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Antioxidant Activity, Total Phenolics and Fatty Acid Profile of *Delonix regia, Cassia fistula, Spathodea campanulata, Senna siamea* and *Tibouchina granulosa*

Abstract

Tropical plants are utilized in traditional medicine and are a storehouse of secondary metabolites, some of which display medicinal properties. Five plants were selected for investigation, namely *Cassia fistula*, *Delonix regia*, *Senna siamea, Spathodea campanulata* and *Tibouchina granulosa*. Extracts of the inflorescence of these plants were prepared and their antioxidant, total phenolics and fatty acid profile were evaluated. Antioxidant and free radical scavenging activities were determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and total phenolics by the Folin-Ciocalteu assay. Lipids were Soxhlet extracted, methylated and analyzed by gas chromatography mass spectrometry. *C. fistula* and *S. siamea* exhibited the highest antioxidant and free radical scavenging activities. *T. granulosa* however contained the highest total phenolic content. Linoleic acid was identified as the predominant unsaturated fatty acid in *C. fistula* and *D. regia*, whereas oleic acid, was the predominant unsaturated fatty acid found in *S. campanulata*, *T. granulosa* and *S. siamea*. Palmitic acid was the major saturated fatty acid in all the lipid extracts with highest percentages being observed in *S. siamea*. Tropical flowers are an untapped source of natural antioxidants with potential health benefits.

Keywords: Antioxidant; Fatty acid; Phenolics; Tropical flowers; *Cassia fistula*; *Delonix regia*; *Senna siamea; Spathodea campanulata; Tibouchina granulosa*

Research Article

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Abbreviations: DPPH: 2,2-diphenyl-1-picryl-hydrazyl; FRSA: Free Radical Scavenging Activity; FAME: Fatty Acid Methyl Esters; NMR: Nuclear Magnetic Resonance Spectroscopy; GC-MS: Gas Chromatography Mass Spectrometry; IV: Iodine Value

Introduction

Jamaica has a diverse distribution of tropical flora. These plants possess a wide array of secondary metabolites which in some cases display medicinal properties which are unknown to the local populace. There is an ever increasing demand to evaluate the antioxidant properties of plant extracts with the aim of finding natural antioxidants which can replace the need for synthetic antioxidants. Five local trees namely *Cassia fistula*, *Senna siamea*, *Delonix regia*, *Spathodea campanulata* and *Tibouchina granulosa* (Figure 1) were selected for investigation. Extracts from the flowers of these plants were investigated as most of the scientific literature focuses on the biological activity of the leaves and stems of these plants.

Figure 1: Tropical flora.

Cassia fistula Linn belongs to the *Leguminosae* family. It is also referred to as Golden shower due to its beautiful yellow blooms. In Ayurvedic classics it is known as Aragvadha (disease killer). Extracts from the tree are widely utilized in Indian traditional medicine. Leaf extracts exhibit a wide range of pharmacological properties which include antibacterial, anti-inflammatory, antioxidant, anti-proliferative and hypoglycemic activities [1]. Rhein is an anthraquinone derivative found in the flower and pod pulp which exhibits anticancer activity [2]. Sun-dried fruit pulp has been utilized for treating constipation, fever, leprosy, diabetes, intestinal disorders and wounds [3].

Delonix regia (Boj. Ex. Hook) (Family: *Caesalpiniaceae*) is native to Madagascar. Flowers consist of five petals, four of which are the same colour (red to orange) with the fifth, having streaks of white. Medicinal properties of the plant include anti-inflammatory, antioxidant and antimicrobial activities [4]. Aqueous extracts from the stem and bark also exhibit moderate antibacterial activity [5]. Flower extracts possess diuretic, hepatoprotective and cytotoxic activities [6,7].

