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**Phytochemical and Antioxidant Composition of Desert Cotton (*Aerva javanica*) Crude Extracts in Solvents with Varying Polarity**

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**Abstract**

Phytochemical and antioxidant composition of crude extracts, obtained from various parts of desert cotton (*Aerva javanica*) using three solvents of varying polarity such as hexane, methanol and water, was determined. Phytochemical screening confirmed the presence of flavonoids, cardiac glycosides, saponins, tannins and ascorbic acid in each part. Total extractable components, total phenolic compounds, total tannins and total flavonoids of different extracts ranged from  $3.1 \pm 0.60$  to  $31.89 \pm 1.76$ ,  $0.11 \pm 0.01$  to  $3.54 \pm 0.36$ ,  $0.041 \pm 0.001$  to  $2.02 \pm 0.150$  and  $0.016 \pm 0.001$  to  $0.93 \pm 0.08$  g/100g dry weight respectively. Trolox equivalent total antioxidant content, Iron reducing capacity and lenoleic acid reduction capacity ranged from  $0.14 \pm 0.007$  to  $3.43 \pm 0.44$  g/100g dry weight,  $0.11 \pm 0.004$  to  $0.61 \pm 0.16$  (Absorbance at  $\lambda=700\text{nm}$ ) and  $25.5 \pm 3.1$  to  $52.45 \pm 3.87\%$  respectively. DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS [2, 2-azino-bis (3-ethylebenzothiazoline-6-sulfonic acid)] and hydroxyl radical scavenging activities ranged from  $22.89 \pm 0.66$  to  $78.16 \pm 0.43$ ,  $37.48 \pm 0.32$  to  $91.5 \pm 0.18$  and  $13.45 \pm 0.64$  to  $52.6 \pm 2.1\%$  respectively. Statistically significant difference in phytochemicals and antioxidant properties was observed among the extracts of varying polarity. Water extract of leaves and stem was found to be comparatively rich in phytochemicals and antioxidants which suggest the preferable use of polar solvents for extraction of such substances from plant material. The results would be a significant contribution regarding phytochemical and antioxidant importance of desert cotton.

Key- Words: Desert cotton, phytochemical screening, phytochemical composition, antioxidant activity, free radical scavenging capacity

**Introduction**

Traditional therapeutic practices of plants provoked the scientists to find new horizons in the field of pharmaceuticals for the use of medicinal plants in the treatment of infectious diseases replacing synthetic medicines<sup>1</sup>. *Aerva javanica*, a member of family (Amaranthaceae), is commonly known as desert cotton and frequently found in sandy and calcareous soil of dry areas of Pakistan. It is an erect perennial herb, with pale green woolly tomentose. It has great nutritional and medicinal importance. It has been used as stuff for pillows, fodder for cattle, fuel for house hold utilities and medicinal remedies for the treatment of infectious diseases in human and veterinary<sup>2</sup>. It has been reported to constitute various biochemical, phytochemical and antioxidant compounds such as carbohydrates, proteins, fiber, fats, steroids, triterpenes, flavonoids, tannins, saponins, alkaloids, sulphates and glycosides<sup>3-4</sup>.

Leaves contain essential oils such as hentriacontane, pentacosane, nonacosane, heptacosane, octacosane, triacontane and hexacosane while stem contains nonacosane, octacosane, heptacosane, hentriacontane, squalene and triacontane<sup>5</sup>. Owing to its good phytochemical composition, desert cotton has been reported to possess antioxidant, antiplasmodial, antihyperglycaemic, antimicrobial, antibacterial, antiviral, anti-ulcer, hypoglycemic and diuretic activities<sup>6-13</sup>. It has been used as folk medicine against skin infections, inflammation, abdominal worms, fever, intestinal gases and rheumatism<sup>2, 7, 14</sup>.

