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Total Phenolics, Total Flavonoids Contents and In Vitro Antioxidant Activity of Methanol Extract and Solvent Fractions of *Desmodium ramosissimum* G. Don †

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Abstract: Oxidative stress has been linked to the pathogenicity of many diseases. This study investigated the total phenolics content (TPC) and total flavonoids content (TFC) of the methanolic extract and solvent fractions (*n*-hexane, ethyl acetate, *n*-butanol, and aqueous) of *Desmodium ramosissimum* using Folin-Ciocalteu and aluminum chloride assays respectively. The extract and solvent fractions were further appraised for their in vitro antioxidant capacity using: total antioxidant capacity (TAC), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) methods at varying concentrations of 25–300 µg/mL. Results revealed that ethyl acetate and *n*-butanol fractions possess elevated levels of TPC and TFC when compared to other solvent fractions and extract in a concentration-dependent manner. The ethyl acetate fraction had the highest TPC (532.36 mg GAE/g), TFC (2843.33 mg QE/g) and ferric reducing potential (56.70 mg GAE/g) at 300 µg/mL. Also, at 300 µg/mL, the TAC (77.33 mg AAE/g) of the *n*-butanol fraction and its DPPH radical scavenging ability (86.04%) were higher. As shown in this study, organic solvents with different chemical natures are capable of extracting chemical constituents with antioxidant components of different polarities and *D. ramosissimum* may also be considered a rich source of natural antioxidants, justifying its pharmacological use in traditional medicine.

Keywords: total phenolics; total flavonoids; oxidative stress; antioxidant activity; *Desmodium ramosissimum*

1. Introduction

Reactive species are mainly derivations of cellular aerobic metabolism and external factors such as radiation, pollution, exposure to certain drugs, heavy metals and toxic chemicals [1]. They play crucial roles in signal transduction, transcription, regulation of cytokine, growth factor and hormone action, neuromodulation, immunological defense and a host of other physiological functions [2,3]. However, a shift in the equilibrium between these molecules and the cellular antioxidant defense mechanism in the interest of the former results in oxidative stress, which compromises the biological system [4]. Oxidative damage to the macromolecular components of the cell is associated with the initiation and development of several maladies such as cancer, diabetes, arthritis, coronary artery and neurodegenerative diseases [1–4]. Recently, the use of natural antioxidants in combating oxidative stress has gained global popularity, as it counteracts the volatile, unstable and toxic nature of synthetic antioxidants [5]. Secondary plant metabolites such as phenolics and flavonoids have been identified as effective, free radical scavengers, chelators of trace metals, inhibitors of enzymes involved in free radical generation and up-regulators of the endogenous antioxidant protection [6]. The presence of hydroxyl groups in their molecular structure accounts for their reducing abilities [6,7]. The antioxidant potentialities inherent in a plant and their various polarities with respect to the solvent used in extraction determine the quantity yield of the extract [8]. *Desmodium ramosissimum* is an erect, slender, and perennial herb used traditionally in the treatment of dysentery, eye disease, and fever in Bauchi State of Nigeria [9]. In vivo and in vitro experiments have indicated that other species of the *Desmodium* plant possess anti-inflammatory, anti-parasitic, antidiabetic, and antibacterial properties and an array of pharmacological principles that improve cardiovascular and cerebrovascular functions and regulate the immune system [10]. The present research focused on evaluating the total phenolics, total flavonoids contents and in vitro antioxidant activity of *D. ramosissimum* methanol extract and its solvent fractions.

2. Materials and Methods

2.1. Chemicals

Methanol was purchased from Sigma-Aldrich (Germany). Sodium hydroxide (NaOH), quercetin, sulphuric acid (H_2SO_4), and ascorbic acid were procured from BDH (England). Folin-Ciocalteu phenol reagent (FCPR) was obtained from Lobal Chemie (India). Sodium nitrite ($NaNO_2$) and gallic acid were procured from Qualikems (India). Sodium carbonate (Na_2CO_3), aluminum chloride ($AlCl_3.6H_2O$), sodium phosphate (NaH_2PO_4), and ammonium molybdate were bought from JHD (China). Potassium ferricyanide ($K_3Fe(CN)_6$), Ferric chloride ($FeCl_3$), and Trichloroacetic acid (TCA) were bought from Lobal Chemie (India). The distilled water used was obtained from the National Center for Energy Research and Development, University of Nigeria, Nsukka.

2.2. Plant Collection

The whole plant of *Desmodium ramosissimum* was obtained from Ede Oballa in Nsukka Local Government Area, Enugu State. The plant was identified and authenticated by a taxonomist, Mr. Alfred Ozioko of the Bioresources Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State, Nigeria.

