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EVALUATION OF ANTIOXIDANT POTENTIAL AND HPTLC, GC-MS ANALYSIS OF PHYTOCOMPOUNDS FROM LEAF EXTRACTS OF *SYZYGIUM JAMBOS* (L.) ALSTON AND *SYZYGIUM TRAVANCORICUM* GAMBLE

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ABSTRACT
The leaf powders of the *Syzygium jambos* and *Syzygium travancoricum* samples were subjected to the screening of phytocompounds (HPTLC and GC-MS) and antioxidant activity. Ethanol extracts of the leaf were subjected to HPTLC in which different mobile phase was tried to separate the bioactive compounds like alkaloids, flavonoids, glycosides, terpenoids, and saponins. GC-MS analysis revealed the presence of 29 compounds in the hexane extract of both the selected samples. From the twenty-nine compounds identified, the most common compounds were Dodecane, 2, 6, 10-trimethyl (11%), 1,2-Benzenedienal acid, mono (2-ethylhexyl) ester (10.76%), Nonane, 3-methyl (8.9%), n-Hexadecanoic acid (15.53%), 2-Heptenal, (z) (14.34%). The antioxidant activity of the leaf samples was determined using *in-vitro* methods. The results of the present study indicated that the ethanol extract of *S. travancoricum* had highest rate of antioxidant activity. However, further pharmacological and clinical studies would be required to investigate *in vivo* mechanism of nutraceuticals effects and toxicity of the selected plant materials.

KEY WORDS: HPTLC analysis, GC-MS, Antioxidant activity, *Syzygium jambos* and *Syzygium travancoricum*.

INTRODUCTION

Syzygium jambos L. (Aloston) and *Syzygium travancoricum* Gamble belong to the family Myrtaceae *S. travancoricum* is an evergreen tree species growing up to 25 m. in height. The bark of the tree is longitudinally fissured and greyish brown in colour. Leaves are simple, petiolate and large in size. Flowers are small, bisexual and white in colour. *S. jambos* is commonly known as rose apple (Lim, 2012) which is widespread in sub-saharan Africa (Adjanohoun, 1989), Central America and Asia (Maskey and Shah,1982). The plant is reported to be used for a variety of ailments and is known for its antipyretic and anti-inflammatory properties. All parts of the plant are indicated to have medicinal value. The leaf decoction is used as a diuretic, a remedy for sore eyes and for rheumatism (Morton, 1987). Seeds and bark are administrated to relieve asthma, bronchitis, diabetes, dysentery,and diarrhea (Morton, 1987). A decoction of the bark is administrated to relieve asthma and bronchitis (Lim, 2012). Previous researchers who have investigated the plant have documented its potential pharmacological value. However, very meager research work has been done of leaf materials of *S. jumbos* and *S. travancoricum*. Hence, in the present research work was carried out to find out its phytocompounds and antioxidant properties in two medicinally important leaf samples of *Syzygium jambos* and *Syzygium travancoricum*.

MATERIALS & METHODS

Collection of plant samples

Fresh plant leaves of *Syzygium jambos* (Alston) L. and *Syzygium travancoricum* Gamble were collected from the natural strands of Nadugani forest, Devala, The Nilgiris, Tamil Nadu. The botanical identity of the collected specimens was properly authenticated by Botanical Survey of India (Southern Circle), Coimbatore. The voucher specimens were deposited at the library of BSI –TNAU campus, Coimbatore, Tamil Nadu, India. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtiught container.

Extract preparation

Fifty grams of air-dried powder was taken along with 200 ml of Hexane, Chloroform, Ethyl acetate, Ethanol and water sequentially in a conical flask, plugged with cotton wool and they were shaken at room temperature for 2 days. After 2 days the supernatant was collected and the solvent was evaporated and stored at 4°C in airtight bottles. The percentage yield of the extracts from each solvent extraction was calculated.

Preliminary phytochemical analysis

Qualitative and quantitative tests for the screening of certain phytochemical compounds were performed on the hydroalcoholic extracts of leaves of *S. jambos* and *S. travancoricum* using standard procedures (Harborne, 1973; Trease and Evans 1989; Sofowara, 1993) as reported by Shad *et al*. (2013).

Estimation of total flavonoids

Total flavonoid content was determined using aluminium chloride ($AlCl_3$) according to the method of (Zhishen *et al*., 1999) using quercetin as a standard. The extract (0.1 ml) was added to 0.3 ml distilled water followed by 5% $NaNO_2$ (0.03 ml). After 5 min at 25°C, $AlCl_3$ (0.03 ml, 10%) was added. After 5 min, the reaction mixture was

treated with 0.2 ml of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. The results were expressed in percentage basis.

Estimation of total alkaloids

The total alkaloid content of plant extracts were estimated according to the method described in Indian Pharmacopoeia (2014). The extracts were weighed (5g each) separately and 100 ml of alcoholic ether mixture (4:1 ratio, v/v) was added with 2 ml of dilute ammonia solution, shaken well and allowed to stand for 1h. Then the solution was filtered with Whatman No. 41 paper and filtrate was collected in a separating funnel and 30 ml of 1 N sulphuric acid was added and shaken well. The acid layer was collected in another separating funnel. Then 25 ml of 0.5N alcoholic sulphuric acid (3:1) was added, extracted for 3 min and the acid layer was collected in the separating funnel. The extraction was repeated until the solution becomes colorless. The collected acid layer was extracted with chloroform in order to remove the extraneous matter and the pH was adjusted to 10 with dilute ammonia solution until alkaloids get precipitated. The chloroform layer was collected into a pre-weighed beaker through a funnel containing sodium sulphate and evaporated to dryness over a water bath. The weight of the residue was measured and the resultswere expressed in a percentage basis.

