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Quantitation of flavonoids in barks of selected taxa of combretaceae

Abstract

The present study was done on the evaluation and quatification of Flavonoids in the barks of selected taxa of Combretaceae viz. *Terminalia (Terminalia alata, Terminalia arjuna, Terminalia bellirica, Terminalia catappa, Terminalia chebula, Terminalia pallida, Terminalia paniculata), Anogeissus acuminata, Anogeissus latifolia, Calycopteris floribunda, Combretum albidum and Quisqualis indica* The selected species such as was randomly selected for the research and the quantitation of flavonoids were detected and identified by paper chromatography technique (R_f values) and standard methodology. The total flavonoid contents (TFC) varied from 77.94 to 238mg/g, expressed as Rutin equivalents. We conclude that all the selected taxa Barks of Combretaceae possess and can be regarded as promising candidates for natural plant sources high value dietary rich flavonoids.

Keywords: combretaceae, flavonoids, flavonoid content, *Terminalia*

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Introduction

Flavonoids are a diverse group of phytonutrients (plant chemicals) found in almost all plant kingdom. Flavonoids are the largest group of phytonutrients, with more than 6,000 types classified into subgroups based on their chemical structure: flavanones, flavones, flavonols, flavan-3-ols, anthocyanins and isoflavones.[1] A variety of *in vitro* and *in vivo* experiments have shown that flavonoids possess antiallergic, anti-inflammatory, antiviral, antioxidant activities, anticancer activity including anticarcinogenic properties, prodifferentiative activity amongst other modes of action and their versatile health benefits reported in various epidemiological studies.[2,3] There has been increasing interest in the research on flavonoids from plant sources because of their versatile health benefits reported in various epidemiological studies. Since flavonoids are directly associated with human dietary ingredients and health, there is a need to evaluate from different plant sources.[4] The growing body of scientific evidence indicates that flavonoids play a beneficial role in disease prevention, however further research in new therapeutic flavonoids should be discovered in different species of plant kingdom and pharmacological assays clinical and epidemiological trials are greatly needed for the dietary benefits.

Taxonomy of *combretaceae*

Combretaceae R. Br. are a major family of flowering plants with trees, shrubs, and lianas in the order Myrtales. This family is commonly called as White mangrove family. The taxonomic characters of this family are given. The leaves are simple, alternate or opposite, entire; stipules small or absent. The flowers are bisexual or sometimes unisexual, usually actinomorphic. The perianth arises from near the summit of a tubular epigynous zone; calyx of usually 4 or 5 distinct to slightly connate sepals; corolla commonly of 4 or 5 distinct petals, occasionally absent. The androecium of 4-10 stamens is adnate to the epigynous zone, commonly in two cycles, often strongly exserted. The gynoecium is a single compound pistil of 2-5 carpels; style and stigma 1; ovary inferior, with 1 locule containing 2(-6) apical ovules pendulous on long funiculi. The nectary is usually a disk (often hairy) above the ovary. The fruit is 1-seeded, often a flattened, ribbed, or winged drupe.[5] It was early reported about the medicinal properties of combretaceae.[6] Many workers reported on specific genus or in particular the parts of the plant. Due to this aspect, we here made an attempt on Combretaceae family. In this article, we evaluated the essential flavonoids in a preliminary way in the selected taxa of Combretaceae. The present study is apparently the first report of quantitative flavonoid profiles for Combretaceae.

Materials and methods

Stem Barks of Combretaceae mentioned below with voucher specimens (Accession number) were collected from different localities growing in their natural habitats with huge interference of external biotic factors were selected in Horsley Hills, Talkona, Tirumala and Tirupati of Chittoor District, Andhra Pradesh in May – July 2015. The Fresh Barks of Combretaceae taxa viz.

i. *Anogeissus acuminata* (Roxb. ex DC.) Guill. & Perr. (Accession Number: SVUTY/CMB-KM-2718);

ii. Anogeissus latifolia (Roxb. ex DC.) Wall. ex Guill. & Perr. (A.No: SVUTY/CMB-KM-2890);

iii. *Calycopteris floribunda* Lam. (A.No: SVUTY/CMB-KM-2864);

iv. *Combretum albidum* G. Don (A.No: SVUTY/CMB-KM-2610);

v. *Combretum indicum* (L.) De Filipps (A.No: SVUTY/CMB--KM-2492); Terminalia species viz;

vi. *Terminalia alata* Heyne ex Roth (A.No: SVUTY/CMB--KM-1429);

vii. *Terminalia arjuna* (Roxb. ex DC.) Wt. & Arn. (A.No: SVUTY/CMB-KM-1430);

viii. *Terminalia bellirica* (Gaertn.) Roxb. (A.No : SVUTY/CMB--KM-1431);

ix. *Terminalia catappa* L. (A.No: SVUTY/CMB-KM-1432);

x. *Terminalia chebula* Retz. (A.No: SVUTY/CMB-KM-1434);

xi. *Terminalia pallida* Brandis (A.No: SVUTY/CMB-KM-1435) and

xii. *Terminalia paniculata* Roth (A.No: SVUTY/CMB-KM-1436) were selected for the study. All the solvents and chemicals in the experiment were of analytical grade obtained from Himedia, Laboratory Pvt. Ltd., India.