Oxidative damage imposed by damaging species (reactive oxygen, nitrogen, chlorine species) may be direct or indirect cause of numerous pathological conditions. Levels of these species are controlled by antioxidant defense system. The components of this system commonly include vitamins, phyto-derivatives of plants and minerals<sup>15</sup>. Disease protective function of phytochemicals is mostly due to their antioxidant activity against oxidative damage<sup>16</sup>. The

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phytochemicals are the non nutritional components of plants which are known to possess antioxidant properties. Antioxidants are the substances which prevent the oxidative damage to biomolecules by trapping endogenous free radical species<sup>17</sup>. The phytochemicals have been extensively used in pharmaceutical field for the preparations of natural medicine. The extraction and purification of these substances from plant material is significantly affected by process conditions. Solvent polarity is one of the significant factors affecting the extraction yield of phytochemicals. Depending on their chemical nature various phytochemical components are extracted in solvents of different polarity. No single solvent may be reliable to extract all of the phytochemicals present in plant material. It is, therefore, necessary to find out extraction efficiencies of various solvents to increase the extraction yield of these compounds<sup>18,19</sup>.

Previously, Sethi and Sharma used petroleum ether, dichloromethane, methanol and water for the extraction and characterization of antioxidants from desert cotton and reported that methanolic extract was comparatively high in total phenolic content and antioxidant activity<sup>6</sup>. Srinivas and Reddy used hexane, chloroform and methanol for the extraction and ethyl acetate, formic acid and water for fractionation of phytochemicals from different parts of desert cotton and reported wide range of phytochemicals with high antibacterial activity in methanol extracts of leaves and flower<sup>10</sup>. Movaliya and Zaveria used alcohol and water for extraction of antioxidants from roots and found the alcoholic extract to possess high scavenging activity against free radicals<sup>20</sup>.

In continuity of previous findings, we planned to investigate the effect of solvent polarity on the extraction and characterization of phytochemicals and antioxidants from root, stem and leaves of desert cotton using hexane, methanol and water. The study will provide valuable data on the extraction efficiency of different solvents for phytochemicals and antioxidants from plant material. The results would be a significant contribution regarding the phytochemical and antioxidant importance of desert cotton.

## Material and Methods

### Sampling

The desert cotton plants were collected from sandy areas of Dunyapur, District Lodhran, Punjab, Pakistan. Leaves, stem and root were separated manually, washed with distilled water, cut into small pieces with a sharp knife and dried in fresh air under shade. The dried samples were ground mechanically using pestle and mortar and sieved through a very fine sieve (400 mesh number) to obtain the sample of uniform particle

size and stored in air tight black coated glass containers for further analysis.

### Phytochemical screening

Phytochemical screening was performed on the aqueous and ethanolic extracts of different parts of desert cotton (*Aerva javanica*) by using the standard procedures described earlier<sup>21,24</sup>.

### Preparation of extracts

The dry powder of root, stem and leaves were extracted individually in three solvents of different polarity (hexane, methanol and water) using soxhlet extractor. The solvent was evaporated to dryness under reduced pressure using rotary evaporator. The solid matter was weighed to calculate the percentage yield of solid extracts. The solid extracts were stored in black coated air tight glass containers at room temperature for further analysis.

### Phytochemical analysis

The quantitative analysis of the phytochemicals was carried out by standard procedures. Method of Taga *et al.*<sup>25</sup> was used for the estimation of total phenolic compounds (TPC). TPC were calculated as g/100 g dry weight using regression equation obtained from the standard curve of gallic acid ( $R^2=0.9946$ ). Total tannins (TT) were estimated by vanillin assay<sup>26</sup> with slight modifications. An aliquot of extract (1 mL) was mixed with 5 mL of reagent solution (equal volume of 4% vanillin solution in methanol mixed with 8% hydrochloric acid in methanol) and allowed to stand at 30°C for 20 min. Absorbance was recorded at 500 nm and TT were calculated as g/100 g dry weight using regression equation obtained from the standard curve of catechin ( $R^2=0.9919$ ). Total flavonoids (TF) were estimated by the method described by Jia *et al.*<sup>27</sup> with slight modification. An aliquot (1 mL) of extract (0.1 mg/mL) was mixed with ethanol (4 mL) and 5% NaNO<sub>2</sub> (0.3 mL) and allowed to stand for 10 min at 25±2°C followed by the addition of 10% AlCl<sub>3</sub> (0.3 mL), 4% NaOH (4 mL) and 30% ethanol (0.4 mL). The contents were thoroughly mixed and allowed to stand for 12 min. Absorbance was measured at 510 nm against blank. TF were calculated as g/100 g dry weight using regression equation obtained from the standard curve of catechin ( $R^2=0.9951$ ).