Estimation of total tannins

The extract (1 g) was digested with 50 ml of water and heated in a water bath for 30 min with frequent stirring (Rajpal, 2011). The supernatant was collected into a volumetric flask and the extraction was repeated until the solution becomes colourless. The solution was cooled and made upto a volume of 100 ml of distilled water from which 25 ml was taken with 750 ml of water and 25 ml of indigo sulphonic acid solution. The contents were titrated against 0.1M potassium permanganate solution with constant stirring until golden yellow colour appears. A blank was also performed without the sample. Each ml of 0.1 M potassium permanganate solution is equivalent to 0.004157 g of tannins. Based on the titration value, the total tannin content was calculated.

Estimation of total phenols

The total phenolic content of the extracts was determined using Folin-Ciocalteu reagent method (McDonal *et al.*, 2001). Different concentrations of standard and samples were prepared and mixed with 1.5 ml of Folin-Ciocalteu reagent, and after 5 min 4 ml of 20% Na_2CO_3 solution was added and made up to thevolume of 100 ml with distilled water. Then the absorbance was recorded at 765 nm. Gallic acid was used as a standard and the results were expressed in a percentage basis.

HPTLC studies

HPTLC studies were carried out using the method of Harbone (1998) to obtain the characteristic finger printing profile of plant extracts. The extract (500mg) was dissolved in water-alcohol (7:3, ratio, v/v) and 20 µl was applied on a pre-coated silica gel plates (60 $F_{254,}$ 0.2 mm thickness, 10 x 10 cm size, Merck, Germany) by using an automizer of HPTLC (CAMAG Linomat-5, Muttenz, Switzerland). The plate was developed in the solvent system to a distance of 8 cm using the mobile phase (Toluene: Ethyl acetate: Formic acid, 2:1:1 ratio). After development, the plate was dried in a hot-air-oven and visualized at 254 and 366 nm. The plate was scanned densitometrically, and the R_f values and color of the resolved bands were recorded.

GC-MS analysis

The volatile compounds of plant extracts were analyzed for phytochemical profiling byusing Gas Chromatographic system coupled with Mass Spectrometry (Perkin Elmer, Model: Clarus-500). Silica capillary column (30 m x 0.25 mm, 0.25 µm film thicknesses, Elite-5 MS non-polar fused 5% Phenyl 95% dimethylpolysiloxane) was used. Oven temperature was programmed with an increase of 6°C/min from room temperature to 150°C, and then an increase of 4°C/min from 150°C to 280°C was set. The injector temperature was 280°C. The carrier gas was helium with the flow rate of 1 ml/min. Sample (1.0 µl) was injected with as plit ratio of 1:10. Ionization energy 70 eV was used in the electron ionization mode; ion source temperature was set at 160-200°C, the mass was scanned in the range of 40-600amu. The instrument was operated using Turbo mass software version 5.2.0. The resulting mass spectrum was compared with inbuilt NIST library (2005) database and fragments of various compounds present in the extracts were identified.

Antioxidant Activity

The antioxidant activity of crude extracts was determined by six different in-vitro standard methods such as DPPH free radical scavenging activity, Phospo- molybdenum assay, Ferric Reducing Antioxidant Power assay (FRAP), Superoxide radical scavenging activity, Hydrogen peroxide scavenging activity and Hydroxyl radical scavenging activitymethods.

DPPH Radical scavenging activity

The DPPH free radical scavenging activity was determined (Yang *et al.*, 2008) with minor modifications. To each different concentration of sample in methanol (0.25 ml) was added 2 ml of methanolic solution containing 4×10^{-4} M DPPH. The mixture was mixed vigorously and left to stand for 30 min in the dark. The absorbance was then determined at 517 nm. The absorbance of the control was obtained by replacing the sample with methanol. DPPH radical scavenging activity of the sample was calculated as follows:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control OD} - \text{Sample OD}}{\text{ControlOD}} \times 100$$

Phospo- molybdenum assay

The total antioxidant capacity of the extracts was evaluated by the Phospho-molybdenum method described by (Ali *et al.*, 2008). The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate /Mo complex at acidic pH.0.3 ml each extract (6%) in triplicates were combined with 3 ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate, and 4mM Ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature.

Ferric reducing antioxidant power assay (FRAP)

FRAP solution (3.6 ml, 0.3 M of Acetate buffer – pH 3.6; 10 Mm of TPTZ in 40 mM of HCl and 10mM of $FeCl_36H_2O$) is added to distilled water (0.4 ml) and incubated at 37°C for 30 min. Then this solution mixed with certain concentration of the plant extract (30 ml) which was measuredat 593 nm. For construction of the calibration curve, five concentrations of $FeSo_4,6H_2O$ (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions (Benzie *et al.,* 1996).

Superoxide radical scavenging activity

The capacity of extracts to scavenge the superoxide anion radical was measured according to the method described by (Zhishen *et al.,* 1999). The reaction mixture was prepared using 3 x 10^{-6} M riboflavin, 1 x 10^{-2} M methionine, 1 x 10^{-4} M nitrobluetetrozoliumchloride and 0.1 mM EDTA in phosphate buffered saline (pH 7.4). For the analysis, 3.0 ml of the reaction mixture was taken with 100 µl of extract in closed tubes and illuminated for 40 min under fluorescent lamp (18 W). The absorbance was then read at 560 nm against the un-illuminated reaction mixture. Results are expressed as superoxide radical scavenging activity on percentage basis.

