

Polarity-dependent Response of Phytochemical Extraction and Antioxidant Potential of Different Parts of *Alcea rosea*

Haq Nawaz^{1,*}, Hira Akram², Qazi Hafiz Muhammad Ishaq¹, Arslan Khalid¹, Briha Zainab¹, Aiman Mazhar¹

ABSTRACT

Objectives: *Alcea rosea* is a good source of medicinally important bioactive phytochemical compounds. The complete extraction of these biochemicals from plant material has remained a problem for the researchers and manufacturers. This study was planned to report the medicinal value of *A. rosea* and find out the suitable extraction solvent to enhance the extraction yield of its phytochemical compounds and their antioxidant potential. **Materials and Methods:** The powdered samples of the selected parts of *A. rosea* were extracted in solvents of varying dipole moments (hexane: 0 D, ethyl acetate: 2.8 D, and methanol: 5.1 D). The extracts were screened for the presence of important phytochemicals and analyzed for their phytochemical content and antioxidant potential. **Results:** The studied parts of *A. rosea* consisted of flavonoids, tannins, terpenoids, saponins, and cardiac glycosides. The regression analysis showed polarity-dependent significant positive effects ($p < 0.05$) on the extract yield, phenolic content, and antioxidant activity in terms of Trolox equivalent total antioxidant activity, ferric reducing power, and nitrogen free radical scavenging activity of the extracts. However, a mixed response of total flavonoids and total tannins content and hydroxyl radical scavenging activity of the extracts was observed against solvent polarity. **Conclusion:** A polarity dependent increase in phytochemical content and free radical scavenging capacity was observed. The study suggests that the polar solvents are more suitable to achieve a good extract yield of phytochemicals possessing a strong antioxidant ability and the solvents of medium polarity may be suitable for the extraction of flavonoids and tannins from the studied parts of *A. rosea*.

Keywords: *Alcea rosea*, Antioxidant potential, Phytochemical composition, Solvent polarity, Free radical scavenging activity.

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History

- Submission Date: 22-07-2022;
- Review completed: 15-08-2022;
- Accepted Date: 01-09-2022.

DOI : 10.5530/fra.2022.2.9

Article Available online

<http://www.antiox.org>

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INTRODUCTION

Alcea rosea, commonly known as Hollyhock or Gulkhaira, is mostly found in Asia and Europe and cultivated in Egypt for medicinal purposes.^{1,2} As a traditional ailment, *A. rosea* has been commonly used for the treatment of fever, respiratory and gastrointestinal problems, sore throat, and skin burns. It has been also found to be effective against bacterial infections and inflammation. It has been also used in wound healing and reducing pain during teething in infants.³⁻⁵ Warm leaves of *A. rosea* have been used to release pus from the affected areas while the flowers are used for prophylaxis and to cure urinary, gastrointestinal, and respiratory diseases. The root extracts have been found effective in the reduction of the calcium oxalate stone formation and its removal from the kidneys in a rat experimental model.⁶⁻⁸ The root of *A. rosea* is also effective in the treatment of cough, skin inflammation, ulcers, hunger loss, and swelling of kidney and respiratory diseases.⁹

Along with good sugar, amino acid, and mineral composition, the *A. rosea* consists of medicinally important bioactive phytochemicals.¹⁰ The

phytochemicals including phenolic acids, tannins, polyphenols, flavonoids, saponins, cardiac glycosides, alkaloids, and terpenoids are medicinally important non-nutritional constituents of plants.¹¹ These phytochemicals have great medicinal value due to their diverse biological activities particularly antioxidant, antimicrobial, antiallergic, cytotoxic, and enzyme inhibitory activities.¹² Based on its phytochemical composition, *A. rosea* possesses great medicinal value due to its anti-urolithiasis, anti-inflammatory, analgesic, antiulcer, and immunomodulatory, immune-stimulatory anticancer, cytotoxic, hepatoprotective activities.¹³⁻¹⁸

Previously, the *A. rosea* extracts have been studied for their phytochemical composition, antioxidant potential, antibacterial activity, and cytotoxic activities against hepatocellular carcinoma.^{10,16,19} However, the polarity-dependent response of phytochemical extraction and antioxidant potential of *A. rosea* has not been reported earlier. Therefore, the present study aimed to investigate the polarity-dependent response of extraction yield and antioxidant potential of bioactive phytochemicals present in various parts

Cite this article: Nawaz H, Akram H, Ishaq QHM, Khalid A, Zainab B, Mazhar A. Polarity-dependent Response of Phytochemical Extraction and Antioxidant Potential of Different Parts of *Alcea rosea*. Free Radicals and Antioxidants. 2022;12(2):49-54.