Spathodea is a monotypic genus in the flowering plant family *Bignoniaceae* that is native to the tropical forests of Africa having been naturalized in the Caribbean, the Pacific and India. It contains a single species, *Spathodea campanulata* and is commonly referred to as the African tuliptree, fountain tree, pichkari, Nandi flame or Flame of the forest. The Greek word Spathodea means 'spathe' (blade), and refers to the ladle-like shape of corolla and calices whereas campanulata describes the bell-shape of the flower. The tree is mainly ornamental with flowers that are reddish-orange, crimson or yellow in colour. The fruit splits open when mature, releasing numerous winged seeds. Flower and bark extracts of *S. campanulata* have been utilized in traditional medicine for the treatment of various maladies [8].

Senna siamea (Lam.) Irwin is a tropical legume belonging to the *Fabaceae* family, subfamily, *Caesalpinioideae*. The tree is native to South and Southeast Asia and has the common name Thailand shower but is also referred to as *Cassia siamea*, *Cassia florida* and *Senna sumatrana*. The tree blossoms yellow flowers throughout the year. Leaves, pods and seeds of the tree are utilized in Burmese and Thai cuisine (Thai KhiLek curry) but must be boiled and the water discarded before consumption to remove toxins. Boiling was found to remove over 90% of barakol, a hepatotoxic compound found in the leaves and flowers [9]. Cassiarins A and B, two novel antiplasmodial alkaloids containing a tricyclic skeleton were isolated from the leaves [10]. Cassiarin A has antimalarial activity [11]. Cassiarins C-E were subsequently isolated from the flowers as well as Cassiarin F which shows potent antiplasmodial activity against *Plasmodium falciparum* [12,13]. Antraquinone glycosides are responsible for the laxative properties of *S. siamea* leaves [14]. Stem bark extracts possess analgesic and anti-inflammatory properties [15].

Tibouchina granulosa also referred to as "Purple glory tree", belongs to the *Melastomataceae* family. Commonly found in the Atlantic forest of Brazil it is also known as Brazilian glory tree or quaresmeira. The tree blossoms purple flowers biannually. The anthocyanidins, pelargonidin and petunidin as well as malvidin-3-(di-*p*-coumaroylxyloside)-5-glucoside and malvidin-3-(*p*-coumaroylxyloside)-5-glucoside have been identified in extracts of the flower [16,17]. Leave infusions have demonstrated wound healing properties [18].

This study was undertaken to investigate the antioxidant properties and fatty acid profile of the flowers of *C. fistula, D. regia, S. campanulata, S. siamea and T. granulosa*.

Materials and Methods

Sample collection

Blooms from *C. fistula, D. regia, S. campanulata, S. siamea and T. granulosa* were harvested from trees growing on the campus of the University of the West Indies, Kingston 7, Jamaica during the summer months of June and July. Petals were oven dried (75°C, 24h, Gallenkamp Laboratory Oven OV-330, England).

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay was performed according to the method of Brand-Williams *et al.* [19]. Samples (200mg) were extracted with ethanol (10mL, 80%) containing hydrochloric acid (1%) at room temperature for 2h on an orbital shaker. Samples were reacted with the stable DPPH radical in an ethanol solution. The reaction mixture consisted of flower extracts (0.5mL), absolute ethanol (3mL) and DPPH (0.5mM, 0.3mL).The reaction was allowed to proceed for 100min after which the absorbance was measured at 517nm using a spectrophotometer (Helios Omega, Thermo Fisher Scientific). A mixture of ethanol (3.3mL) and flower extract (0.5mL) served as the blank. A control solution was prepared by mixing ethanol (3.5mL) with the DPPH radical solution (0.3mL). Samples were analyzed in triplicate. The data obtained was used to calculate the radical scavenging capacity according to the following formula:

$$\% = [1 - A_1/A_0]*100$$

Where

A_1 = Absorbance of sample at 517nm

A_0 = Absorbance of control at 517nm

Total phenolic content

Total phenolics were determined using Folin-Ciocalteu reagent with slight modifications [20]. Samples (200mg) were extracted with ethanol (10mL, 80%) containing hydrochloric acid (1%) at room temperature. Extracts (100µL) were reacted with Folin-Ciocalteu reagent (10%, 750µL) and mixed for 5min followed by addition of Na_2HCO_3 solution (7.5%, 750µL). The solution was incubated at 22°C (1.5h) and the absorbance measured at 760nm using a spectrophotometer (Helios Omega, Thermo Fisher Scientific). A standard calibration curve of gallic acid (0 - 200mg/L) was generated and the results expressed as mg gallic acid/g.