Hydrogen peroxide scavenging activity

The effect of extracts on hydrogen peroxide was analyzed according to the method proposed by (Ruch *et al*., 1989). The extract (100 microliter) was mixed with 5 ml of 45 mM hydrogen peroxide solution in 0.1 M phosphate buffer (pH 7.4). The reaction mixture was vortexed and incubated for 30 min at room temperature and then the absorbency was measured at 230 nm. The extract with phosphate buffer is used as a blank and the level of hydrogen peroxide remaining in the solution was calculated using a calibration curve. The hydrogen peroxide inhibition effect of extract was calculated and expressed on percentage basis.

Hydroxyl radical scavenging activity

The hydroxyl radical quenching activity of extracts was evaluated according to the method of (Hagerman *et al*., 1998). The reaction mixture consists of 10 mMphospate buffer (pH 7.4), 2.8 mMDeoxyribose, 2.8 mM H_2O_2, 0.025 mM $FeCl_3$, 0.1mM EDTA and 0.1 mM ascorbic acid in a total volume of 3 ml. With the reaction mixture, 100 microliter of extract was added and incubated at 37^0C for 15 min. Then the reaction was terminated by the addition of 1 ml of 2.5% ice-cold TCA and 1% TBA. The reactants were mixed well and heated at 90^0C for 15 min in a water bath and cooled to room temperature. The chromogen was extracted with 1-butanol and absorbency was measured at 530 nm. Based on absorbency value, the hydroxyl radical scavenging activity of extracts was calculated and expressed on percentage basis.

RESULTS

Phytochemical screening

Hexane, Chloroform, Ethyl acetate, Ethanol and Aqueous extracts of the selected samples were subjected to qualitative organic analysis (Table 1 & 2). Reducing sugar, carbohydrates, saponins, phenolic compounds, lignin was found in hexane extract of both *S. jambos* and *S. travancoricum* leaf sample. Chloroform leaf extract of the both samples answered positively for carbohydrates, saponins and quinine. Reducing sugar and lignin were found in ethyl acetate extract of both leaf samples. Ethanol leaf extract of both samples of *S. jambos* and *S. travancoricum* answered positively for carbohydrates and lignin. Reducing sugar, glycosides, flavonoids, phenolic compounds and tannin were found to be present in *S. travancoricum*, whereas anthraquinone, proteins and amino acid were found to be present in *S. jambos*. Reducing sugar, carbohydrates, saponins, glycosides, flavonoids, phenolic compounds, tannin, anthraquinone, lignin, proteins and amino acid were found in aqueous extract of both *S. jambos* and *S. travancoricum* leaf samples.

TABLE 1 : Phytochemical screening of various extracts of *Syzygium jambos* leaf

Test	Reagents used	Hexane	Chloroform	Ethyl Acetate	Ethanol	Water
Alkaloids	Dragendroff's	–	–	–	–	H
	Mayer's	–	–	–	–	H
	Wagner's	–	–	–	–	H
	Hager's	–	–	–	–	H
Reducing Sugar	Fehling's	H	–	H	–	H
Carbohydrates	Molisch's	H	H	–	H	H
Saponins	Foam's	H	H	–	–	H
Glycosides	Anthrone	–	–	–	–	H
Steroids	Liebermann burchard	H	–	H	–	–
Flavanoids	Shinado's	–	–	–	–	H
Phenolic Compound	Ferric chloride	H	–	–	–	H
Tannin	Lead acetate	–	–	–	–	H
Quinone	Sulphuric acid	H	H	–	–	–
Anthraquinone	Aqueous ammonia	–	–	–	H	H
Lignin	Phloroglucinol	H	–	H	H	H
Proteins	Million's	–	–	–	H	H
Amino acids	Ninhydrin	–	–	–	H	H

+ referents- positive answer, _ referents-negative answer

TABLE 2 : Phytochemical screening of various extracts of *Syzygium travancorium* leaf

Test	Reagents used	Hexane	Chloroform	Ethyl Acetate	Ethanol	Water
Alkaloids	Dragendroff's	–	–	–	–	H
	Mayer's	–	–	–	–	H
	Wagner's	–	–	–	–	H
	Hager's	–	–	–	–	H
Reducing Sugar	Fehling's	H	–	H	H	H
Carbohydrates	Molisch's	H	H	–	H	H
Saponins	Foam's	H	H	–	–	H
Glycosides	Anthrone	–	–	–	H	H
Steroids	Liebermann burchard	–	–	–	–	–
Flavanoids	Shinado's	–	–	–	H	H
Phenolic Compound	Ferric chloride	H	–	–	H	H
Tannin	Lead acetate	–	–	–	H	H
Quinone	Sulphuric acid	–	H	H	–	–
Anthraquinone	Aqueous ammonia	–	–	–	–	H
Lignin	Phloroglucinol	H	–	H	H	H
Proteins	Million's	–	–	–	–	H
Amino acids	Ninhydrin	–	–	–	–	H

+ referents- positive answer, _ referents-negative answer

Estimation of phytoconstituents

Selected samples using ethanol solvent were prepared to examine the major phytocompound. In the present study flavonoids, alkaloids, lignin, tannin and phenol were quantified. Among the investigated phytocompuonds phenols were exhibited higher levels in both the samples (Table-3).

