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In vitro oxidative stress regulatory potential of *Citrullus colocynthis* and *Tephrosia apollinea*

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The present study investigates the potential role of medicinal plants *Citrullus colocynthis* and *Tephrosia apollinea* in ameliorating the oxidative stress developed during the generation of reactive oxygen species. Organic extracts of different organs (leaf, stem and root) of these medicinal plants obtained in *n*-hexane, chloroform, *n*-butanol and water were assayed for radical scavenging, total antioxidant capacity, anti-lipid peroxidation and reduced glutathione. The total phenolic content (TPC) of both selected medicinal plants was also evaluated. The results indicated that extracts of *T. apollinea* leaf, stem and root have higher TPC compared to those of *C. colocynthis*. Similarly, the results of the present study revealed higher bioactivity of *C. colocynthis* than that of *T. apollinea* in various antioxidant assays. Various plant parts of each plant were also compared.

Keywords: Citrullus colocynthis, Tephrosia apollinea, oxidative stress, total phenolics

Citrullus colocynthis (L.) Schrad of the family *Cucurbitaceae* is widely distributed throughout the world and is reported to possess antifeedant, deterrent, growth-regulating and fertility-reducing properties for insects (1). Furthermore, it is used as an abortifacient, vermifuge, insect repellent and purgative, and also to treat leukemia, cancer, rheumatism jaundice, amenorrhea and fever (2). *Tephrosia apollinea* (Del.) Link is a perennial shrublet of the *Leguminosae* family (subfamily *Papilionaceae*). Genus *Tephrosia* is a genus of the tropical and subtropical regions that comprises about 400 species. Its folk medicinal use has been reported against a number of diseases (3) and various species of the genus are reported to possess insecticidal, piscicidal, antiprotozoal, antiplasmodial, antiviral and anticancer potential (4).

Oxidative stress is generated as a result of the inability of the biological system to detoxify the generation of reactive oxygen species (ROS). Increased generation of ROS causes cancer, diabetes, cataract and neurodegenerative and cardiovascular diseases (5).

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The destructive processes caused by oxidative stress may possibly be inhibited by natural antioxidants, which makes them essential for human health. These natural antioxidants are used in the form of pure chemical constituents or their respective raw extracts (6). The current study is focused on evaluation of the *in vitro* oxidative potential of medicinally important plant species *C. colocynthis* and *T. apollinea*.

EXPERIMENTAL

Collection and extraction of medicinal plants

The selected medicinal plants, *C. colocynthis (CC)* and *T. apollinea (TA)*, were collected from the same area in Jabal Al-Akhdar (Al-Dakhliya Governorate, Oman; N 22°55.976, E 057°40.139) and were identified by a plant taxonomist, and the herbarium sheets were deposited at the Herbarium, UoN Chair of Oman's Medicinal Plants and Marine Natural Products, University of Nizwa, Oman. Both *C. colocynthis (CC)* and *T. apollinea (TA)* were divided into leaf (CCL, TAL), stem (CCS, TAS) and root (CCR, TAR) (Table I), dried in shade, and then subjected to solvent-solvent partition to obtain *n*-hexane (H), chloroform (C), *n*-butanol (B) and water (W) vacuum dried extracts (7). Various concentrations of these extracts were prepared to understand the dose-dependent effects of different extracts from different plant parts. This was done by dissolving 10 mg of each dry extract in 1 mL of methanol to prepare the stock solution, which was then used to make various dilutions (5, 10, 20, 50, 100 and 200 μg mL^{-1}). All the experiments were repeated three times, and the results are reported as the mean of three values ± SD.

Determination of total phenolics and oxidative stress parameters

Folin-Ciocalteu colorimetric method (8) was used to determine the total phenolic content in various parts of the selected medicinal plants. The antioxidant potential was measured using the method of Prieto *et al.* (9) and the results were reported as ascorbic acid equivalents (AAE) in mg g^{-1}. The DPPH radical scavenging potential of the selected medicinal plants was assessed using the method of Hussain *et al.* (10) and the results were reported as AAE (mg g^{-1}). Reduced glutathione activity was measured using a standard curve by Ellman's method, as described by Ezeji *et al.* (11). The level of anti-lipid peroxidation was checked by a modified method of thiobarbituric acid reactive substances (TBARS), as described by Nagarsekar *et al.* (12).