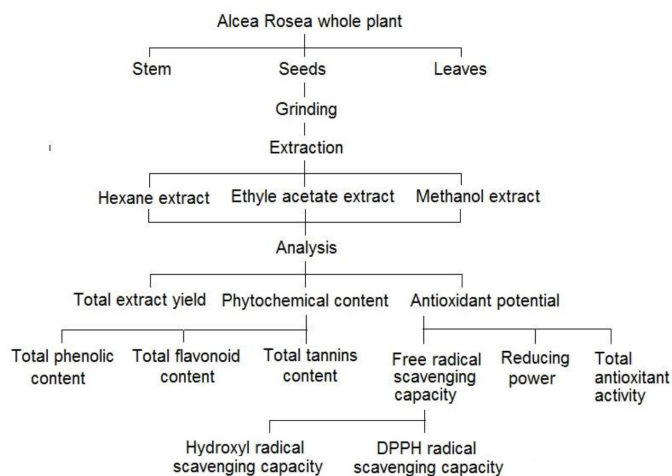


Figure 1: Scheme of study.

of *A. rosea* was investigated in this study. The study would be a significant contribution to the scientific literature regarding the extraction of valuable bioactive compounds from plant materials for pharmaceutical applications.

MATERIALS AND METHODS

Experimental Design

The study was designed to investigate the effect of solvent polarity on the extraction of phytochemicals from different parts of *A. rosea* and their antioxidant activity. The extracts of three parts of *A. rosea* were obtained in solvents of varying polarity (Hexane: 0, ethyl acetate: 2.8, and acetone: 5.1 D). The extracts were screened for the presence of important phytochemicals and analyzed for their phytochemical content and antioxidant potential. The overall scheme of extraction and analysis is presented in Figure 1.

Sampling

The *A. rosea* plant was collected from the local fields in the vicinity of Bahauddin Zakariya University, Multan, Pakistan. The stem, seeds, and leaves were separated manually and washed with clean water. The samples were dried under shade, ground to a fine powder using an electrical grinder, sieved through a fine mesh, and preserved in the airtight glass jars.

Phytochemical Screening

The phytochemical screening was done by the procedures as described earlier.²⁰ The dried sample (0.5 g) was extracted in boiling water (10 ml) and the extract was mixed with a 0.1% FeCl₃ solution. The presence of tannins was confirmed in the appearance of the brownish-green color. The appearance of yellow color by mixing the ethanolic extract of the samples (2 ml) with a few drops of 1% aluminum chloride solution indicated the presence of flavonoids. The mixing of the sample with glacial acetic acid (2 ml) and a drop of ferric chloride solution and the addition of sulfuric acid with the side wall of the test tube resulted in the appearance of a brown-colored ring that indicated the presence of cardiac glycosides in the sample. The presence of terpenoids was tested by mixing the ethanolic extracts of the samples with chloroform (2 ml) and concentrated sulfuric acid (3 ml). The appearance of brownish color confirmed the presence of terpenoids in the sample.

Phytochemicals Composition

The powdered samples were extracted in solvents of varying polarity (Hexane: 0, ethyl acetate: 2.8, and acetone: 5.1 D). The solvents were evaporated to dryness in a water bath and the percentage yield of the extracts was calculated using the following expression.

$$\text{Total extract yield (\%)} = \frac{W_e}{W_s} \times 100$$

where W_e is the weight of the extract and W_s is the weight of the sample.