Lipid extraction and methylation

Lipids were Soxhlet extracted from the dried petals with hexane (reflux) and concentrated *in vacuo*. Lipid extracts (50µL) were *trans*-methylated with methanol/acetyl chloride solution [21]. Fatty acid methyl esters (FAMEs) were determined by Gas Chromatography-Mass Spectrometry (GC-MS).

Gas Chromatography-Mass Spectrometry

Methylated oil in hexane (1.0µL) was chromatographed on an HP6890 series Gas Chromatography interfaced with an HP5973 Mass Selective Detector. Constituent FAMEs were eluted with helium carrier gas (flow rate 1cm^3/min) through a DB1-MS column (20 m x 0.18 mm i.d. x 1.0µm film thickness, Agilent, Santa Clara, CA) in an oven programmed at 60°C for 3min and increased at a ramp rate of 10°C/min up to 250°C for 15min. Samples were injected at 230°C while the detector was maintained at 250°C. Constituents were identified by matching the mass spectra of National Institute of Standards and Technology (NIST) library (match quality > 90%). Iodine values were calculated based on the FAME content utilizing the formula:

$$\text{Predicted IV} = xC1 + yC2 + zC3$$

C1, C2 and C3 corresponds to the relative percentage concentrations of unsaturated fatty acids (one, two and three double bonds, respectively) whereas x, y, and z are coefficients [x = 1, y = 1.5, and z = 2.62] [22].

^{1}H NMR and ^{13}C NMR characterization

^{1}H NMR and ^{13}C NMR characterization were performed on a Bruker Bio Spin 200MHz at 200MHz. Lipid extracts (approx. 20mg) were run in deuterated chloroform ($CDCl_3$) at 25°C, with tetramethylsilane as the internal standard. The ethanolic extract of *D. regia* was also analyzed utilizing deuterated methanol (MeOD).

Statistical analysis

Means and standard deviations of the data are presented. Analysis of Variance (ANOVA) was carried out to determine differences at the significant level of $P<0.05$.

Results and Discussion

Plant extracts rich in phenolic compounds have been found to possess a wide range of pharmacological activity such as antioxidant and anti-inflammatory properties. Antioxidants protect cellular membranes and organelles from the potential damaging effects that may occur due to active oxygen species. The antioxidant, free radical scavenging activities and total phenolic content of the inflorescence of five tropical plants namely *D. regia*, *C. fistula*, *S. campanulata*, *S. siamea* and *T. granulosa* were investigated.

Antioxidant, free radical scavenging activity and total phenolic content

The antioxidant, free radical scavenging activity and total phenolic content of each species of flowers were significantly different from each other ($P<0.05$). Of the five plant species investigated, *C. fistula* and *S. siamea* (Table 1) had the highest free radical scavenging activity followed by *T. granulosa*. Highest antioxidant activity was also observed in *C. fistula.* Prior studies have substantiated the antioxidant potency of *S. siamea* flower extracts [23]. Surprisingly, *D. regia* exhibited low antioxidant and free radical scavenging properties. This may be due to the presence of prooxidants and reducing sugars in the extracts of flower.

Highest total phenolic content was observed in *T. granulosa* (96.35 ± 2.88mg gallic acid/g) followed by *S. siamea* (79.37 ± 4.46mg gallic acid/g). In a study of 12 edible Thai flowers, *S. siamea* had the highest total phenolic content (88mg gallic acid equivalents/g) [23]. There is limited literature regarding the phenolic composition of *T. granulosa* flowers. The major phenolic acids that have been identified in *S. siamea* flower extracts include gallic acid, ferulic acid and sinapic acid with the predominant flavonoids being quercetin and rutin [23]. Fistulic acid was isolated from the flowers and pods of *C. fistula* [24]. Other phenolics identified in *C. fistula* flower extracts include kaempferol and rhein [25]. A total phenolic content of 15.12 ± 0.47mg/g gallic acid equivalents has been reported for *S. campanulata* inflorescence extracts [26]. The flavonoids, rutin and catechin have been detected in ethanolic extracts of *S. campanulata* [26].