Statistical analysis

Data was analyzed by Student's *t*-test ($p < 0.05$) to detect significant differences between extracts. To understand the significance of the effects of plant extracts, a two-way ANOVA with repeated measures and Bonferroni *post-hoc* analysis was made using the Graph Pad Prism. Unscrambler (Camo version 9.0 CA, USA) was used for principal component analysis (PCA) to determine the correlation of extracts and their effects in various antioxidant activities. The PCA model was built with different concentrations and a full cross-validation was used to validate the oxidative stress mitigation potential of the two plants.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis regarding TPC was performed. Also, various concentrations (5-200 μg mL^{-1}) of dry extracts from different parts of *C. colocynthis* and *T. apollinea* were assessed for reducing the reactive oxygen species using different *in vitro* bioassay techniques.

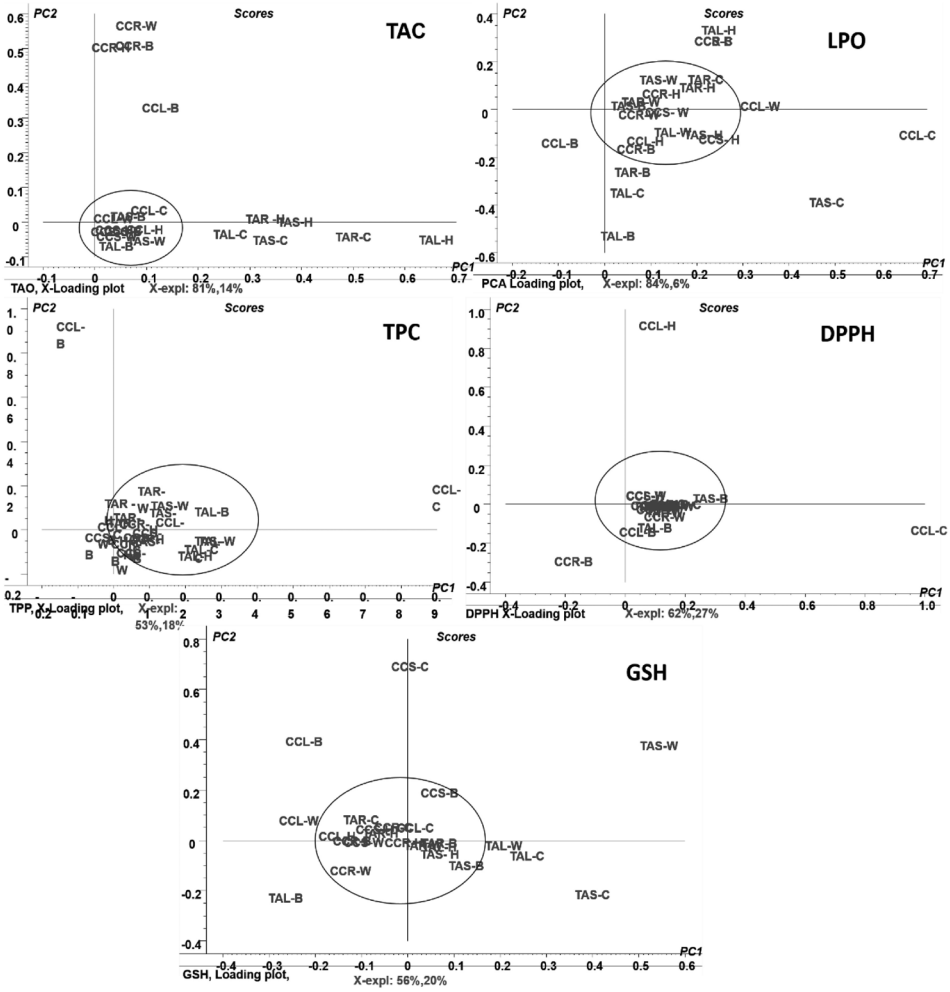

Fig. 1. Principal component analysis (PCA) showing the correlation between different *in vitro* oxidative stress-related parameters (TAC – total antioxidant capacity, LPO – lipid peroxidation, TPC – total phenolic content, DPPH – 2,2′-diphenyl-1-picrylhydrazyl scavenging radical potential, GSH – reduced glutathione content) and extracts of different parts of medicinal plants. For other acronyms see Table I.