### Antioxidant analysis

Trolox equivalent total antioxidant contents (TETAC) of extracts of different parts of desert cotton was evaluated by phosphomolybdenum assay<sup>28</sup>. TETAC was calculated as g/100 g dry weight using regression equation obtained from the standard curve of Trolox ( $R^2=0.9829$ ). Reducing ability of extracts was determined in terms of iron reducing capacity (IRC) and lenoleic acid reduction capacity (LARC) by the

methods described by Oyaizu<sup>29</sup>, and Osawa and Namiki<sup>30</sup> respectively. DPPH (2, 2-diphenyl-1-picrylhydrazyl), ABTS [2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] and hydroxyl radical scavenging activities were determined by the methods described by Moreno *et al.*, Re *et al.* and Smirnoff *et al.* respectively<sup>31-33</sup>.

#### Statistical analysis

The whole procedures from extraction to analysis were repeated in triplicate. The results were expressed as means  $\pm$  standard deviation of three parallel replicates. The means were separated by one way analysis of variance (ANOVA) at confidence level  $p \leq 0.05$  by applying Tukey's multiple range tests using statistical software SPSS version 19.0.

### Results and Discussion

#### Phytochemical composition

Phytochemical screening confirmed the presence of flavonoids, cardiac glycosides, saponins, tannins and ascorbic acid in each part of the plant (Table 1). Present results of phytochemical screening are comparable to those reported earlier<sup>3</sup>. The results for phytochemical analysis of different extracts from different parts of desert cotton are presented in Table 2. TEM of different extracts from root, stem and leaves ranged from  $3.1 \pm 0.60$  to  $16.95 \pm 0.82$ ,  $3.7 \pm 0.15$  to  $24.30 \pm 0.18$  and  $17.74 \pm 0.67$  to  $31.89 \pm 1.76$  g/100 g dry weight respectively. TPC of different extracts from root, stem and leaves ranged from  $0.11 \pm 0.01$  to  $1.37 \pm 0.06$ ,  $0.24 \pm 0.013$  to  $1.5 \pm 0.32$  and  $1.52 \pm 0.07$  to  $3.54 \pm 0.36$  g/100 g dry weight respectively. TF of different extracts from root, stem and leaves ranged from  $0.04 \pm 0.001$  to  $0.55 \pm 0.10$ ,  $0.138 \pm 0.02$  to  $1.022 \pm 0.13$  and  $0.819 \pm 0.02$  to  $2.02 \pm 0.15$  g/100 g dry weight respectively. TT of different extracts from root, stem and leaves ranged from 0.015 to  $0.33 \pm 0.02$ ,  $0.05 \pm 0.005$  to  $0.43 \pm 0.17$  and  $0.28 \pm 0.008$  to  $0.93 \pm 0.08$  g/100 g dry weight respectively. Statistically significant difference ( $p \leq 0.05$ ) was observed in TEM, TPC, TF and TT of extracts in different polarity solvents as well as different parts of desert cotton. TEM, TPC, TF and TT were found to be comparatively high in water extract of leaves and low in hexane extract of root. The present results for TPC, TF and TT were found to be comparable with those reported earlier for *Aerva lenta*<sup>34</sup>. Araujo *et al.*<sup>35</sup> reported that 50% of the plants with higher tannin contents showed good antimicrobial activity. A direct correlation between total phenolic compound and antioxidant activities has been reported which introduce phenols as the main antioxidant of *Aerva tomentosa*<sup>6</sup>.