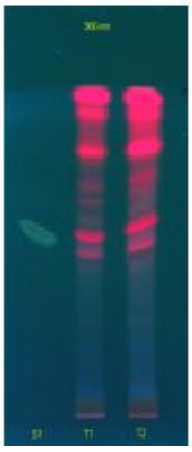
TABLE 3: Estimation of major phytoconstituents of *Syzygium jambos* and *Syzygium travancoricum* leaf

S.No.	Phytoconstituents	*Syzygium jambos*	*Syzygium travancoricum*
		Content (g/100 g sample)	
1	Flavanoid	1.33	3.60
2	Alkaloid	1.12	2.35
3	Lignin	0.74	1.22
4	Tannin	0.49	0.59
5	Phenol	2.60	4.72

HPTLC studies

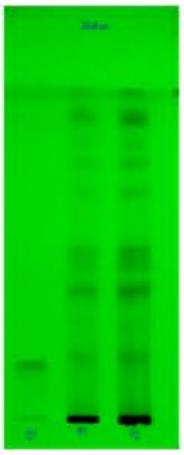
The HPTLC analysis was performed to obtain the characteristicfinger printing profile of selected plant extracts. A better separation with water-alcohol (7:3, ratio, v/v) was observed. The results for the HPTLC run were captured on the visualized, and the photograph of the chromatographic plate is presented in Fig-1 & 2 and Table 4 & 5).

HPTLC plate seen at 254 nm

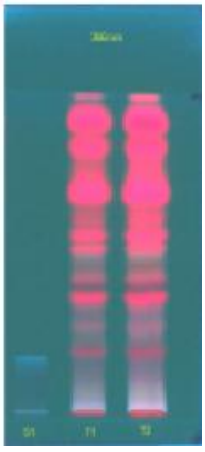


HPTLC plate seen at 366 nm

FIGURE 1: HPTLC profile of ethanolic extract of *Syzygium jambo* leaf



HPTLC plate seen at 254 nm



HPTLC plate seen at 366 nm

FIGURE 2 : HPTLC profile of ethanol extract of *Syzygium travancoricum* leaf

TABLE 4: Various secondary metabolites observed in HPTLC of ethanolic extract of *S. jambos* leaf

S.No	Name of the compound	Mobile phase	Spray reagent	Colour of the spot/band	
				Visibile light	UV (366nm)
1	Alkaloids	Ethyl acetate-methanol - water (10:1:35:1)	Dragendorff's reagent followed by 10 ethanolic sulphuric acid reagent	Yellow, Orange/Yellow	Range
2	Flavonoids	Toluen-acetone-formic acid (4.5:4.5:1)	1% Ethanolic aluminium chloride reagent	Ash/yellow	Ash-
3	Glycosides	Ethyl acetate-ethanol-water (8:2:1.2)	Anisaldehyde sulphuric acid reagent	Pinkish violet	-
4	Saponins	Chloroform-glacial acetic acid-methanol-water (6.4:3.2:1.2:0.8)	Anisaldehyde sulphuric acid reagent	Blue/Yellow/Green/violet	Violet
5	Terpenoids	n-Hexane-ethyl acetate (7.2:2.9)	Anisaldehyde sulphuric acid reagent	Blue	Blue

TABLE 5: secondary metabolites observed in HPTLC of ethanolic extract of *S. travancoricum* leaf

S.no	Name of the compound	Mobile phase	Spray reagent	Colour of the spot/band	
				Visibile light	UV(366nm)
1	Alkaloids	Ethyl acetate-methanol - water (10:1:35:1)	Dragendorff's reagent followed by 10 ethanolic sulphuric acid reagent	Yellow, Orange/Yellow	Orange
2	Flavonoids	Toluen-acetone-formic acid (4.5:4.5:1)	1% Ethanolic aluminium chloride reagent	Ash/yellow	Ash-
3	Glycosides	Ethyl acetate-ethanol-water (8:2:1.2)	Anisaldehyde sulphuric acid reagent	Pinkish violet	-
4	Saponins	Chloroform-glacial acetic acid-methanol-water (6.4:3.2:1.2:0.8)	Anisaldehyde sulphuric acid reagent	Blue/Yellow/Green /violet	Violet
5	Terpenoids	n-Hexane-ethyl acetate (7.2:2.9)	Anisaldehyde sulphuric acid reagent	Blue	-

GC-MS studies

The hexane extract of selected plants was analyzed for different classes of organic chemical constituents by using Gas Chromatographic system coupled with Mass Spectrometry (Perkin Elmer, Model: Clarus-500). The GC-MS results showed that medicinally valuable phytochemicals were present in the plant extracts (Fig. 3 & 4 and Table 6 & 7).