Preparation of extracts

The fresh barks were air dried at room temperature under shade for 3weeks and grinded to 60 mm mesh size by using Willy Mill. Powder of 100 g of each bark was soaked in 200 mL of 95% methanol (3 times) and filtered the extract with Whatman No.1 filter paper. Filtrate was dried under vacuum by using rotary evaporator. Extracts were dried by using rotary evaporator and preserved at 4°C.[7] The crude hydro-methanolic extract was used for the present study.

Quantitative analysis of flavonoids

Two-dimensional paper chromatography technique was done for the rapid separation of mixtures of flavonoids from hydro-methanolic extract of processed bark materials.[8] R_f values in conjugation with UV spectra and the color under UV light with or without NH_3 indicate that the flavonoids. This absorption peak of UV Spectra (λ_{max}=510nm) was used to measure the quantity of flavonoids.[9,10]

Quantification of total flavonoid content (TFC)

The total flavonoid content of crude extract was determined by the Aluminium chloride colorimetric method with some modification.[11,12] 50 µL of crude extract (1mg/mL ethanol) were made up to 1mL with methanol, mixed with 4mL of distilled water and then 0.3mL of 5% $NaNO_2$ solution; 0.3mL of 10% $AlCl_3$ solution was added after 5min of incubation, and the mixture was allowed to stand for 6min. Then, 2mL of 1mol/L NaOH solution were added, and the final volume of the mixture was brought to 10mL with double-distilled water. The mixture was allowed to stand for 15min, and absorbance was measured at λ_{max}=510nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The content and concentrations of flavonoids in extracts was expressed in terms of Rutin equivalent (mg of RU/g of extract)

Results and discussion

In this study we quantified the dietary flavonoids in the selected plant taxa of combretaceae using calorimetric assay and with paper chromatography technique (Figure 1) for detection of flavonoid compounds based of R_f values of the color developing spray for detection of the specific flavonoid compound in hydromethanolic extract. Dietary rich Flavonoids viz. Rutin, Myricetin, Quercetin, Kaempferol, Luteolin, Apigenin, Orientin, Vitexin were identified according to their R_f values in the plant taxa (Table 1) (Table 2). We referred the literature which quoted Two-dimensional paper chromatography represents one of the best methods for the rapid separation of mixtures of flavonoids from crude methanol or methanol-water extracts of dried plant material.[8] Paper partition chromatography have been used as a preliminary test for the detection of flavonoids as suggested by Wender and Gage.[13]

Selected plants

i. *Anogeissus acuminata*

ii. *Anogeissus latifolia*

iii. *Calycopteris floribunda*

iv. *Combretum albidum*

v. *Quisqualis indica*

vi. *Terminalia arjuna*

vii. *Terminalia bellirica*

viii. *Terminalia catappa*

ix. *Terminalia chebula*

x. *Terminalia pallida*

xi. *Terminalia paniculata*

xii. *Terminalia tomentosa*

Detected flavonoids

a) Rutin,

b) Myricetin,

c) Quercetin,

d) Kaempferol,

e) Luteolin,

f) Apigenin,

g) Orientin,

h) Vitexin,

i) Uni Identified compounds

Solvents used for detection of flavonoids

I. Iso-propyl alcohol: Ammonia (25%): Water (8:1:1)

II. n-Butanol: Acetic acid: Water (4:1:5).

III. Conc. Hydrochloric acid: Acetic acid: Water (3:30:10)

IV. Phenol: Water (3:1)

Table 1 R_f values of flavonoid compounds detected on paper chromatogram

Flavonoid	Rf values in solvent			
	I	2	3	4
Rutin	0.03	0.57	0.35	0.2
Myricetin	0.07	0.43	0.28	0.13
Quercetin	0.26	0.64	0.41	0.28
Kaempferol	0.37	0.85	0.54	0.58
Luteolin	0.44	0.78	0.66	0.67
Apigenin	0.61	0.91	0.83	0.87
Orientin	0.78	0.31	0.02	0.42
Vitexin	0.91	0.42	0.06	0.62
(UI)	0.14	0.25	0.2	0.35

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Table 2 Qualitative analysis of flavonoid compounds detected in selected taxa of combretaceae