#### Antioxidant activity

TETAC of different extracts of root, stem and leaves ranged from  $0.14 \pm 0.007$  to  $1.28 \pm 0.03$ ,  $1.36 \pm 0.034$  to  $3.43 \pm 0.44$  and  $1.57 \pm 0.07$  to  $2.12 \pm 0.34$  g/100 g dry weight respectively. The reducing abilities in terms of IRC and LARC of different extracts of root, stem and leaves ranged from  $0.11 \pm 0.004$  to  $0.16 \pm 0.0005$ ,  $0.21 \pm 0.005$  to  $0.61 \pm 0.16$ ,  $0.24 \pm 0.006$  to  $0.3 \pm 0.01$  (Absorbance at  $\lambda = 700\text{nm}$ ) and  $28.19 \pm 2.39$  to  $52.39 \pm 4.1$ ,  $37.06 \pm 4.57$  to  $52.45 \pm 3.87$ ,  $25.5 \pm 3.10$  to  $48.3 \pm 2.92$  respectively. TETAC was found to be high in water extract of stem and low in hexane extract of root. Statistically significant difference ( $p \leq 0.05$ ) was observed in TETAC and reducing capacities of different extracts as well as different parts of desert cotton (Figure 1 and Figure 2). Methanolic extract of stem was found to possess comparatively good reducing properties. LARC of hexane extracts of root, stem and leaves was found to be higher than those of methanolic and water extract.

The free radical scavenging ability of extracts of different parts of desert cotton was determined using DPPH, ABTS and hydroxyl radical (Figure 3). The inhibition capacity of the DPPH radical of different extracts from root, stem and leaves ranged from  $22.89 \pm 0.66$  to  $46.12 \pm 1.12$ ,  $27.94 \pm 0.9$  to  $54.35 \pm 1.53$  and  $38.13 \pm 1.52$  to  $78.16 \pm 0.43\%$  respectively. ABTS radical scavenging activity of different extracts from root, stem and leaves ranged from  $37.48 \pm 0.32$  to  $90.73 \pm 2.1$ ,  $71.22 \pm 0.32$  to  $91.50 \pm 0.18$  and  $88.50 \pm 0.32$  to  $90.73 \pm 0.32\%$  respectively. The hydroxyl radical scavenging activity of different extracts from root, stem and leaves ranged from  $13.45 \pm 0.64$  to  $40.76 \pm 1.04$ ,  $22.65 \pm 2.5$  to  $52.60 \pm 2.1$  and  $24.75 \pm 1.8$  to  $49.7 \pm 0.13\%$  respectively.

Statistically significant difference ( $p \leq 0.05$ ) was observed among free radical scavenging activities of different extracts as well as different parts desert cotton. The inhibition capacity of the DPPH radical of extracts of different parts of desert cotton showed that leaves of desert cotton have highest inhibition capacity in water extract. While methanolic extract of root and stem showed high DPPH radical inhibition capacity. ABTS radical scavenging activity of extracts of different parts of desert cotton were found to be high in methanolic extracts of root, stem and leaves followed by water and hexane extracts. Hydroxyl radical scavenging activity of different extracts of stem, leaves and roots of desert cotton were found to be high in water extracts followed by methanolic and hexane extracts respectively. However, the DPPH, ABTS, Hydroxyl radical scavenging activity of different extracts of each part of desert cotton were found to low

than that of Trolox. The water and methanolic extracts of desert cotton showed good radical scavenging capacities and results are comparable with those reported earlier for *Aerva lenta*<sup>36</sup>. Previously, Murtaza *et al.*<sup>37</sup> also reported that methanolic extract of desert cotton show comparatively high antiradical activity than acetone extract.

Plant derived antioxidants provide direct or indirect disease preventing effect against oxidative stress responsible for numerous pathological conditions<sup>15</sup>. Antioxidants inhibit the cellular damage by capturing the free radical intermediates of oxidative chain reactions and can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides<sup>30</sup>. They are often reducing agents that inhibit oxidation by being oxidized themselves<sup>38</sup>.

