT AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF *SYZYGIUM CALOPHYLLIFOLIUM* WALP. LEAF EXTRACT

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ABSTRACT

To assess the total phenolic and flavonoid contents and to evaluate a systemic record of the relative antioxidant activity of the methanol extract of *Syzygium calophyllifolium* Walp leaf. Total phenolic content was estimated by folin-ciocalteau method and flavonoids were determined by Aluminium chloride method. The antioxidant activity was evaluated by the DPPH radical scavenging activity, hydroxyl radical scavenging activity, $ABTS^{+}$, Super Oxide radical scavenging activity and reducing power. Antioxidant activity of methanolic extract was compared with standard antioxidant ascorbic acid. The total phenol and flavonoid content of the methanol extract of *S. calophyllifolium* leaf were found to be 1.12g/100g and 1.42g/100g respectively. The result of antioxidant study of *S. calophyllifolium* methanolic extract exhibited a highest activity in DPPH (IC50-34.63), followed by Superoxide dismutase (IC50-33.64), ABTS (IC50-33.17) and least activity was seen in the Hydroxyl study. The result indicated that the methonolic leaf extract of *S. calophyllifolium* exhibited potent *In vitro* antioxidant activity.

KEYWORDS: *Syzygium calophyllifolium*, *Eugenia calophyllifolia*, Myrtaceae, *In vitro* antioxidant, DPPH, $ABTS^{+}$, reducing power.

INTRODUCTION

The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyland thus inhibit the oxidative mechanisms that lead

to degenerative diseases.[1] Free radicals are the major cause of chronic and degenerative diseases such as coronary heart diseases, inflammatory stroke, diabetes and cancer.[2] Free radicals are fundamentals to any biochemical process and represent an essential part of aerobic life and metabolism.[3]

Antioxidant compounds can decrease oxidative stress and minimize the incidence of these diseases. The mechanism of the action of these antioxidant compounds include suppression of reactive oxygen species formation either by inhibition of the enzymes or by chelating of trace elements involved in free radical production, scavenging of reactive species and up-regulating or protecting antioxidant Defence.[4] It is possible to reduce the risk of chronic diseases and prevent disease progression by either enhancing the body's natural antioxidant defences or by supplementing with proven dietary antioxidants.[5] Recently, there has been an upsurge of interest in the therapeutic potentials of plant-derived antioxidants in reducing free radical-induced tissue injury and the current trend is to substitute synthetic with naturally occurring antioxidants.[6] Several biologically active compounds of plant origin have been found to possess antioxidant, free radical scavenging activity and many are being applied therapeutically for free radical associated disorders.[7]

Recent evidences suggested the involvement of oxidative stress in the pathogenesis of various diseases and have attracted much attention of the scientists and general public on the role of antioxidants in the maintenance of human health and prevention and treatment of diseases.[8] Natural antioxidants are found in various parts of plants such as leaves, fruits, seeds, roots and bark.[9, 10] In the present study the total phenolic and flavonoid contents were estimated and the antioxidant activity of methonolic leaf extract of *Syzygium calophyllifolium* was evaluated by the DPPH radical scavenging activity, hydroxyl radical scavenging activity, and Antioxidant activity by radical cation ($ABTS^{\cdot+}$), Super Oxide radical scavenging activity and reducing power.

MATERIALS AND METHODS

The leaves of *S. calophyllifolium*Walp were collected from Doddabetta, The Nilgiris, Tamilnadu. The leaves were dried under shade at room temperature to a constant weight. The dried leaves were ground with the help of mixer grinder, pulverized and the powder obtained was stocked in a plastic container.

Preparation of Plant Extract: The varying amount of the coarse powder (100 µg/ml, 200 µg/ml, 400µg/ml, 800µg/ml, 1600µg/ml) was extracted successively with 250 ml methanol in a Soxhlet apparatus for 24 hrs. The extract was filtered through Whatman No.41 filter paper and concentrated in a rotary evaporator. All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity.