The total phenolic content (TPC) of the extracts obtained from the selected parts of *A. rosea* was estimated by the Folin Ciocalteu method²¹ as described earlier.²² The TPC (g/100 g dry weight) was calculated using the following equation obtained from the linear regression curve of the Gallic acid standard ($R^2 = 0.9923$).

$$\text{TPC (g/100 g dry weight)} = \frac{\text{Abs. of the sample}}{5.1948}$$

The total flavonoid content (TFC) was determined by the method reported by Michalaska *et al.* as described earlier.^{23,24} The TFC (g/100 g dw) was calculated using the following equation obtained from the linear regression curve of Catechin ($R^2 = 9951$).

$$\text{TFC (g/100 g dry weight)} = \frac{\text{Abs. of the sample}}{7.1517}$$

The tannins contents (TTC) in each extract were evaluated following the method of Fagbemi *et al.* using Folin Ciocalteu's reagent.²⁵ The TTC was calculated as g/100 g Tannic acid equivalent using the following equation obtained by the standard regression curve of Tannic acid ($R^2 = 9945$).

$$\text{TTC (g/100 g dw)} = \frac{\text{Abs. of the sample}}{12.592}$$

Antioxidant Analysis

The Trolox

Equivalent total antioxidant activity (TETAOA) was estimated following the previously reported phosphomolybdenum method.^{20,26} The regression equation obtained from the regression curve of Gallic acid ($R^2 = 0.9977$) was used to calculate the TETAOA.

$$\text{TETO A (g/100 g dw)} = \frac{\text{Abs. of the sample}}{8.3257}$$

The ferric reducing power (RP) was estimated using the previously described method.²⁷ The absorbance of the reaction mixture at 720 nm was used to represent the reducing power.

2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH RSA) was evaluated by the previously reported DPPH assay.²⁸ The following expression was used to calculate the DPPH RSA.

$$\text{DPPH RSA (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Hydroxyl radical scavenging activity (HRSA) of each extract was evaluated by the method of Smirnoff and Cumbes.²⁹ The following expression was used to calculate the HRSA.

$$\text{HRSA (\%)} = 1 - \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Statistical Analysis

The results were expressed as mean \pm standard deviation of three replicates. The significance of variance in the results was determined by a one-way analysis of variance using Tukey's multiple range test at $p \leq 0.05$.

The regression analysis was applied to analyze the polarity-dependent variation in the phytochemical content and antioxidant parameters.

RESULTS

The phytochemical screening of the selected parts of *A. rosea* confirmed the presence of saponins, flavonoids, terpenoids, tannins, and cardiac glycosides (Table 1). The results of the total extractable components and total phytochemical content of the selected parts of *A. rosea* as extracted in different solvents and analyzed in terms of TEC, TPC, TFC, and TTC are presented in Table 2. The TEC of the stem, seed, and leaves in various solvents ranged from 1.294±0.065 to 14.200±0.710 g/100g dw. The TEC of the selected parts of *A. rosea* as extracted in various solvents was found to be significantly different ($p < 0.05$). The results of regression analysis showed a polarity-dependent exponential increase in TEC of each part

Table 1: Phytochemical screening of stem, seed and leaf *A. rosea*.

Plant part	Tannins	Saponins	Flavonoids	Terpenoids	Cardiac glycosides
Stem	+	+	+	+	+
Seed	+	+	+	+	+
Leaves	+	+	+	+	+

Table 2: Total extractable components, phytochemical content, and antioxidant potential of *A. rosea* extracts obtained in solvents of varying polarity.