TABLE 6: GC-MS profile of hexane extract of *Syzygium jambos* leaf

S. No.	Phytocompounds	Formula	Molecular weight	Retention time	Peak area	%Peak area
1	Heptane, 2,2,3,5-tetramethyl-	$C_{11}H_{24}$	156	8.21	12097773	1.8861
2	2-Heptenal, (Z)-	$C_7H_{12}O$	112	8.35	10185596	1.5880
3	Heptane, 2,2,3,5-tetramethyl-	$C_{11}H_{24}$	156	8.50	7029960	1.0960
4	Octane, 3,3-dimethyl-	$C_{10}H_{22}$	142	8.92	9756205	1.5210
5	Heptane, 5-ethyl-2,2,3-trimethyl-	$C_{12}H_{26}$	170	9.47	51033712	7.9563
6	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212	9.77	70577472	11.0032
7	Heptane, 5-ethyl-2,2,3-trimethyl-	$C_{12}H_{26}$	170	10.18	49973040	7.7909
8	Nonane, 3-methyl-	$C_{10}H_{22}$	142	10.34	57669852	8.9909
9	Undecane, 3-methyl-	$C_{12}H_{26}$	170	10.61	49248872	7.6780
10	Undecane, 3,8-dimethyl-	$C_{13}H_{28}$	184	10.81	17569720	2.7392
11	Decane, 2,3,4-trimethyl-	$C_{13}H_{28}$	184	11.06	14434844	2.2504
12	Decane, 3-methyl-	$C_{11}H_{24}$	156	11.46	7821664	1.2194
13	2,4-Decadienal	$C_{10}H_{16}O$	152	15.20	7066797	1.1017
14	Caryophyllene	$C_{15}H_{24}$	204	17.81	7644363	1.1918
15	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	26.05	32527574	5.0711
16	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	27.46	14354317	2.2379
17	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264	28.52	82469656	12.8572
18	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	33.74	69070088	10.7682

TABLE-7 GC-MS profile of hexane extract of *Syzygium travancoricum* leaf

S. No.	Phytocompounds	Formula	Molecular weight	Retention time	Peak area	%Peak area
1	Octane	C_8H_{18}	114	4.64	15451957	2.2506
2	Hexanal	$C_6H_{12}O$	100	4.75	74878912	10.9060
3	2-Heptenal, (Z)-	$C_7H_{12}O$	112	8.00	98459864	14.3405
4	Hexanoic acid	$C_6H_{12}O_2$	116	8.94	7048877	1.0267
5	2-Decenal, (E)-	$C_{10}H_{18}O$	154	14.67	27702498	4.0348
6	2,4-Decadienal	$C_{10}H_{16}O$	152	15.41	69594144	10.1363
7	2,4-Decadienal, (E,E)-	$C_{10}H_{16}O$	152	15.92	89079616	12.9743
8	Benzene,(1-pentylheptyl)-	$C_{18}H_{30}$	246	23.01	9686806	1.4109
9	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	24.62	35051964	5.1053
10	n-Hexadecanoic acid	C16H32O2	256	27.40	106674760	15.5370
11	9,12-Octadecadienoicacid (Z,Z)-	$C_{18}H_{32}O_2$	280	31.79	77697776	11.3166