Estimation of Total Phenolics: Total phenolic content was estimated using Folin-Ciocalteau reagent based assay as previously described method[11] with little modification. To 1 mL of each extract (100 µg/mL) in methanol, 5 mL of Folin-Ciocalteau reagent (diluted ten-fold) and 4mL (75g/L) of Na_2CO_3 were added. The mixture was allowed to stand at 20ºC for 30min and the absorbance of the developed colour was recorded at 765nm using UV-VIS spectrophotometer. 1mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of Flavonoids: The flavonoids content was determined according to.[12] An aliquot of 0.5 ml of sample (1 mg/mL) was mixed with 0.1 ml of 10% aluminium chloride and 0.1ml of potassium acetate (1 M). In this mixture, 4.3 ml of 80% methanol was added to make 5mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415 nm. The value of optical density was used to calculate the total flavanoid content present in the sample.

DPPH Radical Scavenging Activity: The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H.[13] The free radical scavenging activity of all the extracts was evaluated by 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method.[13] Briefly, 0.1 mM solution of DPPH in methanol was prepared, and 1ml of this solution was added to 3 ml of the solution of the *S.calophyllifolium* extracts at different concentration (100, 200, 400, 800 & 1600 µg/ml). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10s UV, Thermo Electron Corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the

DPPH radical was calculated by using the following formula. DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0) X100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Hydroxyl Radical Scavenging Activity: The scavenging capacity for hydroxyl radical was measured according to the modified method of.[14] Stock solutions of EDTA (1 mM), FeCl3 (10 mM), Ascorbic Acid (1 mM), H_2O_2 (10 mM) and Deoxyribose (10 mM), were prepared in distilled deionized water. The assay was performed by adding 0.1 ml EDTA , 0.01 ml of $FeCl_3$, 0.1 ml H_2O_2, 0.36 ml of deoxyribose, 1.0 ml of the extract of different concentration (50, 100, 200, 400, 800 & 1600 µg/ml) dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH 7.9) and 0.1 ml of ascorbic acid in sequence. The mixture was then incubated at 37oc for 1 h. About 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10% TCA and 1.0 ml of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using the following equation. Ascorbic acid was used as reference.

Hydroxyl radical scavenging activity= $\{(A_0 - A_1)/A_0) X100\}$

Where, A_0 is the absorbance of the control reaction and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Superoxide Radical Scavenging Activity: The superoxide anion scavenging activity was measured as described by.[15] The superoxide anion radicals were generated in 3.0 ml of Tris – HCl buffer (16 mM, pH 8.0), containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract of different concentration (100, 200,400, 800 & 1600 µg/ml), and 0.5 ml Tris – HCl buffer (16mM, pH 8.0). The reaction was started by adding 0.5 ml PMS solution (0.12 mM) to the mixture, incubated at 25^oC for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation.

Superoxide radical scavenging activity= $\{(A_0 - A_1)/A_0) X 100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Antioxidant Activity by Radical Cation ($ABTS^+$): ABTS assay was based on the slightly modified method of.[16] ABTS radical cation (ABTS+) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium per sulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS+ solution was diluted with ethanol to an absorbance of 0.70 + 0.02 at 734 nm. After addition of 100 µl of sample or trolox standard to 3.9 ml of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 min. The results were expressed as ascorbic acid equivalent antioxidant capacity.

ABTS radical cation activity = $\{(A_0 - A_1)/A_0) \text{ X}100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

Reducing Power: The reducing power of the extract was determined by the method of.[17] About 1.0 ml of the extract of different concentration (100, 200, 400, 800 & 1600 µg/ml) was mixed with sodium phosphate buffer (5.0 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 ml, 1.0%). The mixture was incubated at 50^{o}C for 20 min. Then 5 ml of 10% trichloroacetic acid was added and centrifuged at 980 g (10 min at5^{o}C) in a refrigerator centrifuge. The upper layer of the solution (5.0 ml) was diluted with 5.0 ml of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed in triplicate determination and the results were averaged.

RESULTS

The total phenol and flavonoid content of the methanol extract of *S. calophyllifolium* leaf were found to be 1.12g/100g and 1.42g/100g respectively.

The scavenging effect increased with the concentration of standard and samples. At 1600 µg/ml concentration the leaves of *S. calophyllifolium* possessed 125.81± 0.25% scavenging activity on DPPH. Similarly, the highest scavenging activity of hydroxyl (103.87± 0.74), SOD (118.84± 0.68) and ABTS (117.85± 0.36) were found at higher concentration (1600

µg/ml) of the leaf extract of *S. calophyllifolium*. With regards to activity of reducing power at the highest concentration exhibited high when compared to standard activity (Table 1).