Table 1: Antioxidant properties of tropical flowers *D. regia*, *C. fistula*, *S. campanulata*, *S. siamea* and *T. granulosa*

Tropical Flora	Antioxidant Activity/ mg gallic acid/g	^{1}FRSA/%	Total Phenolic/mg gallic acid/g
Cassia fistula	12.14 ± 2.16	92.06 ± 1.25 %	54.05 ± 5.09
Tibouchina granulosa	10.76 ± 0.92	85.84 ± 5.64 %	96.35 ± 2.88
Senna siamea	9.59 ± 0.91	89.07 ± 6.35 %	79.37 ± 4.46
Spathodea campanulata	7.71 ± 2.49	68.37 ± 4.84 %	12.50 ± 1.97
Delonix regia	0.015 ± 0.02	18.33 ± 1.18 %	60.77 ± 4.65

^{1}FRSA: Free radical scavenging activity
Values presented are the mean ± SD (n=3)

Eleven phenolic compounds have been identified in aqueous extracts from *D. regia* flowers [27]. These include the phenolic acids, protocatechuic acid, gallic acid, and 2-hydroxy 5-[3,4,5 trihydroxyphenyl) carbonyl oxy] benzoic acid [27]. Additionally, *trans*-cinnamic, salicylic and chlorogenic acids were reported by Shabir et al. [28]. The flavonols rutin, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin trihexoside, quercetin 3-*O*-robinobioside, kaempferol rhamnoside hexoside and isorhamnetol rhamnosyl-hexoside were also identified of which quercetin 3-*O*-rutinoside and quercetin 3-*O*-glucoside occurred in

highest concentrations [27]. A comparison of the nuclear magnetic resonance (NMR) spectral data reported by Adje et al. [27], with NMR data from ethanolic extracts of *D. regia* flowers, showed evidence of the presence of both quercetin 3-*O*-β-glucoside and quercetin 3-β-O-rutinoside. A signal at δ170.24 confirmed the presence of hydroxyl functionalities. In the ^{1}H NMR the presence of sugar moieties due to glucose and rhamnose was evident from signals resonating between δ3 to δ4. The presence of aromatic compounds was confirmed by a peak at δ6.82 [27].

Fatty acid profile and iodine value

Polyunsaturated fatty acids may indirectly serve as antioxidants, thereby reducing the risk of cardiovascular disease, atherosclerosis and inflammation [29]. Linoleic acid was identified as the predominant unsaturated fatty acid in *C. fistula* (41.25%) and *D. regia* (14.69%) flower lipid extracts whereas oleic acid, was the predominant unsaturated fatty acid in *S. campanulata* (23.50%), *T.* granulosa (29.55%) and *S. siamea* (19.34%). Gondoic acid was identified in *S. campanulata* (11.17%) and *T. granulosa* (8.57%). Erucic acid was identified in small quantities (Table 2). Palmitic acid was the major saturated fatty acid in all the lipid extracts with the highest percentages being observed in *S. siamea* (54.43%). Stearic acid was the next most abundant saturated fatty acid identified in these extracts. Lauric acid was only detected in *D. regia*. The lipid extract expected to be most stable to oxidation is that from *S. campanulata* with a predicted iodine value of 39 (Table 3).