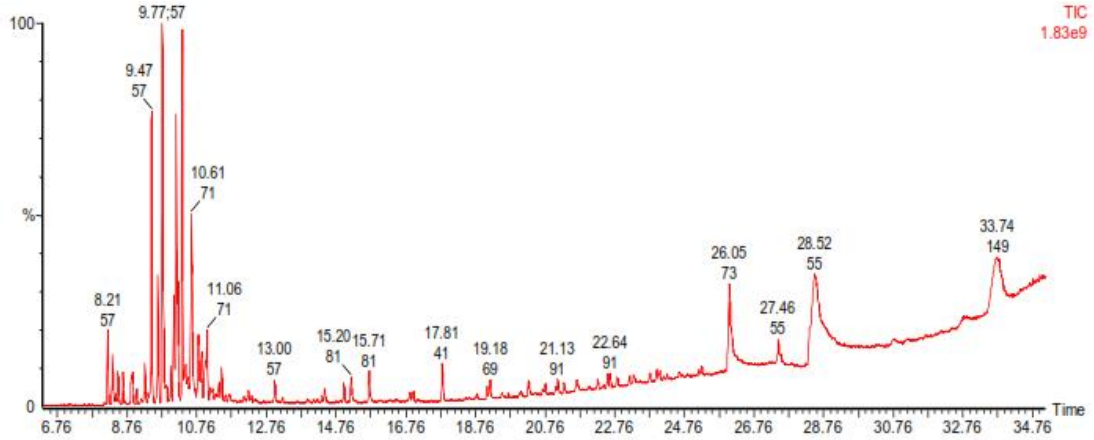


FIGURE 3: GC-MS profile of hexane extract of *Syzygium jambos* leaf

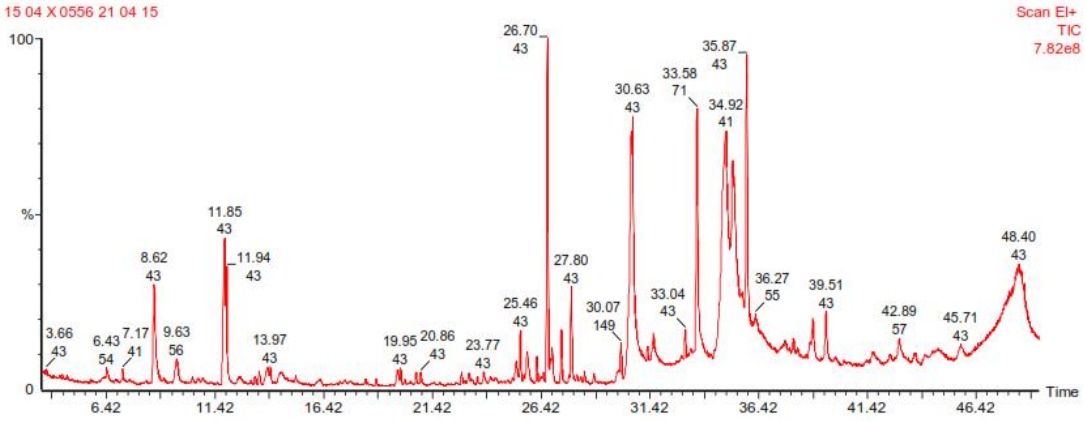


FIGURE 4: GC-MS profile of hexane extract of *Syzygium travancoricum* leaf

Antioxidant activity

The antioxidant activity of the extracts was determined using a DPPH scavenging activity, Phospo- molybdenum assay, Ferric Reducing Antioxidant Power assay (FRAP), Superoxide radical scavenging activity, Hydrogen peroxide scavenging activity and Hydroxyl radical scavenging activity.

The extracts of the leaf tissues possessed free radical scavenging properties, but to varying degrees, ranging from 28.44 to 43.44 % DPPH scavenging. Using the organic solvent extraction, ethanol showed the better DPPH scavenging activity. A maximum scavenging activity was offered by ethanol extract of *S. travancoricum* 43.44 % (Fig-5). The result of total antioxidant activityby phospho-molbdenum assayis shown in the Fig-6. It is based on the reduction of Mo (VI) to Mo (V) by the extract andsubsequent formation of green phosphate /Mo (V) complex at acidic pH. Total antioxidant activity of the phospho- molybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity (total antioxidant

capacity). The results indicate a concentration dependent total antioxidant capacity. It means that ethanol extract of *S. jambos* and *S. travancoricum* will have as much quantity of antioxidant compounds as equivalents of ferulic acid ofeffectively reduce the oxidant in the reaction matrix. Antioxidant capacity of ferulic acid has been as a reference standard from which plant extract with potential antioxidant activity is compared. The extracts of all the tested plant specimens possess total antioxidant activity, but varying degrees, ranging from 141.50 to 228.mg/ml. Using the organic solvent extraction, generally, ethanol showed better total antioxidant activity. A maximum total antioxidant activity was offered by ethanol extract of *S. travancoricum* (228. mg/ml), followed by ethanol extract of *S. jambos* (141.50 mg/ ml), at 1000 mg/ ml concentration (Fig-6).