	Plant parts	Hexane	Ethyl acetate	Methanol	p-value
TEC* (g/100 g dw)	Stem	2.250±0.112 ^b	2.400±0.120 ^b	8.600±0.430 ^a	0.000
	Seed	1.294±0.065 ^c	4.00±0.200 ^b	10.700±0.535 ^a	0.000
	Leaves	3.200±0.160 ^c	6.00±0.300 ^b	14.200±0.710 ^a	0.000
TPC (g/100 g dw)	Stem	0.066±0.002 ^c	0.304±0.015 ^b	1.037±0.067 ^a	0.000
	Seed	0.455±0.034 ^c	0.751±0.056 ^b	1.223±0.029 ^a	0.000
	Leaves	0.738±0.061 ^c	1.137±0.043 ^b	4.206±0.041 ^a	0.000
TFC (g/100 g dw)	Stem	0.028±0.001 ^b	0.05±0.002 ^b	0.315±0.027 ^a	0.000
	Seed	0.015±0.00 ^c	0.09±0.004 ^b	0.189±0.00 ^a	0.000
	Leaves	0.024±0.007 ^c	0.089±0.01 ^b	0.065±0.008 ^a	0.000
TTC (g/100 g dw)	Stem	0.022±0.00 ^b	0.020±0.003 ^b	0.044±0.002 ^a	0.000
	Seed	0.026±0.001 ^b	0.11±0.024 ^{ab}	0.044±0.00 ^a	0.057
	Leaves	0.007±0.001 ^c	0.04±0.002 ^b	0.09±0.014 ^a	0.000
TETAOA (g/100 g dw)	Stem	0.103±0.016 ^b	0.090±0.002 ^b	0.372±0.046 ^a	0.000
	Seed	0.094±0.024 ^c	0.259±0.003 ^b	0.969±0.028 ^a	0.000
	Leaves	0.080±0.033 ^b	0.215±0.008 ^b	2.126±0.260 ^a	0.000
Reducing power (Abs. at 720 nm)	Stem	0.176±0.004 ^c	0.092±0.004 ^b	0.269±0.025 ^a	0.000
	Seed	0.149±0.007 ^a	0.120±0.030 ^a	0.208±0.070 ^a	0.119
	Leaves	0.065±0.014 ^b	0.371±0.028 ^a	0.365±0.021 ^a	0.000
DPPH RSA (%)	Stem	64.270±0.570 ^c	59.940±0.630 ^b	74.280±0.710 ^a	0.000
	Seed	71.420±0.720 ^c	68.560±0.860 ^b	65.700±0.510 ^a	0.000
	Leaves	61.420±0.780 ^c	55.700±0.890 ^b	79.990±0.400 ^a	0.000
HRSA (%)	Stem	38.770±1.110 ^c	81.530±5.690 ^b	64.080±6.670 ^a	0.000
	Seed	42.650±0.900 ^b	89.080±2.750 ^a	85.400±5.770 ^a	0.000
	Leaves	41.730±0.990 ^c	63.360±4.270 ^b	94.280±2.200 ^a	0.000

The values are mean ± standard deviation of three replicates.

*TEC: Total extractable components, TPC: Total phenolic content, TFC: Total flavonoid content, TTC: Total tannin content, TETAOA: Trolox equivalent total antioxidant activity, DPPH RSA: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity, HRSA: Hydroxy radical scavenging activity

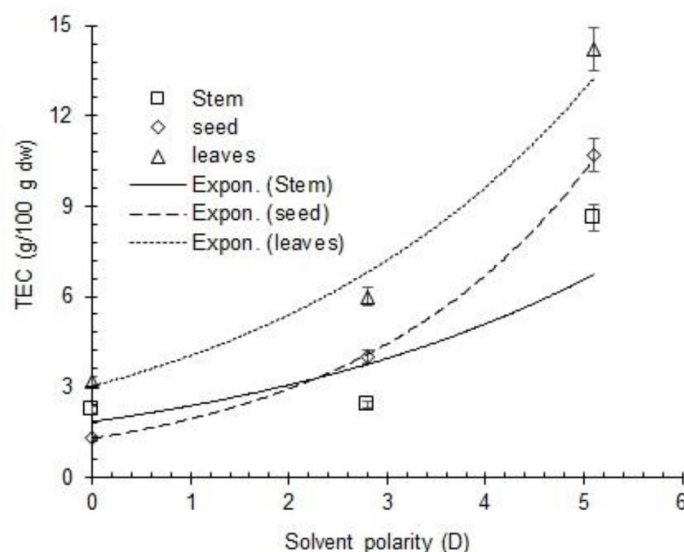


Figure 2: Polarity-dependent response of TEC of various parts of *A. rosea*.

Table 3: Parameters of regression analysis of the experimental data to study the polarity-dependent response of the studied parameters of various parts of *A. rosea*.

