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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 *Desmodium ramosissimum* methanol extract and its solvent fractions (n-hexane, ethyl acetate, n-butanol, and aqueous) 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 higher 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*

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Results and Discussion

Table 1: Total Phenolic 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

Table 2: 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 ($p>0.05$).

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Table 3: 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)

Table 4: 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 ($p>0.05$).

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Table 5: 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 ($p>0.05$).

- Solvent-solvent extraction is commonly used to isolate plant antioxidant compounds and solvent type determines extract yield and antioxidant activity.
- The presence of substantial amounts of Phenols and flavonoids in both the extract and fractions may also be contribute to the antioxidant activity of the plant.

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- The solvent fractions obtained from ethyl acetate and *n*-butanol revealed a significantly ($p < 0.05$) higher TAC compared to other fractions.

- Also, 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.

- Among the solvent fractions considered, *n*-butanol fraction revealed an overall highest DPPH scavenging activity.

- The scavenging property 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.

- 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.

Conclusion

Desmodium ramosissimum may be considered a potential source of natural antioxidants since its methanol extract, ethyl acetate and *n*-butanol fractions exhibited interesting antioxidative properties. This validates its use in traditional medicine. Also, It could serve as an alternative source of therapeutics.

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