Lipid extracts were also analyzed by NMR spectroscopy. Peaks observed were characteristic of triglycerides, which are the predominant forms that fatty acids exist in plant extracts [30]. Olefinic protons (-CH=CH-) due to the presence of linoleic or oleic acid, resonated between δ5.27 - δ5.36ppm as a multiplet (Table 4). These protons were not baseline resolved from the H-2 proton of the glyceride backbone which resonated between δ5.05 - δ5.29ppm. H-1 and H-3 of the glyceride backbone resonated between δ4.05 - δ4.65ppm (Figure 2). Bis-allylic protons (polyunsaturated acyl chain) and allylic methylenes (unsaturated acyl chain) resonated at δ2.70 - δ2.80ppm and δ1.90 - δ2.06 ppm respectively. Protons from the acyl moiety of the triglyceride resonated in the range of δ2.24 - 2.33ppm (H-2) and δ1.60 - δ1.63ppm (H-3). Methylene protons were observed at δ1.20ppm and terminal methyl groups were observed at δ0.88 (Table 4). The ^{13}C NMR spectrum of *C. fistula* is illustrated in Figure 3. Peaks characteristic of triglycerides were also observed. These included carbons on the glyceride backbone, olefinic carbons and carbons from the fatty acid side chains (Table 5).

Table 2: Fatty acid profile of *C. fistula*, *S. siamea, D. regia*, *S. campanulata* and *T. granulosa* flower extracts.

Fatty Acid		*C. fistula* (%)	*S. siamea* (%)	*D. regia* (%)	*S. campanulata* (%)	*T. granulosa* (%)
Lauric	C12:0	[1]ND	ND	4.73 ± 2.84	ND	ND
Myristic	C14:0	2.13 ± 0.24	2.91 ± 0.69	4.82 ± 3.31	2.09 ± 0.41	ND
Palmitic	C16:0	36.48 ± 4.20	54.43 ± 1.10	36.04 ± 4.03	23.32 ± 6.73	22.64 ± 1.60
Palmitoleic	C16:1	ND	ND	ND	2.07 ± 0.36	3.13 ± 0.43
Stearic	C18:0	15.21 ± 2.09	13.86 ± 2.01	9.72 ± 1.33	17.47 ± 5.16	12.68 ± 5.39
Oleic	C18:1	ND	19.34 ± 8.66	18.24 ± 0.20	23.50 ± 9.58	29.55 ± 7.84
Linoleic	C18:2	41.25 ± 5.97	15.82 ± 12.4	14.69 ± 0.33	ND	9.97 ± 0.10
Arachidic	C20:0	2.78 ± 0.87	5.00 ± 0.09	7.67 ± 1.58	13.10 ± 0.80	10.01 ± 3.17
Gondoic	C20:1	ND	ND	ND	11.17 ± 7.98	8.57 ± 5.98
Behenic	C22:0	0.95 ± 0.29	ND	11.72 ± 1.79	3.64 ± 1.85	2.87 ± 1.95
Erucic	C22:1	ND	ND	ND	2.40 ± 0.97	ND
Lignoceric	C24:0	ND	ND	4.79 ± 1.06	2.79 ± 1.05	ND

[1]ND: Not detected

Table 3: Lipid content and predicted iodine value of *S. campanulata*, *T. granulosa, D. regia*, *C. fistula* and *S. siamea* flower extracts.

Tropical Flora	% Lipid	[1]IV
Spathodea campanulata	1.67 ± 0.33	39
Tibouchina granulosa	4.10 ± 0.50	57
Delonix regia	5.04 ± 0.01	42
Cassia fistula	5.25 ± 0.05	63
Senna siamea	5.87 ± 0.03	45

[1]IV: Iodine value
Values presented are the mean ± SD (n=3).

Table 4: [1]H NMR spectroscopy of lipid hexane extracts of *C. fistula*, *S. siamea, D. regia, S. campanulata* and *T. granulosa* flower lipid extracts.