The result of antioxidant study of *S. calophyllifolium*methanolic extract exhibited a highest activity in DPPH (IC50-34.63), followed by Superoxide dismutase (IC50-33.64), ABTS (IC50-33.17) and least activity was seen in the Hydroxyl study.

Table 1: Effect of methonolic leaves extract of *S. calophyllifolium* on the in vitro study of different antioxidant assay.

Concentration (µg/ml)	DPPH		HYDROXL		SOD		ABTS		Reducing power	
	Extract	STD	Extract	STD	Extract	STD	Extract	STD	Extract	STD
	(AA)		(AA)		(AA)		(AA)		(AA)	
100	28.33 ±0.12	21.64 ±0.18	23.54 ±0.91	19.42 ±0.93	34.66 ±0.71	28.26 ±0.19	36.68 ±0.22	28.45 ±0.57	0.321 ±0.042	0.298 ±0.014
200	48.24 ±0.76	33.41 ±0.49	46.47 ±0.18	36.26 ±0.29	53.34 ±0.72	47.94 ±0.30	48.33 ±0.57	43.57 ±0.63	0.356 ±0.087	0.330 ±0.054
400	83.18 ±0.72	69.14 ±0.34	74.66 ±0.16	58.30 ±0.86	71.28 ±0.39	63.29 ±0.84	69.24 ±0.95	64.14 ±0.28	0.386 ±0.025	0.367 ±0.013
800	104.49 ±0.34	84.24 ±0.74	89.37 ±0.64	86.68 ±0.18	92.01 ±0.33	88.37 ±0.65	88.94 ±0.48	86.28 ±0.49	0.411 ±0.082	0.389 ±0.085
1600	125.81 ±0.25	98.12 ±0.18	103.87 ±0.74	98.71 ±0.24	118.84 ±0.68	102.47 ±0.49	117.85 ±0.36	98.34 ±0.44	0.438 ±0.027	0.403 ±0.079
IC 50	**34.63**	**22.58**	**31.45**	**28.13**	**33.64**	**30.54**	**33.17**	**29.82**	-	-

AA-Ascorbic acid

Each value is expressed as percentage of activity mean ± standard deviation (n=3).

DISCUSSION

The free radicals mediated toxicity can be effectively eliminated by plant derived antioxidant compounds[18] such as flavonoids, tannins, alkaloids, quinines, amines, vitamins and other secondary metabolites possess anti-inflammatory, anti-carcinogenic, antibacterial and antiviral activities.[19] Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plants.[20] The beneficial effects of these molecules are related to their antioxidant activity.[21] The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties.[22] The present study revealed that the methanol extract of *S.calophyllifolium* leaf contains phenol and flavonoids in considerable amount.

The previous study[23] reported the total phenolic, total flavonoid and the antioxidant activity of *Syzygium polyanthum* (Wight) Walp leaves (Myrtaceae). Their study revealed the presence of gallic acid and caffeic acid in the methanolic extract of the leaves could lead to the mild antioxidant property of *S. polyanthum*. The total phenol and antioxidant activity of *Syzygium*

cuminii(L.) fruits (Myrtaceae) were previously investigated.[24] According to their findings the fruit was the richest source of total phenol and possessed highest antioxidant activity.

The methanolic leaves extract of *Cassia tora* Lin. (Caesalpiniaceae) was evaluated *invitro*[25] and the result showed that the plant has potent *invitro* antioxidant activity. The author(s) suggested that the antioxidant activity may be attributed to the phenolics present in methanolic extract of the leaves of *C.tora.* The high antioxidant activity and the very important values for polyphenols were noted in the methanolic leaves extract of *Phoenyx dactylifere* (Arecaceae).[26] The *invitro* free radical scavenging activity of aqueous and methanolic leaf extracts of *Aegle tamilnadensis* Abdul Kader (Rutaceae) was studied earlier.[27] The study indicated that the methanolic leaf extract showed better antioxidant activity than the aqueous leaf extract and the activity observed may be due to the presence of phenolic and flavanoid content in the methanolic extract.

CONLUSIONS

From the present study it can be concluded that the leaves of *S. calophyllifolium* contain phenol and flavonoid. The methanolic leaf extract of *S.calophyllifolium* possessed the potent free radical scavenging ability.

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