Table I. Total phenolics and antioxidant activity of different extracts (200 μg mL^{-1}) of T. apollinea *and* C. colocynthis

Control[a]	TPC (mg GAE g^{-1} extract)	LPO (%)	DPPH (%)	GSH (mmol g^{-1} glutathione)	TAC (mg AAE g^{-1} extract)
	91.8 ± 1.8	82.7 ± 1.4	98.3 ± 3.8	2128.1 ± 119.8	2082.8 ± 93.6
CCR-H	3.4 ± 0.0	10.9 ± 0.2	95.3 ± 8.6	1261.2 ± 19.0	1816.0 ± 161.8
CCR-C	3.5 ± 0.0	6.8 ± 0.3	13.0 ± 2.5	1323.4 ± 7.2	1093.4 ± 11.3
CCR-B	5.8 ± 0.0	9.5 ± 0.3	88.5 ± 9.1	1340.1 ± 22.3	1865.5 ± 185.4
CCR-W	14.1 ± 0.0	7.8 ± 0.6	24.6 ± 47.4	1274.2 ± 21.5	1852.1 ± 220.1
CCS-H	4.3 ± 0.6	14.0 ± 0.4	63.2 ± 41.2	1177.0 ± 51.0	6.0 ± 4.4
CCS-C	14.8 ± 2.7	16.6 ± 1.8	43.0 ± 10.4	1375.9 ± 12.0	22.3 ± 2.4
CCS-B	20.2 ± 0.9	16.6 ± 1.8	9.6 ± 0.7	1402.9 ± 26.3	36.2 ± 6.5
CCS-W	21.4 ± 0.2	7.1 ± 0.1	12.2 ± 10.6	1119.6 ± 81.1	22.2 ± 14.4
CCL-H	12.0 ± 0.2	9.4 ± 0.1	71.5 ± 195.2	1570.5 ± 105.4	96.0 ± 18.5
CCL-C	12.7 ± 1.0	40.7 ± 0.6	80.2 ± 6.0	1838.3 ± 9.8	88.6 ± 14.2
CCL-B	3.7 ± 0.0	9.3 ± 0.1	20.1 ± 25.5	1239.8 ± 22.6	1974.0 ± 197.5
CCL-W	24.1 ± 0.7	18.3 ± 1.1	63.2 ± 41.2	1850.1 ± 47.2	8.1 ± 6.1
TAR-H	27.1 ± 2.0	12.6 ± 2.6	11.6 ± 6.4	246.6 ± 23.4	512.7 ± 70.5
TAR-C	32.4 ± 1.0	12.1 ± 0.4	15.7 ± 2.3	280.3 ± 19.0	850.6 ± 101.2
TAR-B	29.9 ± 1.3	8.3 ± 2.2	32.7 ± 2.9	65.5 ± 10.6	22.2 ± 6.7
TAR-W	15.0 ± 0.8	6.8 ± 0.7	24.6 ± 4.5	90.2 ± 8.0	7.6 ± 5.1
TAS-H	41.0 ± 15.3	13.1 ± 0.5	66.3 ± 3.0	513.7 ± 33.6	665.7 ± 56.1
TAS-C	67.8 ± 9.9	20.3 ± 0.4	98.3 ± 11.8	416.9 ± 50.1	561.8 ± 3.5
TAS-B	28.6 ± 1.0	7.4 ± 0.5	97.0 ± 18.2	407.8 ± 53.5	65.1 ± 10.8
TAS-W	17.0 ± 1.1	8.1 ± 3.7	86.0 ± 14.1	72.6 ± 4.4	47.7 ± 35.2
TAL-H	65.5 ± 6.1	16.1 ± 0.6	50.2 ± 11.8	629.1 ± 60.6	1322.2 ± 63.2
TAL-C	68.9 ± 24.6	10.2 ± 1.4	65.7 ± 10.3	381.1 ± 29.8	428.2 ± 59.2
TAL-B	84.5 ± 2.6	8.7 ± 0.7	8.3 ± 7.2	643.7 ± 11.2	44.7 ± 51.7
TAL-W	83.3 ± 14.4	9.8 ± 3.6	17.4 ± 30.2	507.8 ± 15.4	12.1 ± 3.0