2.3. Preparation of Plant Material and Extraction Procedure

The whole plant of *D. ramosissimum* was used. The plant material was air-dried at ambient temperature for 12 days and pulverized into fine particles using a mechanical milling machine (Thomas Wiley, USA). One hundred and forty-six grams (146.0 g) of pulverized *D. ramosissimum* were extracted with 2.3 L of methanol for 24 h by cold maceration at ambient temperature. The mixture was filtered and the filtrate concentrated *in vacuo* at reduced temperature (40 °C) and pressure to obtain the dried extract.

2.4. Solvent-Solvent Partitioning of the Extract

Eight grams (8.0 g) of methanol extract of *D. ramosissimum* were dissolved in 400 mL of 20% methanol in water and the resulting mixture successively partitioned against *n*-hexane (5 × 250 mL), ethyl acetate (4 × 250 mL) and *n*-butanol (4 × 250 mL) as previously reported [11]. The solvent fractions were concentrated *in vacuo* to obtain *n*-hexane, ethyl acetate, *n*-butanol and water fractions, respectively.

2.5. Quantitative Phytochemical Screenings

Quantitative phytochemical assessment of the extracts and fractions were done to estimate the total phenolics content (TPC) using the Folin-Ciocalteu method as previously reported [12] and total flavonoids content (TFC) using the aluminum-chloride colorimetric assay as described by [13].

2.6. In Vitro Antioxidant Assay

The in vitro antioxidant analyses of the extract and fractions were carried out using total antioxidant capacity (TAC), which was determined by the phosphomolybdate method, as previously reported [14], DPPH radical scavenging method previously reported by [15] and ferric reducing antioxidant power (FRAP) determined in accordance with the method described by [16].

2.7. Statistical Analysis

Results were presented as mean ± standard deviation (SD) of three replicate measurements. The statistical analyses were performed by one-way ANOVA, followed by a *Post hoc* test (Least significant difference) using SPSS version 20 (IBM). Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Percentage Extract and Fraction Yield

Percentage yield of extract was determined from dried plant material while that of the fractions was based on the dried extract as shown in Table 1.

Table 1. Percentage extract and fraction yield.

Extract/Fractions	Mass of Extract (g)	Extraction Yield (%)
Extract	9.48	6.48
n-hexane	1.90	2.69
Ethyl acetate	1.88	2.62
n-butanol	1.81	1.78

3.2. Total Phenolics Content

The present study showed that TPC increased as the concentration of extract and fractions increased, with ethyl acetate fraction of *D. ramosissimum* exhibiting the highest levels of phenolics when compared with all other fractions, as shown in Table 2.

Table 2. Total Phenolics Content (mg GAE/g dry weight of plant extract).

Conc. (µg/mL)	Extract	*n*-hexane	Ethyl acetate	*n*-butanol
25	ND	ND	53.88 ± 4.67 ^{ab}	ND
50	6.3 ± 1.39 ^{ba}	ND	109.03 ± 5.17 ^{bb}	3.58 ± 1.05 $^{ba(l)}$
100	20.55 ± 0.91 ^{ca}	ND	212.97 ± 10.14 ^{cc}	36.30 ± 1.89 ^{cd}
200	49.33 ± 2.29 ^{da}	12.67 ± 1.39 ^{db}	396.30 ± 15.00 ^{dc}	107.82 ± 11.92 ^{dd}

250	63.27 ± 2.40 ^{ea}	19.33 ± 3.19 ^{eb}	469.64 ± 3.28 ^{ec}	141.15 ± 10.92 ^{ed}
300	77.82 ± 2.73 ^{fa}	28.73 ± 0.00 ^{fb}	532.36 ± 20.79 ^{fc}	153.88 ± 6.39 ^{fd}

n = 3. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts across rows and columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across rows and columns are considered non-significant ($p > 0.05$).

3.3. Total Flavonoids Content

The present study showed that TFC increased as the concentration of extract and fractions increased, with ethyl acetate fraction of *D. ramosissimum* exhibiting the highest levels of flavonoids when compared with all other fractions, as shown in Table 3.

Table 3. Total Flavonoids Content (mg QE/g plant extract).