Parameters	Plant parts	Regression Equation	Trend of variation	R ²
TEC (g/100 g dw)	Stem	y=1.8398e ^{0.2543x}	Exponential	0.7379
	Seed	y=1.2819e ^{0.4138x}	Exponential	0.9997
	Leaves	y=3.0233e ^{0.2898x}	Exponential	0.9787
TPC (g/100 g dw)	Stem	y=0.0666e ^{0.539x}	Exponential	1
	Leaves	y=0.6307e ^{0.3346x}	Exponential	0.8893
TFC (g/100 g dw)	Stem	y=0.0211x ² - 0.0511x + 0.028	Polynomial	1
	Seed	y=0.0032x ² + 0.0179x + 0.015	Polynomial	1
TTC (g/100 g dw)	Leaves	y=-0.0066x ² + 0.0417x + 0.024	Polynomial	1
	Stem	y=0.0022x ² - 0.0068x + 0.022	Polynomial	1
TETAOA (g/100 g dw)	Seed	y=-0.0039x ² + 0.0234x + 0.026	Polynomial	1
	Leaves	y=0.0078e ^{0.5051x}	Exponential	0.9773
	Stem	y=0.0802e ^{0.2404x}	Exponential	0.6134
Reducing Power (Abs. at 720 nm)	Seed	y=0.1676x - 0.0006	Linear	0.8474
	Leaves	y=0.0625e ^{0.6336x}	Exponential	0.923
	Stem	y=0.021x ² - 0.0887x + 0.176	Polynomial	1
DPPH RSA (%)	Seed	y=0.0095x ² - 0.037x + 0.149	Polynomial	1
	Leaves	y=0.0825e ^{0.3485x}	Exponential	0.7907
	Stem	y=1.5257x ² - 5.8185x + 64.27	Polynomial	1
HRSA (%)	Seed	y=-1.118x + 71.504	Linear	0.9968
	Leaves	y=2.4713x ² - 8.9626x + 61.42	Polynomial	1
	Stem	y=-4.482x ² + 27.821x + 38.77	Polynomial	1
TTC (g/100 g dw)	Seed	y=-3.5651x ² + 26.564x + 42.65	Polynomial	1
	Leaves	y=41.358e ^{0.1594x}	Exponential	0.9982

*TEC: Total extractable components, TPC: Total phenolic content, TFC: Total flavonoid content, TTC: Total tannin content, TETAOA: Trolox equivalent total antioxidant activity, DPPH RSA: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity, HRSA: Hydroxy radical scavenging activity

($R^2 = 0.7379-0.9997$) (Figure 2). The regression parameters of polarity-dependent regression curves of TEC of the selected parts of *A. rosea* are presented in Table 3.

The TPC, TFC, and TTC of the stem, seed, and leaves in various solvents ranged from 0.066 ± 0.002 to 4.206 ± 0.041 , 0.015 ± 0.00 to 0.315 ± 0.027 , and 0.007 ± 0.001 to 0.11 ± 0.024 g/100g dw respectively. The results were found to be statistically different ($p < 0.05$) in the selected parts of *A. rosea* as extracted in various solvents except that of TTC of the seeds of *A. rosea*. An increase in the solvent polarity resulted in an exponential positive effect on TPC of each part and TTC of leaves, a polynomial positive effect on TFC of stem and seeds and TTC of the stem, and a polynomial negative effect on TFC of leaves and TTC of seeds ($R^2 = 0.8893-1$) (Figure 3a-c). The regression parameters of the polarity-dependent regression curves of the studied phytochemicals in the selected parts of *A. rosea* are presented in Table 3.