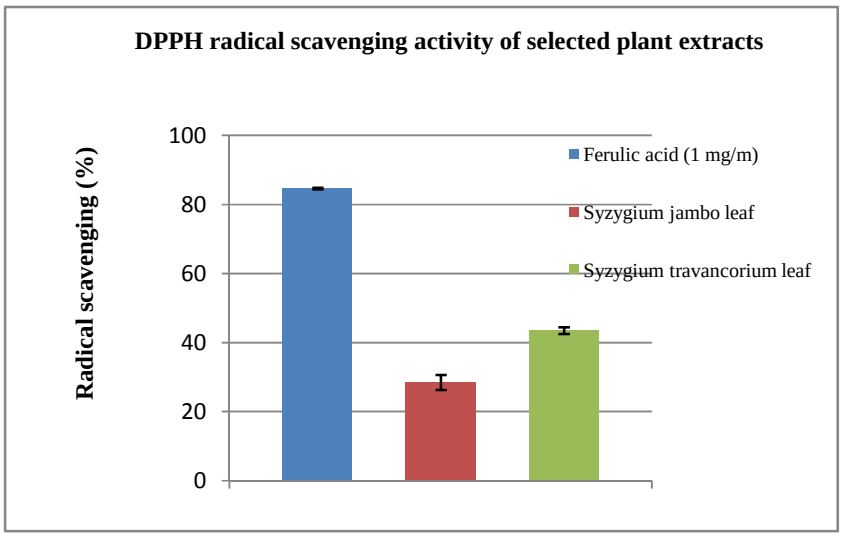


FIGURE 5: DPPH radical scavenging activity

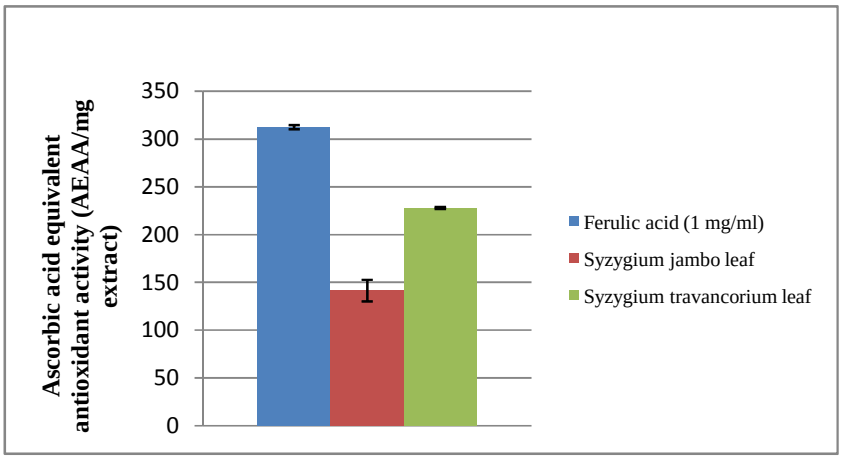


FIGURE 6: Phosphomolybdate reducing power

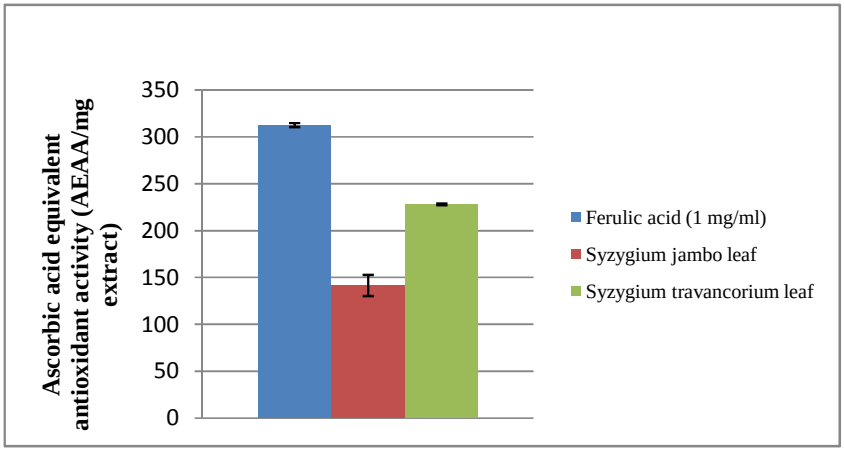


FIGURE-7 Ferric reducing power assay

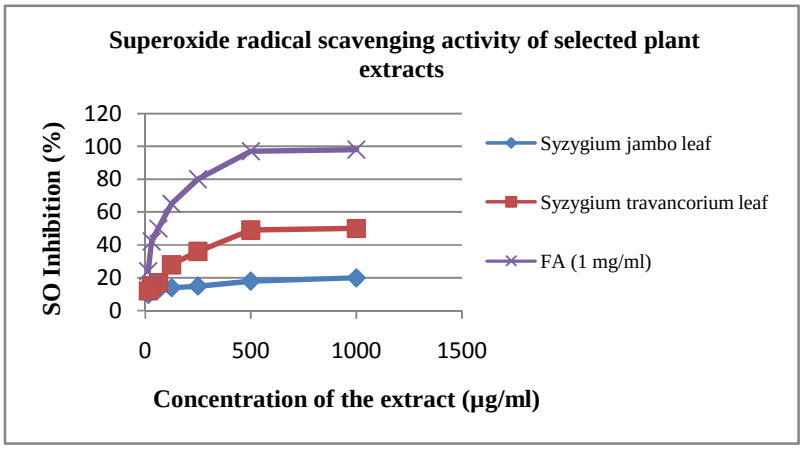


FIGURE 8: Superoxide radical scavenging activity

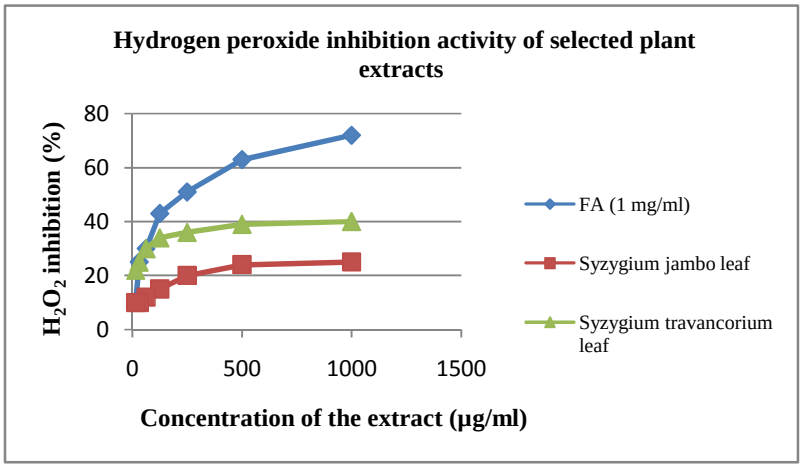


FIGURE 9: Hydrogen peroxide inhibition

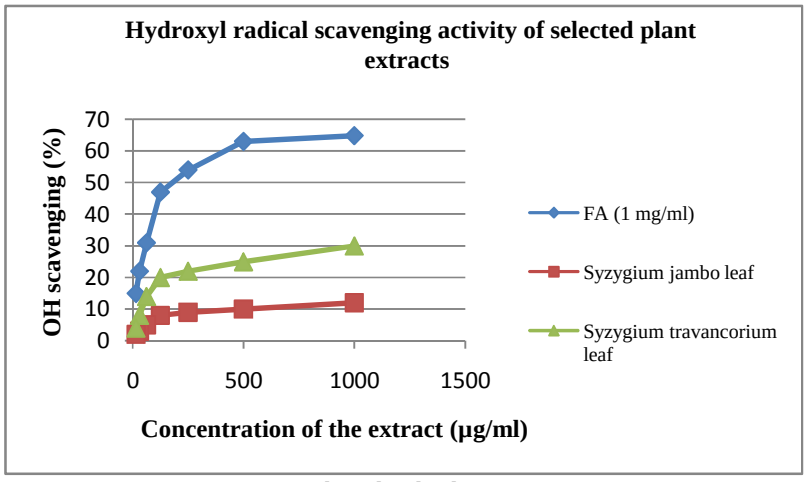


FIGURE-10 Hydroxyl radical scavenging activity

The total antioxidant activity of the plant sample is recorded in figure 7. The extracts showed considerable antioxidant activity ranging from 0.313 to 0.587mg/ml. The maximum total antioxidant activity was observed in ethanol extract of *S. travancoricum*. The superoxide free radical scavenging assay of plant sample was given (Fig-8), and it was found to be ranged from 20 to 50 %. Among the selected samples, the ethanol extract of *S. travancoricum* (50%) has exhibited the highest rate of free radical scavenging activity. The hydrogen peroxide scavenging activity of plant samples was given in (figure-9). It was found to be ranged from 25.02 to 40.06%. Among the selected