Conc. (μg/mL)	Extract	*n*-hexane	Ethyl Acetate	*n*-butanol
25	153.33 ± 15.28 $^{aa(k)}$	100.00 ± 52.92 $^{aa(l)}$	330.00 ± 17.32 $^{ab(i)}$	296.67 ± 106.93 $^{ab(m)}$
50	193.33 ± 5.77 $^{ba(k)}$	116.67 ± 45.09 $^{bb(l)}$	593.33 ± 40.41 $^{bc(i)j}$	433.33 ± 11.55 $^{bd(m)}$
100	273.33 ± 15.28 ^{ca}	150.00 ± 26.46 ^{cb}	1176.67 ± 66.58 $^{cc(p)j}$	793.33 ± 20.82 ^{cd}
200	446.67 ± 45.09 ^{da}	226.67 ± 25.17 ^{db}	2146.67 ± 90.74 $^{dc(p)}$	1580.00 ± 131.15 ^{dd}
250	553.33 ± 11.55 ^{ea}	303.33 ± 56.86 ^{eb}	2376.67 ± 200.33 $^{ec(p)}$	1946.67 ± 120.14 $^{ed(r)}$
300	640.00 ± 34.64 ^{fa}	303.33 ± 15.28 ^{fb}	2843.33 ± 340.78 $^{fc(p)}$	2090.00 ± 75.50 $^{fd(r)}$

n = 3. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts across rows and columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across rows and columns are considered non-significant ($p > 0.05$).

3.4. Antioxidants Activity of Extracts and Fractions by Phosphomolybdate Method

The solvent fractions obtained from ethyl acetate and *n*-butanol revealed a significant ($p < 0.05$) rise in TAC compared to other fractions in a concentration-dependent manner.

Table 4. Antioxidants activity of extracts and fractions by Phosphomolybdate method (mg AAE/g of plant extract).

Conc. (μg/mL)	Extract	*n*-hexane	Ethyl acetate	*n*-butanol
25	35.00 ± 3.61 $^{aa(i)}$	46.00 ± 0.00 $^{ab(j)}$	67.00 ± 1.73 $^{ac(k)}$	47.67 ± 1.52 $^{ab(l)}$
50	34.00 ± 0.00 $^{ba(i)}$	47.00 ± 0.00 $^{bb(j)}$	68.00 ± 0.00 $^{bc(k)}$	49.00 ± 1.00 $^{bd(l)}$
100	36.00 ± 2.00 $^{ca(i)}$	48.67 ± 1.15 $^{cb(j)}$	68.33 ± 1.15 $^{cc(k)}$	51.00 ± 1.00 $^{cb(l)}$
200	61.33 ± 2.08 ^{da}	68.33 ± 1.15 ^{db}	74.00 ± 1.00 ^{db}	71.00 ± 2.00 ^{db}
250	65.33 ± 1.15 $^{ea(r)}$	72.67 ± 0.55 ^{eb}	76.00 ± 1.53 $^{ec(n)}$	75.33 ± .15 $^{ec(p)}$
300	68.00 ± 1.00 $^{fa(r)}$	75.00 ± 0.00 ^{fb}	77.00 ± 1.00 $^{fc(n)}$	77.33 ± 0.58 $^{fc(p)}$

n = 3. Results are expressed in mean ± standard deviation with mean values with the different **letters** as superscript across rows and columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across rows and columns are considered non-significant ($p > 0.05$).

3.5. DPPH Scavenging Free Radical Activity of Extract and Fraction

Among the solvent fractions, ethyl acetate and *n*-butanol fractions exhibited a distinctive increase in free-radical scavenging ability. Furthermore, DPPH scavenging ability is concentration-dependent for methanol extract and all solvent fractions except ethyl acetate and *n*-butanol fractions.

Table 5. Antioxidants activity of extracts and fractions by DPPH scavenging free radical capacity (%).

Conc. (μg/mL)	Extract	*n*-hexane	Ethyl Acetate	*n*-butanol
25	31.21 ± 2.72 ^{aa}	33.86 ± 7.40 $^{aa(i)}$	80.43 ± 0.43 $^{ab(j)}$	72.79 ± 0.22 ^{ac}
50	42.01 ± 1.09 ^{ba}	38.68 ± 2.67 $^{bb(i)}$	81.30 ± 0.31 $^{bc(j)}$	84.78 ± 1.15 $^{bd(k)}$
100	57.60 ± 1.20 ^{ca}	49.26 ± 2.81 ^{cb}	80.13 ± 0.64 $^{cc(j)}$	85.32 ± 0.59 $^{cd(k)}$
200	80.43 ± 1.48 $^{da(l)}$	70.91 ± 2.11 $^{db(m)}$	83.57 ± 0.94 ^{dc}	85.54 ± 0.49 $^{dc(k)}$
250	79.36 ± 1.58 $^{ea(l)}$	76.40 ± 2.67 $^{eb(m)}$	84.71 ± 0.28 $^{ec(n)}$	85.92 ± 0.42 $^{ec(k)}$
300	80.56 ± 1.41 $^{fa(l)}$	80.99 ± 1.08 $^{fa(m)}$	85.39 ± 0.68 $^{fb(n)}$	86.04 ± 0.24 $^{fb(k)}$

n = 3. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts across rows and columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across rows and columns are considered non-significant ($p > 0.05$).