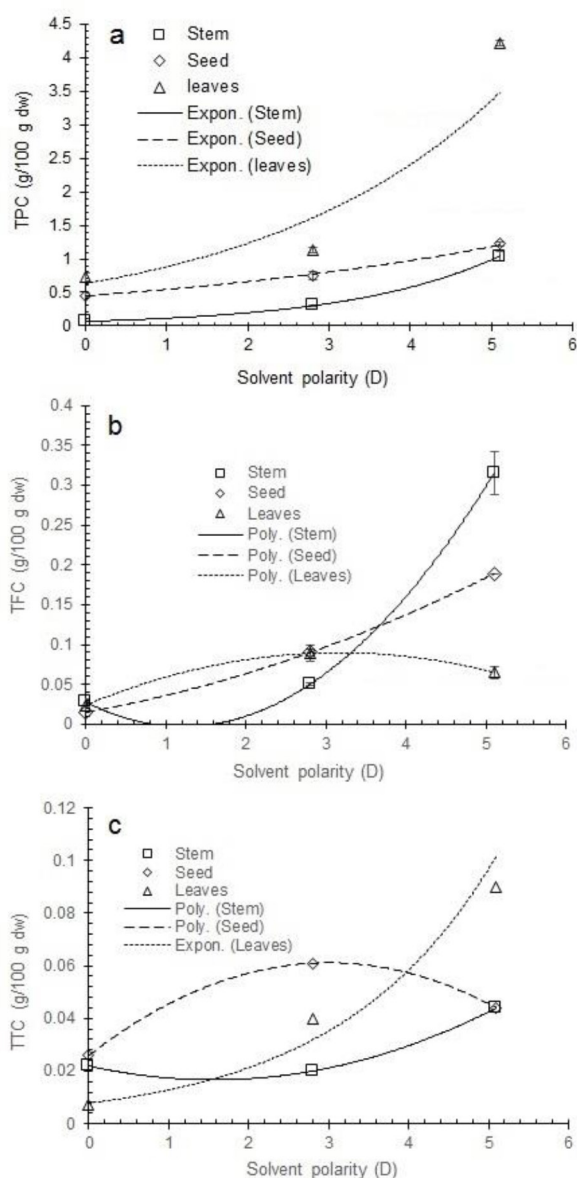


Figure 3a-c: Polarity-dependent response of TPC, TFC, and TTC of extracts obtained from various parts of *A. rosea*. TPC: Total phenolic content, TFC: Total flavonoid content, TTC: Total tannins content

The experimental values of antioxidant potential of various extracts of the selected parts of *A. rosea* in terms of TETAOA and RP are presented in Table 2. TETAOA and RP of the stem, seed, and leaves in various solvents ranged from 0.080 ± 0.033 to 2.126 ± 0.260 g/100g dw and 0.065 ± 0.014 to 0.371 ± 0.028 Abs. at 720 nm respectively. The TETAOA and RP of the selected parts of *A. rosea* as extracted in various solvents were found to be statistically different ($p < 0.05$) except for the RP of the seeds of *A. rosea*. The solvent polarity showed an exponential positive effect on TETAOA of stem and leaves and RP of leaves, a polynomial positive effect of solvent polarity on RP of stem and seeds, and a linear positive effect on TETAOA of seed ($R^2 = 0.6134-0.923$) (Figure 4a, b). The regression parameters of the polarity-dependent regression curves of TETAOA and RP of the selected parts of *A. rosea* are presented in Table 3.

The results of the free radicals of the selected parts of *A. rosea* as extracted in different solvents and analyzed in terms of DPPH RSA and HRSA are presented in Table 2. DPPH RSA and HRSA of the stem, seed, and leaves in various solvents ranged from 55.700 ± 0.890 to 79.990 ± 0.400 and 38.770 ± 1.110 to 94.280 ± 2.200 % respectively. The DPPH RSA and HRSA of various extracts of the selected parts of *A. rosea* were found to be statistically different ($p < 0.05$). The regression analysis showed a polynomial positive effect of solvent polarity on DPPH RSA of stem and leaves, a negative polynomial effect on HRSA of stem and seed, a linear negative effect on DPPH RSA of seeds, and an exponential positive effect on HRSA of leaves ($R^2 = 0.9982-1$) (Figure 5a, b). The regression parameters of the polarity-dependent