Proton	Functionality	*C. fistula* δ (ppm)	*S. siamea* δ (ppm)	*D. regia* δ (ppm)	*S. campanulata* δ (ppm)	*T. granulosa* δ (ppm)
CH_3	Terminal methyl	0.88	0.87	0.87	0.86	0.86
CH_2	Methylene	1.25	1.18	1.25	1.18	1.28
CH_2-CH_2-COO	Acyl chains	1.60	1.61	1.61	1.61	1.63
CH_2-CH=CH	All unsaturated fatty acids	2.06	1.90	2.04	1.97	2.03
CH_2-COO	All acyl chains	2.31	2.28	2.30	2.24	2.33
C=C-CH_2C=C	Protons attached to bisallylic carbon	2.77	2.70	2.80	-	2.79
$CH_2O(\alpha)$	Glycerol (triglycerides)	4.14	4.08	4.05	4.59	4.17
$CH_2O(\alpha)$	Glycerol (triglycerides)	4.30	4.21	4.07	4.65	4.32
CHO(β)	Glycerol (triglycerides)	5.27	5.27	5.11	5.05	5.29
CH=CH	Olefinic protons	5.35	5.28	5.35	5.27	5.36

Table 5: [13]C NMR spectroscopy of *Cassia fistula* flower lipid extracts.

Carbon	Assignment	*C. fistula* δ (ppm)
α-CH_3	Acyl chains	14.09; 14.14
β-CH_3	Acyl chains	22.58; 22.70
C3	Acyl chains	24.87
C11	Diallylic	25.63
C8-11 (oleyl)	Allylic	27.20
CH_{2n}	Acyl chains	29.13 - 29.71
C16	Linoleyl	31.53; 31.93
β-C2	Acyl chains	34.06; 34.20
α-CH_2O	Glycerol moiety	62.10
β-CH_2O	Glycerol moiety	68.87
C12; C13	Linoleyl	127.89; 128.06
C10	Linoleyl	130.00; 130.24
C1	Glycerol moiety	173.35

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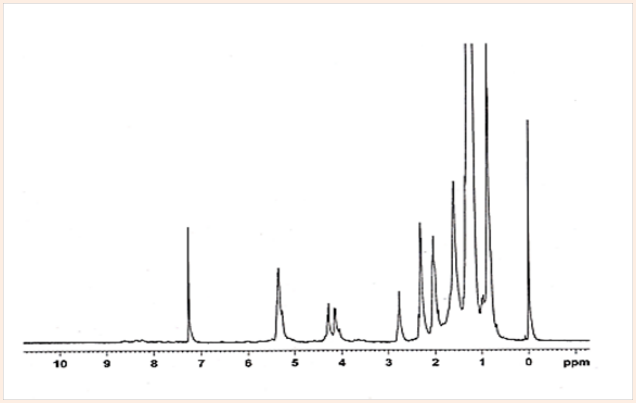

Figure 2: ^{1}H NMR spectral data of *C. fistula* flower lipid extracts.

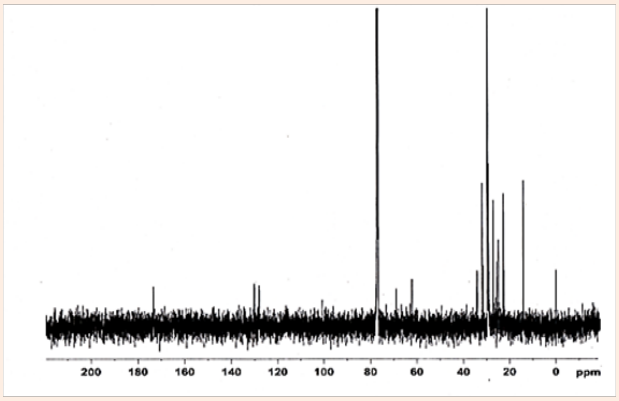

Figure 3. ^{13}C NMR spectral data of *C. fistula* flower lipid extracts.

Conclusion

The consumption of diets rich in antioxidants and polyunsaturated fatty acids has potential health benefits decreasing susceptibility to certain diseases, for example cardiovascular disease. The results from this study illustrates that the flowers investigated are a rich source of phenolic compounds with antioxidant and DPPH radical-scavenging activity. *C. fistula* flower extracts exhibited highest antioxidant activity. *D. regia*'s low antioxidant and free radical scavenging activities may be due to the presence of prooxidants and reducing sugars. The flowers are a minor source of lipids. These flowers are a valuable source of bioactives with potential applications in the pharmaceutical and food industries serving as a source of natural antioxidants.

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