3.6. Ferric Reducing Antioxidant Power of the extract and fractions

The methanol extract and ethyl acetate fraction showed higher ferric reducing power than other fractions. The potential of extract and fractions in reducing ferrous was concentration-dependent.

Table 6. Antioxidants activity of extracts and fractions by ferric reducing antioxidant power (mg GAE/g plant extract).

Conc. (μg/mL)	Extract	*n*-Hexane	Ethyl Acetate	*n*-Butanol
25	16.91 ± 0.78 $^{aa(i)}$	0.76 ± 0.14 $^{ab(j)}$	26.67 ± 0.82 ^{ac}	1.24 ± 0.37 $^{ab(p)}$
50	18.30 ± 1.00 $^{ba(i)}$	1.09 ± 0.18 $^{ba(j)k}$	32.42 ± 0.90 ^{bc}	2.88 ± 0.43 $^{ba(p)}$
100	21.18 ± 0.36 ^{ca}	1.73 ± 0.24 $^{cb(k)}$	38.39 ± 0.84 ^{cc}	5.73 ± 1.79 $^{cb(p)}$
200	22.35 ± 0.76 ^{da}	2.27 ± 0.36 $^{db(k)}$	49.88 ± 1.46 ^{dc}	17.82 ± 6.12 ^{da}
250	24.45 ± 0.51 $^{ea(n)}$	3.09 ± 0.18 ^{eb}	52.48 ± 1.19 ^{ec}	32.64 ± 7.82 ^{ed}
300	25.33 ± 0.37 $^{fa(n)}$	5.55 ± 0.74 ^{fb}	56.70 ± 1.09 ^{fc}	41.27 ± 1.64 ^{fd}

n = 3. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts across rows and columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts across rows and columns are considered non-significant ($p > 0.05$).

4. Discussion

The bioactive constituents in plants are ubiquitously distributed in various tissues of plants [17]. Therefore, the whole plant of *Desmodium ramosissimum* was used for this study. Solvent-solvent extraction is frequently used to isolate plant antioxidant compounds but the extract yields and antioxidant activities are dependent on the chemical structure of the solvent type [18].The high phenolics content recorded in ethyl acetate fraction of *D. ramosissimum* is consistent with the research of [19] who reported that the high phenolics content in the ethyl acetate extract of *Desmodium gangeticum* was responsible for the scavenged free radicals in a concentration-dependent manner in the in vitro antioxidant assay [19].The presence of substantial amounts of flavonoids in both the extract and fractions may also contribute to the antioxidant activity of the plant. The solvent fractions obtained from ethyl acetate and *n*-butanol revealed a significantly ($p < 0.05$) higher TAC compared to other fractions. Recent studies revealed that flavonoids and polyphenolic compounds account for the phosphomolybdate scavenging property of medicinal plants [20]. Also, ethyl acetate and *n*-butanol fractions tend to have similar measures of TAC, as the concentration of both fractions increased. However, the ethyl acetate fraction has high TAC even at lower concentrations compared to an *n*-butanol fraction. This observation could be attributed to the solvent type. The various rates of DPPH scavenging activity of the methanolic extract and solvent fractions at different concentrations may be a function of phenolics (polyphenols) and flavonoids which are phytochemical constituents in *D. ramosissimum* that serve as reductants, donating a single electron or a hydrogen atom to DPPH radical [17]. Also, ethyl acetate and *n*-butanol fractions compete closely with each other in their ability to scavenge DPPH radicals. Among the solvent fractions considered, *n*-butanol fraction revealed an

overall highest DPPH scavenging activity. This result is in line with the review of antioxidants in medicinal plants [21]. In reducing power assay, the antioxidants present in the extract and solvent fractions of *D. ramosissimum* prompted the conversion of Fe^{3+}/ferricyanide complex to the ferrous (Fe^{2+}) state, demonstrating its reducing power. Previous report by [22] hinted that the reducing properties exert antioxidant response by providing hydrogen atom(s) to dissociate the free radical chain.

5. Conclusions

Generally, antioxidants exert their action either by neutralizing the reactive intermediates or protecting the antioxidant defense network. *Desmodium ramosissimum* may be considered a rich source of natural antioxidants since its methanol extract, ethyl acetate and *n*-butanol fractions exhibited interesting antioxidative properties. This justifies its use in traditional medicine and makes it a promising source of pharmaceuticals and other therapeutics.

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