AAE – ascorbic acid equivalents, *CC – C. colocynthis*, DPPH – 2,2′-diphenyl-1-picrylhydrazyl scavenging radical potential, GAE – gallic acid equivalents, GSH – reduced glutathione content, H, C, B , W – *n*-hexane, chloroform, *n*-butanol, water extract, resp., L – leaf, LPO – lipid peroxidation, S – stem, R – root, *TA – T. apollinea,* TAC – total antioxidant capacity TPC – total phenolic content; Values are mean ± SD (n = 3).

[a] Control: DPPH and TAC – ascorbic acid, anti-LPO – butylhydroxy toluene (BHT), GSH – glutathione, TPC – gallic acid.

Total phenolics

The results of the present study showed that the total phenolic content was significantly higher ($p < 0.0035$) in the stem of *C. colocynthis*, especially in *n*-butanol and water extracts, and in leaf water extract (Table I) than in other samples. In the case of *T. apollinea, all leaf* extracts, especially the *n*-butanol and water ones were significantly ($p < 0.0338$) more active than those of stem and root (Tables I and II). The PCA analysis showed that CCL-C and CCL-B were outliers in the PCA plot, whereas most of *T. apollinea* extracts formed a closed cluster. In conclusion, the results suggest that TPC was markedly higher in *T. apollinea compared to C. colocynthis* (Fig. 1, Table I).

Total antioxidant capacity

TAC of all the extracts revealed that both medicinal plants possess significant ($p < 0.05$) antioxidant potential or comparable to that of the control (Table I). The present results indicated that the maximum concentration of all the root extracts (200 μg mL^{-1}) possessed significantly higher ($p < 0.007$) total antioxidant potential compared to other extracts of *C. colocynthis*, except for leaves in *n*-butanol (Tables II and III). In the case of *T. apollinea,* the *n*-hexane and chloroform extracts of stem, root, and leaf showed significantly higher ($p < 0.0345$) antioxidant activity compared to other extracts. However, this activity was lower than that of commercially available ascorbic acid, which was used as a standard. The PCA model also revealed that CCR-W, CCR-B and CCR-H formed a distinctive group among the plants, their extracts revealing the bioactivity potential of *C. colocynthis* (Fig. 1).

DPPH radical scavenging potential

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activities of all the extracts were assessed and the results are shown in Table I. The root and leaf extracts of *C. colocynthis* showed significant ($p < 0.004$) results for all the plant extracts (Table II). Similarly, stem extracts of *T. apollinea* also showed good DPPH potential but the effect of root extracts was independent of increasing concentration (Tables II and III). Overall, the *n*-hexane and chloroform extracts revealed a dose-dependent impact of scavenging the DPPH radical compared to *n*-butanol and water extracts (Tables II and III). This is in accord with the PCA analysis. The PCA model showed that CCR-B, CCL-C and CCL-H were outliers whereas the other extracts were mostly adjoined to each other (Fig. 1). Overall, the *C. colocynthis* plant showed a significant pattern of effects.