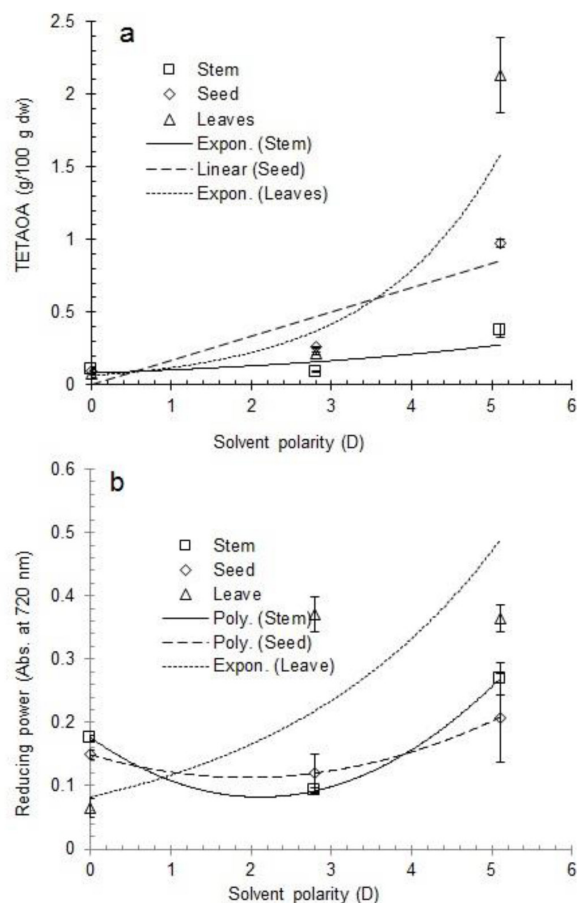


Figure 4 a-b: Polarity-dependent response of TETAOA and RP of extracts obtained from various parts of *A. rosea*. TETAOA: Trolox equivalent total antioxidant activity, RP: Reducing power

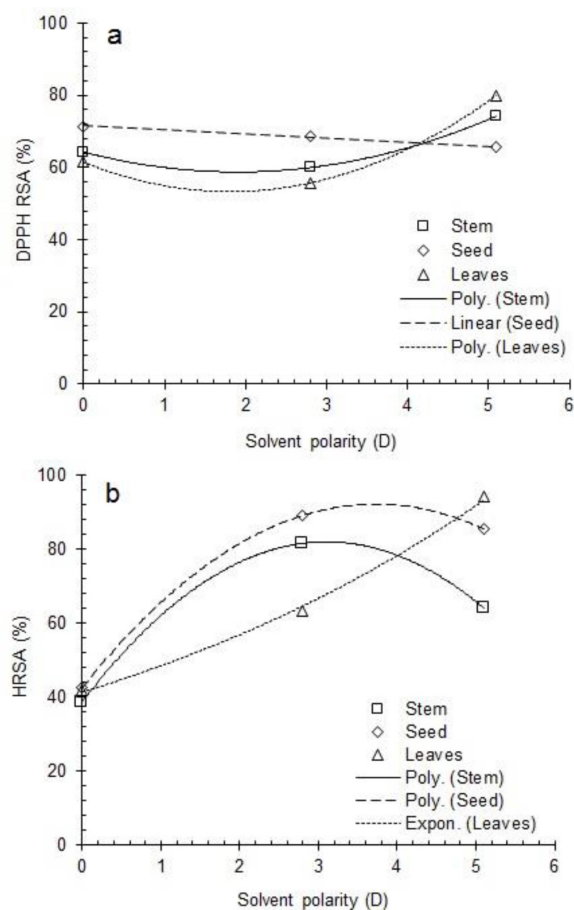


Figure 5a-b: Polarity-dependent response of DPPH RSA and HRSA of extracts obtained from various parts of *A. rosea*.

DPPH RSA: 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity, HRSA: Hydroxyl radical scavenging activity.

regression curves of DPPH RSA and HRSA of the selected parts of *A. rosea* are presented in Table 3.

DISCUSSION

The results of phytochemical screening of seed, stem, and leaves of *A. rosea* showed the presence of some bioactive phytochemical compounds including saponins, flavonoids, terpenoids, tannins, and cardiac glycosides. The presence of these phytochemicals makes *A. rosea* a good source of bioactive phytochemical compounds for pharmaceutical applications.