S. no.	Name of the taxa	Flavonoid								
		R	M	Q	K	L	A	O	V	U.I
1	Anogeissus acuminata	+	+	+	+	-	+	+	+	+
2	Anogeissus latifolia	+	+	+	+	-	+	+	-	-
3	Calycopteris floribunda	+	+	+	+	-	-	+	+	-
4	Combretum albidum	+	+	+	+	-	-	+	+	-
5	Quisqualis indica	+	+	-	+	-	+	-	+	-
6	Terminalia arjuna	+	+	-	+	+	+	+	+	+
7	Terminalia bellirica	+	+	-	+	+	+	+	+	-
8	Terminalia catappa	+	+	-	+	+	+	+	-	+
9	Terminalia chebula	+	+	+	+	+	-	+	+	+
10	Terminalia pallida	+	+	+	+	+	+	+	+	-
11	Terminalia paniculata	+	+	+	+	+	-	+	-	-
12	Terminalia tomentosa	+	+	+	+	+	+	+	+	+

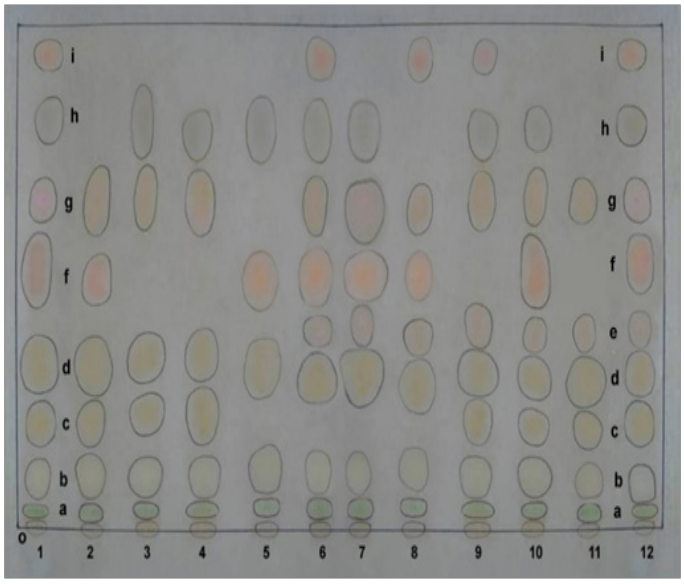

Figure 1 Determination of flavonoids through paper chromatographic plate in the methanolic extract of the barks of combretaceae

It was reported that the concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation.[14] Our results predicted the same. Different Flavonoid compounds detected in the selected 12 taxa for hydromethanolic extract. Unidentified compounds were also detected which has to be identified and characterized. R-Rutin, M-Myricetin, Q-Quercetin, K-Kaempferol, L-Luteolin, A-Apigenin, O-Orientin, V-Vitexin, U.I-Uni Identified compounds. Denotion: (+)-Present; (-)-Absent. The yield of extract obtained from 10g of dry plant material was measured for each extract (Table 3) Values are the means of three biological replicates±standard deviation. (n=3). TFC-mg of RU/g of dry hydro methanolic extract. Terminalia species of Combretaceae contains the highest flavonoid content compared to *Anogeissus species* and *combretum albidum* (Table 3). For the experimented hydromethanolic extract, the extractive values are in the order*: Terminalia tomentosa>Terminalia arjuna>Terminalia paniculata>Anogeissus acuminata>Terminalia pallida>Terminalia catappa>Terminalia chebula>Anogeissus latifolia>Combretum albidum >Terminalia bellirica>Quisqualis indica>Calycopteris floribunda.*

Table 3 The extractive yield (g) and total flavonoid content (TFC-mg rutin equivalent/g Dry extract)

S. No.	Name of the taxa	Extractive yield(g)	Total flavonoid content (mg rutin equivalent/g DW)
1	Anogeissus acuminata	2.04	211.15±4.33
2	Anogeissus latifolia	1.42	140.18±0.26
3	Calycopteris floribunda	0.88	77.94±1.14
4	Combretum albidum	1.32	92.30±0.05
5	Quisqualis indica	0.96	61.43±1.16
6	Terminalia arjuna	2.6	219.966±4.71
7	Terminalia bellirica	1.08	108.10±1.09
8	Terminalia catappa	1.74	86.84±0.77
9	Terminalia chebula	1.24	165.58±9.75
10	Terminalia pallida	1.84	134.47±0.06
11	Terminalia paniculata	2.26	192.69±0.72
12	Terminalia tomentosa	2.86	238.25±2.01

In a recent study, Flavonoid compounds have been isolated characterized by ultra-high performance liquid chromatography-electrospray ionization-mass spectrometry (UHPLC-ESI-MS).[15] Flavonoid has been used as chemotaxonomic markers in the genus *Drosera*.[16]

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Conclusion

In this study, hydro methanol extracts of *selected plant taxa of combretaceae* have high flavonoid contents. All the combretaceae taxa investigated in this article may contribute high valued amount of flavonoid as possible sources for future novel compounds in food and pharmaceutical formulations.

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Conflict of interest

Author declares that there is no conflict of interes

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