Reduced glutathione content

In the present assay, *C. colocynthis* was found to possess a significantly higher ($p < 0.001$) reduced glutathione content compared to *T. apollinea* (Tables II and III). Among different plant parts of *C. colocynthis*, leaf extracts had a significantly higher ($p < 0.001$) glutathione content compared to those of stem and root, and these results were found to be dose-dependent (Table III). Stem and leaf extracts of *T. apollinea* also showed a better reduced glutathione content potential compared to its root extracts (Table I). Bioactivity results are also in correlation with the PCA analysis. The PCA scores showed that CCS-C and CCL-B from *C. colocynthis* and TAS-W, TAS-C and TAL-B from *T. apollinea* formed a distinctive location whereas the

Table II. Two-way ANOVA of the bioassays performed for different extracts of T. apollinea *and* C. colocynthis

Medicinal plant Root		*T. apollinea*			*C. colocynthis*		
		Stem	Leaf	Root	Stem	Leaf	
TPC	MS	1677	1139	773.8	3.362	75.51	5.09
	p-value	0.0071	0.0001	0.0338	0.0001	0.0035	< 0.0001
TAC	MS	67823	43891	216160	2137	439.2	5.09
	p-value	< 0.0001	< 0.0001	0.0345	0.007	0.005	< 0.0001
LPO	MS	9.482	18.08	18.53	24.22	37.67	254.0
	p-value	< 0.0001	< 0.0001	< 0.0001	0.0046	0.0205	0.0170
DPPH	MS	57.55	3358	655.2	248353	72.51	418773
	p-value	0.2372	0.0062	0.3289	< 0.0001	0.0035	0.004
GSH	MS	8267	93515	16116	4299	54326	< 0.0001
	p-value	0.0239	0.0205	0.3447	0.1178	0.0009	0.0001

Values in each column are the mean of three replicates. MS – mean of the squares; for other acronyms see Table I. Two-way ANOVA was performed for two factors (plant's part * type of extract). *p*-value < 0.05 indicates statistical significance.

Table III. Two-way ANOVA of the bioassays performed for different concentrations (5–200 μg mL^{-1}) of T. apollinea *and* C. colocynthis *extracts*

Medicinal plant Root		*T. apollinea*			*C. colocynthis*		
		Stem	Leaf	Root	Stem	Leaf	
TPC	MS	568.7	1781	2200	4.617	86.32	2557
	p-value	< 0.0001	0.0001	0.0002	0.0002	0.0006	0.0002
TAC	MS	71950	59743	115160	3507	361.2	3057
	p-value	< 0.0001	< 0.0001	0.0013	0.0001	0.0001	0.0001
LPO	MS	6.712	24.19	5.709	4.307	10.33	43.52
	p-value	< 0.0001	< 0.0001	< 0.0001	0.007	0.1435	0.032
DPPH	MS	144.2	6719	1093	2105	86.32	28040
	p-value	0.0175	<0.0001	0.0001	0.0001	0.0001	0.5861
GSH	MS	6043	48994	9499	13680	20993	0.0001
	p-value	0.0001	0.0001	0.0001	0.0012	0.0166	0.0001

For acronyms see Tables I and II.
Values are the mean of three replicates.
Two-way ANOVA was performed for two factors (plant's part * different concentrations of extracts).
p-value < 0.05 indicates statistical significance.

others were mostly clustered to each other (Fig. 1). This suggests the prominence of variations among different plant parts of both species and various organic extracts in glutathione levels. Low levels of GPx activity may reflect decreased defense against oxidant-mediated cardiovascular damage (13).

Anti-lipid peroxidation

Chloroform extract of *C. colocynthis* leaf showed significantly higher ($p < 0.017$) anti-lipid potential compared to the other extracts (Tables I and II), whereas different extracts of *T. apollinea* showed a dose-dependent response for avoiding lipid peroxidation (Tables I and III). The PCA analysis also suggests a scattering effect of almost all extracts and their concentrations. In comparison with other bioassays, in anti-lipid peroxidation, few extracts formed a cluster, while most of them were outliers.

CONCLUSIONS

The present results show that *C. colocynthis and T. apollinea* carry a potential against oxidative stress and can be further explored as a source of potent therapeutic agents against free radical related pathological damages. The value of these natural resources would be even higher if the bioactive fractions from the present study were subjected to advanced chromatographic and spectroscopic techniques to isolate and identify the purified and potentially bioactive chemical constituents. Therefore, further characterization of the phenolic composition is needed. Future studies should be also focused on enzyme inhibition and *in vivo* assays to further explore the biological potential.

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