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Total polyphenol and flavonoid content of *Syzygium jambos* (L) Alston leaf extracts and its *in vitro* DPPH radical scavenging activity

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ABSTRACT

Background: To investigate the total polyphenol and flavonoid content of *Syzygium jambos* leaf extracts and to correlate DPPH quenching by *in vitro* method. **Methods:** Shade dried leaves were extracted by cold maceration method and spectrophotometric method was adopted to evaluate the DPPH radical scavenging potential using standard procedures. **Results:** The total phenolic content (TPC) of ethanolic and chloroform leaf extracts were predicted as 42.72±0.07and 31.06±1.4 GAE/g of extract respectively. The TPC of ethanolic and chloroform leaf extracts accords with the radical destroying mechanism possessing strongest antioxidant activity against DPPH free radical with IC_{50} values 114.56±0 and 132.91±0.007 µg/mL respectively. A moderate radical shooting activity was observed in methanolic leaf extract exhibiting an IC_{50} 159.57±0.003 µg/mL, which was not up to the significance compared to the standard ascorbic acid with IC_{50} 66.5±0 µg/mL. **Conclusion:** A positive radical scavenging correlation was exhibited by polyphenolic contents of ethanolic, chloroform and methanolic extracts whereas, the flavonoids of the *S.jambos* leaf extract displayed a low correlation in radical quenching. Studies suggest that apart from plant phenols as a major phytometabolites in free radical scavenging, other secondary metabolites in plants may also be accountable for antioxidant mechanism. Further studies are obligatory to identify the exact compound for the antioxidant potential in *S.jambos* leaf extracts.

Keywords: Antioxidation, Free radicals, Polyphenols, Scavenging potential

1. INTRODUCTION

The lifestyle of communities has steered with an inordinate variation in physiology and metabolism exposed to an extensive diseases and disorders. Free radicals and reactive oxygen species generates oxidative damage of tissues leading to atherosclerosis, aging, coronary and neurodegenerative diseases.[1] Considerable interest has now been developed in the discovery of naturally occurring antioxidants as an alternative to synthetic radical scavengers, which has been delimited due to their carcinogenic effects on biological system.[2] The generation of natural antioxidant polyphenols, flavonoids, vitamin C and E from the terrestrial plants are considered with an excessive importance as an alternative source of synthetic antioxidant molecules. Many species of edible and inedible plants has been renowned with important medicinal properties exhibiting beneficial impact on health, antioxidant, digestive stimulation, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic and anticarcinogenic potential.[3]

Syzygium jambos (L) Alston (Family: Myrtaceae) called as Champai, is a folklore Indian traditional medicinal plant native to Southeast Asia naturalized in India. The plant is grown in villages of Western Ghats, Coimbatore and in Kerala for its fruits as well as medicinal importance. Literature reviews on *S.jambos* fruit tonic, flowers, seeds, leaves, bark and root preparations either in a form of decoctions or extracts has been used in different ailments such as central nervous system disorders, diuretic, gastrointestinal, diabetes, rheumatism, microbial infections, lung diseases and disorders.[4-5] The tribal community in Western Ghats of Coimbatore district empirically use *S.jambos* extracts for health needs, which has been acquired among them as an undocumented oral communication from generation to generation. The above literature review enlightens the importance and medicinal value of plant polyphenols, flavonoids and their significance in ailments. In viewing the medicinal uses of *S. jambos* in folklore and traditional oral histories, the present investigation was made to evaluate the total polyphenol and flavonoid content of *S.jambos* leaf extract and its correlation with free radical scavenging potential.

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2. MATERIALSAND METHODS

2.1. Collection of plant material

The plant sample was collected from Western Ghats, Coimbatore, Tamil Nadu, India and authenticated as *Syzygium jambos* (L) Alston (Family: Myrtaceae) by Dr. G.V.S. Murthy, Scientist 'F' and Head, Botanical Survey of India, Southern Regional Centre, Coimbatore. The voucher specimen (No: BSI/SRC/5/23/ 2012-13/Tech.1269) was de-

posited in the Department of Microbiology, RVS College of Arts & Scince for future references. The plant leaves were collected based on the information's among tribal such as Irulas, Konars and Malasar community living in and around the Western Ghats.