Desert cotton is an important medicinal plant which contains bioactive compounds which have therapeutic and curative action against various diseases due to their good antioxidant properties<sup>12</sup>. Alcoholic and aqueous extracts of *Aerva javanica* root has been found to show potent scavenging abilities against free radicals, reducing abilities for iron and inhibitory properties against lipid peroxidation<sup>20</sup>. Owing to its good antioxidant activity, *Aerva javanica* root has been reported to be used for the treatment of rheumatism, swelling, toothache, inflammation, headache and kidney problems. The hexane extract of *Aerva javanica* root has been also found to be effective against cisplatin induced renal toxicity due its nephroprotective activity. The hexane extract was found to show significant recovery in serum protein level and restore renal antioxidant defense system in experimental rats with cisplatin induced renal injury<sup>38</sup>.

The presence of tannins, flavonoids, ascorbic acid and glycosides in desert cotton provides a strong evidence for its good antioxidant composition. Comparatively high values of TEM, TEC, TT and TF in water extract of leaves, stem and root suggest the polar nature of most of the phytochemicals present in desert cotton. Water extract of leaves showed comparatively high value of DPPH radical scavenging ability while hexane extract of root showed high value of linoleic acid reducing capacities. Water extract of stem was found to show high value of TEM and hydroxyl radical scavenging ability. These results suggest that significant amount of phytochemicals may be extracted from desert cotton stem and leaves using water as extracting solvent. Moreover, the linoleic reducing capacities and free radical scavenging abilities of extracts from various parts of desert cotton as compared to Trolox (standard antioxidant) favor the

preferable use of desert cotton extracts for the prevention of oxidative stress caused by free radicals.

### Conclusion

Present results suggest the suitability of water and methanol as extracting solvents for phytochemical and antioxidant analysis of plant materials. A significant amount of phytochemicals may be extracted from desert cotton using these solvents. The results also favor the preferable use of water extract and methanol extract of desert cotton as a source of antioxidant in pharmaceutical formulations particularly for the prevention of oxidative stress caused by free radicals.

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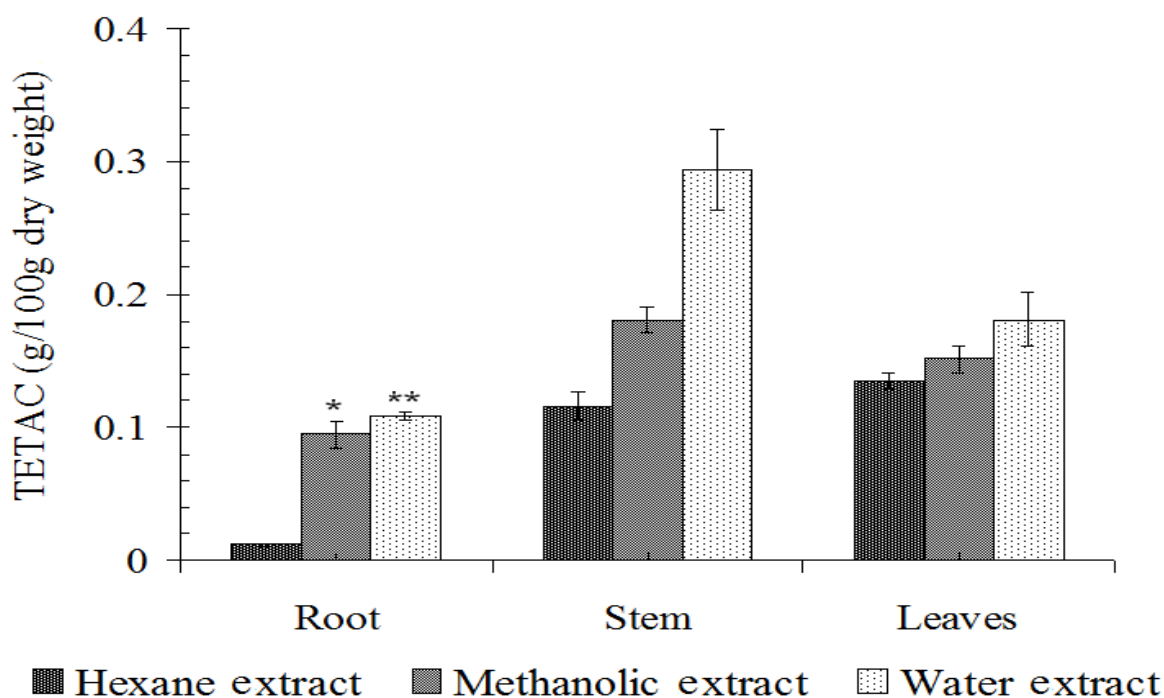