samples, the ethanol extract of *S. travancoricum* (40.06%) has exhibited the highest rate of free radical scavenging activity, followed by 20.02 % of *S. jambos*in ethanol extract. The hydroxyl radical scavenging activity of plant sample was studied (Fig-10), and it wasfound to be ranged from 12.01 to 30. 03 %. Among the selected samples, the ethanol extract of *S. travancoricum* (30.03%) has exhibited the highest rate of radical scavenging activity.

DISCUSSION

In nature, all plants are synthesized some chemicals by themselves to perform their physiological activities. In the present study, the investigated plants have exhibited different kinds of secondary metabolites. Especially phenols and flavonoids are the majorphytocompounds of the selected plant sample. The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these substances include alkaloids, glycosides, steroids, flavonoids, fatty oils, resins musilages, tannins, gums phosphorus and calcium for cell growth, replacement, and body building (Kubmarawa *et al*., 2008). Chemical constituents have been isolated from an aqueous extract of *Tinospora cordifolia* showed the presence active ingredients which is similar to that of current studies (Javeed Ahmed *et al*., 2011). *S. travancoricum* has exhibited the highest level of polyphenol (4.72g/100g) content. This results comparable to the earlier reports on *Adhatoda vasica* (leaves) (Vijayanandraj *et al.,* 2014); *Goniothalamus velutinus* (Erum Iqbal *et al.,* 2015) and *Achyranthes coynei* (Upadhya *et al.,*2015).

HPTLC fingerprint studies confirmed the results of phytochemical screening by the presence of various colored bands at different wavelengths (254nm, 366nm) with specific solvent systems, symbolizing the presence of particular phytocompounds. These results comparable to the previous study of methanolic leaf extract *Cassia fistula* reported the presence of alkaloids, flavonoids, triterpenoids, carbohydrates, glycosides, saponins, protein and aminoacid (Sujogya *et al.,* 2011). *Cassia auriculata* reported some of the phytocompounds in HPTLC fingerprint profile (Jyothi *et al.,* 2013). Different classes of organic chemical constituents were separated by Gas Chromatography and structure of the components was identified by MS Spectrophotometer.

The GC-MS results shows medicinally valued phytochemicals are present in the selected plant extracts of *S. jambos* and *S. travancoricum.* These compounds are well known as plant derived antioxidant agents. The GC-MS analysis of present samples revealed the presence of a number of bioactive compounds *viz*. 9, 1 Octadecadienal, (Z), Dodecane, 2,6, 10-trimethyl-,n-Hexadecanoic acid , 2,4-Decadienal, (E,E)-were determined, which is comparable to the earlier report on Syzygium aromaticum (Murugan, 2011) and *Commelina nudiflora* (Natanamurugaraj Govindan , 2015).

The antioxidant ability and radical scavenging properties of plants are associated with its medicinal values. The antioxidant activity of the extracts was determined using a DPPH scavenging activity, Phospo- molybdenum assay, Ferric Reducing Antioxidant Power assay (FRAP), Superoxide radical scavenging activity, Hydrogen peroxide scavenging activity and Hydroxyl radical scavenging activity. In antioxidant assays, Superoxide radical scavenging activity was found to be more effective free radical inhibition activity. Ethanol extract of *S. travancorium* were found to exhibit more effective free radical inhibition activity against superoxide radical scavenging activity. The free radical inhibition activity of crude ethanolic extract of selected powder samples was ranged between 20 % and50 %, which is in agreement with that of the previous reports on *Stachyslavan dulifolia* (9.99%), *Ocimum basilicum* (44%) (Abdul-Lateef Molan *et al*., 2012). Among the selected samples, the *S. travancoricum* showed the highest free radical scavenging activity. The free radical scavenging abilities of the phenolic extracts of both *S. jambos* and *S. travancoricum* leaves were investigated using different *in-vitro* models. The stable radical DPPH has been widely used for the determination of primary anti-oxidant activity (Brand-Williams *et al.,* 1995: Katalinic *et al.,* 2004). The DPPH and OH radical scavenging abilities of the phenolic extracts and the results are presented in Figure 5 to 10. The results of the present study showed that *S. travancoricum* exhibited highest scavenging ability than *S. jambos.* However the values revealed that the DPPH (43.44%) free radicals, ethanol extract had a significantly highest scavenging ability than the scavenged OH (30.03%) radicals which was compared to earlier report on *Cichorium intybus* leaves (Shalini Saggu , 2015) and would be an important candidate in pharmaceutical formulations and play an important role in improving the human health by participating in the antioxidant defense system against free radical generation.

CONCLUSION

S. travancoricum have highest potential of antioxidant activity comparable with that of *S. jambos*. The *S. jambos* leaves contains high levels of riboflavin that plays a major role in energy production and forms an essential nutrient in diet and protect human beings from sickle-cell disease and related ailments. However, pharmacological and clinical studies would be required to investigate *in vivo* mechanism of nutraceuticals effects. Further toxicity studies using human cell line are needed.

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