2.2. Plant extract preparation

The fresh disease free powdered leaves were defatted with petroleum ether for 24 hours, filtered and allowed to dry for complete evaporation of solvent. About 200 gms of defatted plant powder was extracted with 800 mL of organic solvents *viz*. methanol, ethanol, chloroform, and distilled water by cold maceration method. After a week of socking, filtration was conducted with whatmann filter paper no.1, and concentrated *via*. rotary vacuum evaporator. The concentrated crude extracts were stored at 4°Cfor further analysis.

2.3. Qualitative and quantitative phytochemical analysis

The crude leaf extracts were subjected to qualitative phytochemical tests.[6] The quantitative tests for alkaloids, tannins, saponins and flavonoids were accomplished by standard procedures,[7-9] and the residue of each filtrate was calculated as percentage of the dried fraction.

2.4. Determination of total polyphenol and flavonoid content (TPC and TFC)

The TPC was determined by Folin-Ciocalteu method[10] calculated from a standard gallic acid calibrated curve, recording the spectrophotometric absorbance of solutions (10-180 µg/mL) at 765 nm. The results of TPC were expressed as mg gallic acid equivalent (mg GAE/g)/ gram of extract. The flavonoid content of the extracts was determined following the method.[20] The TFC of the extracts were determined by a standard procedure[11] with rutin standard and TFC was expressed as rutin equivalent (RuE/g)/gram of extract.

2.5. *In vitro* free radical scavenging assay

Antioxidant activity of *S.jambos* leaf extracts were executed by a standard procedure[12] using free radicals 2, 2-diphenyl-1-picrylhydrazyl (DPPH). About 3 mL of test plant extract (5-150 µg/mL) was mixed with 1 mL of absorbance (<1.00 at 517 nm in spectrophotometer) adjusted 0.1mM DPPH solution remained at room temperature in dark and the absorbance was recorded after 30 minutes incubation. Standard ascorbic acid and absorbance adjusted DPPH solution was used as positive and negative controls in the assay. Spectrophotometric absorbance of the mixtures was measured at 517 nm. The percentage inhibition of DPPH radical quenching by the sample as well as standard was calculated as follows:

$$\text{DPPH Scavenging (\%)} = \frac{\text{OD of negative control-OD of plant sample treated}}{\text{OD of negative control}} \times 100$$

The DPPH reducing ability was considered to be directly proportional to the quantity of antioxidants present in extracts. Concentrations yielding 50% inhibition (IC_{50}) was calculated by interpolation from linear regression analysis. The $1/IC_{50}$ coefficient was calculated to determine the correlation between antioxidant activity of the constituents such as total polyphenol and flavonoid content in the plant extracts.

2.6. Statistical analysis

The data obtained in the present study are mean±SEM of three replicate determinations. Statistical comparisons employed one way ANOVA by Dunnett's multiple comparison test with control by GraphPad Prism software (Version 5.0), $P<0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Qualitative and quantitative phytochemical screening

The preliminary phytochemical screening test of *S.jambos* leaf extracts revealed the presence of alkaloids as well as flavonoids in SJMLE, SJELE and SJCLE. Besides SJALE, qualitatively positive tannin presence was observed in SJELE. A specific group of saponins called triterpenoid was observed in SJMLE and SJELE. Along with SJMLE a dietary essential phytosterol presence was predicted positive in SJCLE and besides polyphenols the presence of saponins were also confirmed in all the four extracts.

The quantitative analysis of *S.jambos* leaf extracts (Table 1) revealed the presence of alkaloids in SJCLE (0.213±0%), SJELE (0.191±0.01%), SJMLE (0.151±0.01 %) and SJALE (0.008±0%). The perception of flavonoid was higher in SJCLE (0.4±0%), compared to SJELE (0.283±0.02%), SJMLE (0.267±0.01%) and SJALE (0.025±0%). The water soluble polyphenols called tannins were higher in SJALE (0.251±0.01 %), with its least presence in SJELE (0.108±0.01%), SJMLE (0.079±0.03%), and SJCLE (0.057±0%). An appreciable quantity of saponin was estimated in SJALE (0.467±0.02%) with lower its predictability in SJELE (0.061±0.01%), SJMLE (0.045±0%) and SJCLE (0.032±0.01%).