Fig. 1: Trolox equivalent total antioxidant capacity (g/100 g dry weight) of different extracts of root stem and leaves of desert cotton

\*Means±standard deviation of three parallel replicates

\*\*The results for extracts of different parts of desert cotton in each solvent are significantly different at the confidence level  $p \leq 0.05$  using Tukey's multiple range test.

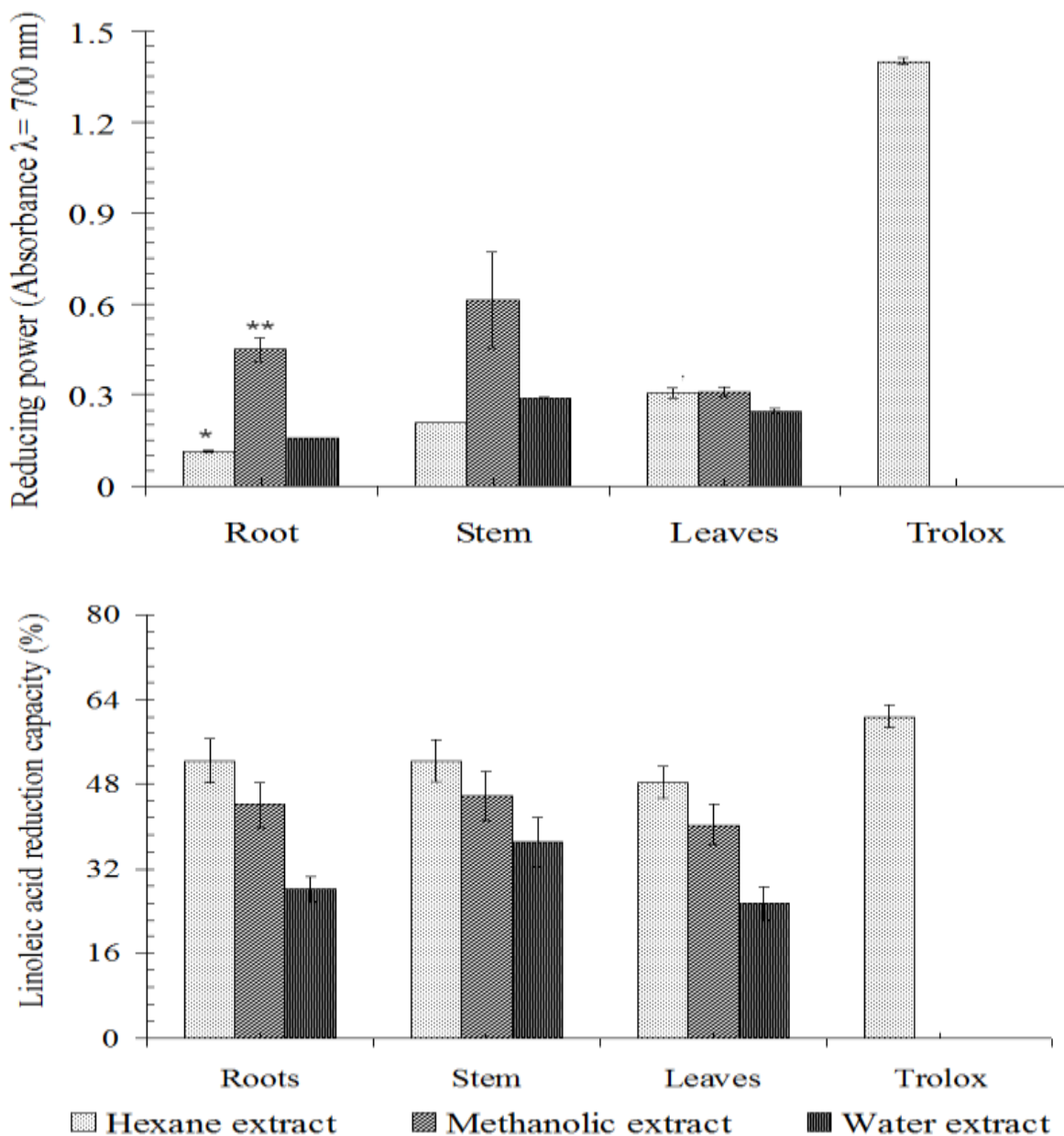
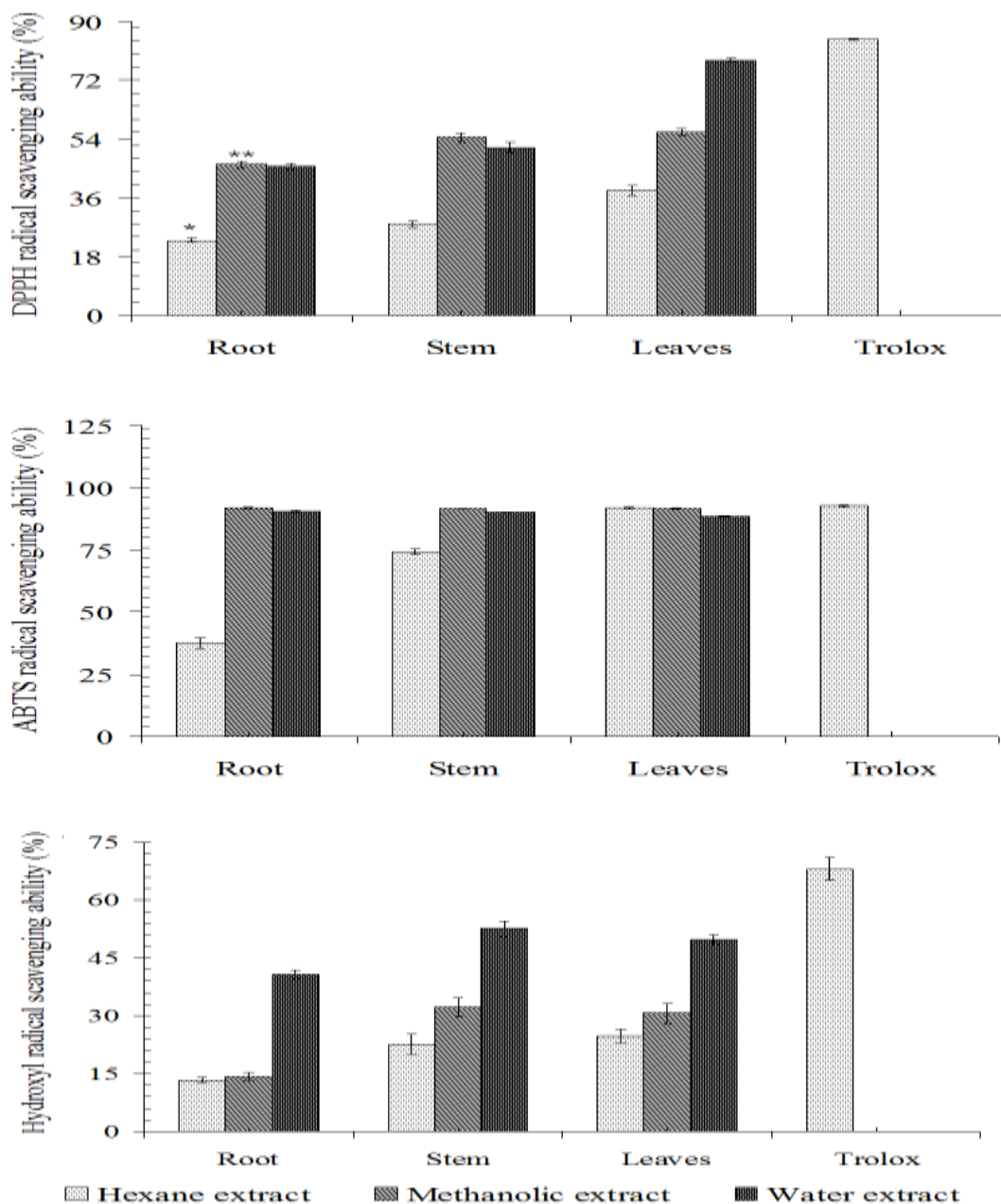