Table 1. Percentage phytochemical constituents, total polyphenol content and flavonoid content (TPC & TFC) of *S. jambos* leaf extracts

Plant Extract	Alkaloids	Flavonoids	Tannins
SJALE	0.008±0^{d}	0.025±0^{d}	0.251±0.01^{a}
SJMLE	0.151±0.01^{c}	0.267±0.01^{c}	0.079±0.03^{c}
SJELE	0.191±0.01^{b}	0.283±0.02^{b}	0.108±0.01^{b}
SJCLE	0.213±0^{a}	0.4±0^{a}	0.057±0^{d}

Plant Extract	Saponins	TPC (mg GAE /g)	TFC (RuE/g)
SJALE	0.467±0.02^{a}	10.5±2.01^{d}	21.67±1.01^{d}
SJMLE	0.045±0^{c}	24.94±0.02^{c}	60.56±0.32^{b}
SJELE	0.061±0.01^{b}	42.72±0.07^{a}	39.44±0.11^{c}
SJCLE	0.032±0.01^{d}	31.06±1.4^{b}	69.44±2.15^{a}

SJALE- *S.jambos* aqueous leaf extract; SJMLE- *S.jambos* methanolic leaf extract; SJELE- *S.jambos* ethanolic leaf extract; SJCLE- *S.jambos* chloroform leaf extract. Data's were expressed as mean±SEM (n= 3). Superscript letters ranging from a to d indicate difference at least significant level.

The determination of total polyphenol content was accomplished by a standard gallic acid curve with a linearity ranging between 10 to 180 µg/mL concentrations. The occurrence of polyphenol was higher in SJELE (42.72±0.07 mg GAE/g) compared to SJCLE, SJMLE and SJALE with 31.06±1.4, 24.94±0.02, and 10.5±2.01 mg GAE/g of extract respectively. Similar existence of total flavonoid was calibrated by standard rutin curve with linearity ranging between 10 to 250- µg/mL concentrations. The SJCLE revealed higher flavonoid content with 69.44±2.15 mg RuE/g of extract, followed by SJMLE, SJELE, and SJALE with 60.56±0.32, 39.44±0.11 and 21.67±1.01 mg RuE/g extract respectively. The observation suggests that the polyphenols and flavonoids are highly docketed by ethanol and chloroform fractions respectively.

3.2. Antioxidation correlation with polyphenol and flavonoid content

A dose dependent DPPH radical quenching of *S.jambos* extracts were depicted in Table 2 and fig- 1. With response to free radical destruction at 180 µg/mL, the percentage of antioxidant activity in SJELE (77.61%), SJCLE (73.27%) and SJMLE (54.85%) were predicted with IC_{50} values of 114.56, 132.91 and 159.57µg/mL respectively, ascertaining the scavenging ability of antioxidant metabolites in the extracts. The presences of plant secondary metabolites are generally correlated for their antioxidant potential in the *in vitro* experiments using DPPH.[13] In the present study the $1/IC_{50}$ was predicted towards the IC_{50} values of *S.jambos* extracts and a correlation was generated for the antioxidant potential with TPC and TFC using a linear relationship curve accessible in Fig.2. The *S.jambos* phenolic contents revealed a highly positive correlation by effectively shooting the free radicals and the correlation coefficient was expressed as $R^2 = 0.9642$. On the other hand a low correlation was observed between total flavonoid contents ($R^2 = 0.4572$) indicating the flavonoids are less significant in free radical scavenging. We also accomplish that the antioxidant activity of *S.jambos* extracts are not limited to phenolic and flavonoid compounds but may also be related to the presence of other antioxidant secondary metabolites that may present in *S.jambos* leaves.

Table 2. DPPH radical scavenging activity of *S.jambos* leaf extracts and its IC_{50} concentrations

Conc. µg/ml	Ascorbic acid	SJALE	SJMLE	SJELE	SJCLE
10	6.45±0	0	3.82±0	4.37±0	4.23±0
20	16.19±0.001	0	7.512±0	13.38±0	18.05±0.02
40	38.51±0	1.56±0.001	12.18±0.007	25.3±0	20.42±0.003
80	55.87±0.001	3.17±0	16.26±0	41.42±0.001	29.79±0.001
120	69.87±0.002	6.09±0.01	40.59±0.005	51.34±0	43.61±0.001
180	88.4±0.002	8.35±0.02	54.85±0.001	77.61±0	73.27±0.001
IC_{50}	**66.5±0****	**ND**	**159.57±0.003^{ns}**	**114.56±0***	**132.91±0.007^{ns}**

Data's were expressed mean±SEM (n= 3). Values in the parenthesis represent the percentage DPPH scavenging. ND-Not detected; Inhibitory concentration (IC50) with *, ** represents the values are significantly different at p<0.05 and p<0.01 respectively analyzed by Dunnett's multiple comparison test; ns- not significant.

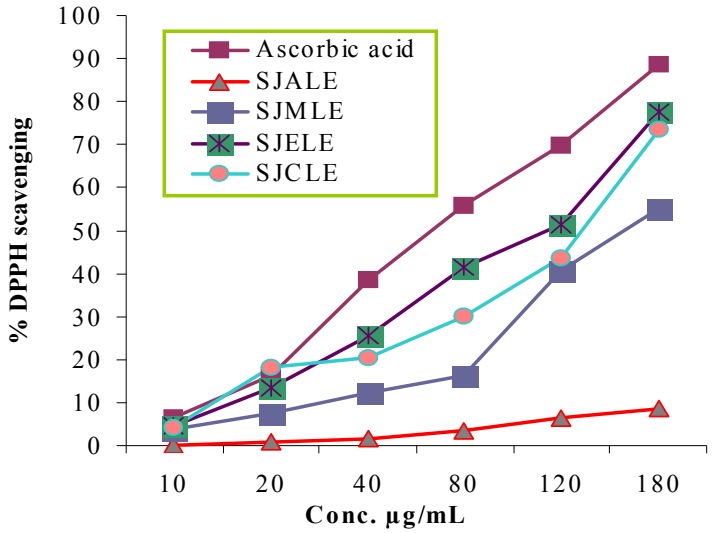

Fig.1. DPPH scavenging activity of *Syzygium jambos* leaf extracts

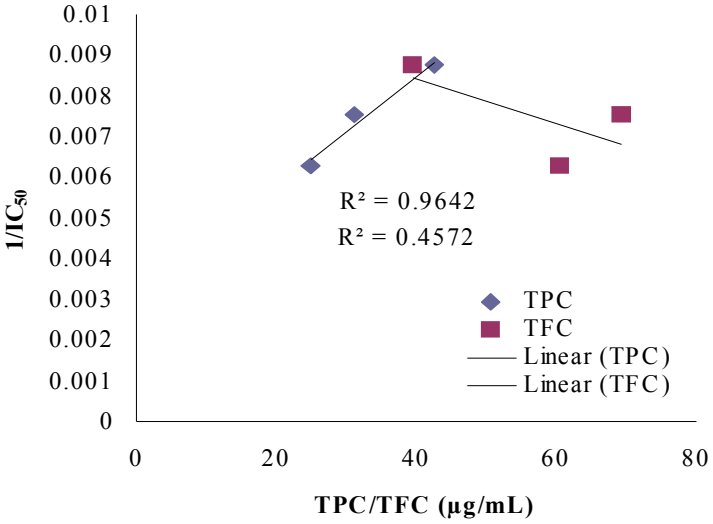

Fig. 2 Correlation of total polyphenol and flavonoid content of *Syzygium jambos* leaf extracts

3. CONCLUSION

In the present investigation higher content of phenolics and flavonoids were observed in ethanolic and chloroform extracts of *S.jambos*. The phenolic correlation with antioxidation was found to be higher compared to the flavonoids. Apart from the scavenging potential of phenolics, the non-phenolic substances may also responsible for antioxidant activity in the species. Furthermore, the quality of extracts depends principally by the solvent used in extraction procedure. The ethanol was found to be the best solvent in liberation of phenolics, besides the chloroform and methanol proved to be potential in flavonoid release. Our study correlates with the previous reports related to antioxidant activity, hence, we conclude that the extracts from leaves of *S.jambos* are good sources of antioxidants and their antioxidant properties are encouraging relevant to previous report.[14]

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