Fig. 2: Reducing abilities of different extracts of root stem and leaves of desert cotton

\*Means  $\pm$  standard deviation of three parallel replicates

\*\*The results for extracts of different parts of desert cotton in each solvent are significantly different at the confidence level  $p < 0.05$  using Tukey's multiple range test.



**Fig. 3: Free radical scavenging activities of different extracts of root stem and leaves of desert cotton**  
 \*Means±standard deviation of three parallel replicates  
 \*\*The results for extracts of different parts of desert cotton in each solvent are significantly different at the confidence level  $p \leq 0.05$  using Tukey's multiple range test.

**Table 1: Phytochemical screening of root, stem and leaves of desert cotton**



Tests for screening	Root	Stem	Leaves
Tannins	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Anthocyanins	-	-	-
Saponins	+	+	+
Ascorbic acid	+	+	+

‘+’ Present

‘-’ Absent

**Table 2: Phytochemical composition (g/100 g dry weight) of different extracts of root stem and leaves of desert cotton**

	Hexane Extract	Methanol Extract	Water Extract	P-value**
<b>TEM</b>				
Root	3.10±0.60*	8.61±1.88	16.95±0.82	<b>0.00</b>
Stem	3.70±0.15	16.42±1.54	24.30±0.18	<b>0.00</b>
Leaves	17.74±0.67	18.67±0.17	31.89±1.76	<b>0.00</b>
P-value***	0.00	0.00	0.00	
<b>TPC</b>				
Root	0.11±0.01	0.60±0.03	1.37±0.06	<b>0.00</b>
Stem	0.24±0.013	1.49±0.11	1.5±0.32	<b>0.00</b>
Leaves	1.55±0.054	1.52±0.08	3.54±0.36	<b>0.00</b>
P-value	0.00	0.00	0.00	
<b>TT</b>				
Root	0.0155±0.001	0.024±0.002	0.33±0.02	<b>0.00</b>
Stem	0.055±0.005	0.08±0.05	0.43±0.17	<b>0.20</b>
Leaves	0.283±0.008	0.134±0.01	0.93±0.08	<b>0.00</b>
P-value	0.00	0.02	0.001	
<b>TF</b>				
Roots	0.041±0.001	0.33±0.020	0.55±0.10	<b>0.00</b>
Stem	0.138±0.022	0.95±0.010	1.022±0.13	<b>0.00</b>
Leaves	0.819±0.020	0.93±0.12	2.02±0.15	<b>0.00</b>
P-value	<b>0.00</b>	<b>0.00</b>	<b>0.00</b>	

\*Means±standard deviation of three parallel replicates

\*\*p-values show the significant difference between extracts of different parts of desert cotton in a single solvent at the confidence level  $p \leq 0.05$  using Tukey's multiple range test.

\*\*\*p-values show the significant difference between extracts of a single part of desert cotton in different solvents at confidence level  $p \leq 0.05$  using Tukey's multiple range test.

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