The phytochemical compounds present in the selected parts of *A. rosea* were extracted in solvents of varying polarity and the polarity-dependent response of phytochemical extraction from *A. rosea* was noted in terms of TEC. The regression analysis of the experimental data showed a polarity-dependent exponential increase in TEC of the selected parts of *A. rosea* that may be attributed to the presence of a majority of the polar compounds in different parts of *A. rosea* that are extracted in highly polar solvents. The present results of polarity-dependent variations in TEC agree with those reported earlier for bean seeds.²⁴

The results also showed the trends of polarity-dependent increase in TPC which may be attributed to the presence of a majority of the polar phenolic compounds in the studied parts of *A. rosea* that are extracted in highly polar solvents. These trends of polarity-dependent variations in TPC agree with those reported earlier for corn silk²⁴ but disagree

with those reported for bean seeds.³⁰ However, the polarity-dependent polynomial decrease in TFC of leaves and TTC of seeds suggests the presence of less polar flavonoids and tannins in leaves and seeds that are extracted mostly in the less polar solvent. These polynomial variations in TFC agree with those reported earlier for bean seeds.³⁰

The phytochemicals such as phenolics, flavonoids, and tannins are the medicinally important bioactive compounds naturally synthesized in plants. These compounds possess strong antioxidant potential due to their hydrogen donating ability. These compounds are known to protect the cells and tissues in the living organisms by reducing the oxidative stress caused by the endogenous and exogenous free radicals including reactive oxygen and reactive nitrogen species. These compounds possess the ability to terminate the free radical chain reactions occurring in the living system by their electron donating potential. Based on their antioxidant potential, the bioactive phytochemicals have been reported to reduce the risk of cancer and hepatic, cardiovascular, and neurological damage.

The results showed that the TETAOA and RP of the selected parts of *A. rosea* were increased with an increase in the solvent polarity in an exponential fashion. The polarity-dependent increase in TETAOA and RP may be correlated with the TPC and TFC which suggests the presence of polar phytochemical compounds with strong antioxidant potential in the studied parts of *A. rosea*. These results are also in agreement with those reported for bean seeds³⁰ and in partial agreement to those reported for cabbage seeds.³¹

The results showed a polarity-dependent polynomial increase in DPPH RSA of stem and leaves. These results suggest the prevalence of polar phytochemical compounds in stem and leaves of *A. rosea* that possess strong scavenging potential against nitrogen free radicals. The results also showed an exponential increase in HRSA of leaves which suggests the presence of some polar phytochemical compounds possessing strong antiradical activity against oxygen free radicals. However, the polarity-dependent linear decrease in DPPH RSA of seed and polynomial decrease in HRSA of stem and seed suggests the presence of relatively less polar phytochemical compounds possessing good scavenging activity against reactive nitrogen and reactive oxygen species respectively. The polarity-dependent variations in DPPH RSA agree with those reported for corn silk²⁴ but disagree with those reported for bean seeds.³⁰

The presence of phenolic acids, flavonoids, and tannins in considerable amounts advocates *A. rosea* as a valuable source of bioactive phytochemical compounds for pharmaceutical applications. The polarity-dependant increase in the extraction and antioxidant activity of these phytochemicals suggest the use of polar solvents along with some nonpolar solvent to increase the extract yield of the bioactive phytochemicals possessing good antioxidant potential.

CONCLUSION

In conclusion, all of the studied parts of *A. rosea* consisted of valuable phytochemicals including flavonoids, saponins, terpenoids, tannins, and cardiac glycosides. The increase in polarity of the extracting solvent showed an exponential increase in the extract yield of the phytochemicals present in various parts of *A. rosea*. The increase in the solvent polarity also showed positive effects on the phenolic content, antioxidant activity, ferric reducing power, and DPPH radical scavenging ability of the extracts that indicated the presence of polar phytochemical compounds with good antioxidant potential in the studied parts of *A. rosea*. However, a mixed response of total flavonoids and total tannins content and hydroxyl radical scavenging ability of the extracts was observed against solvent polarity. The study suggests that the polar solvents are more suitable to achieve a good extract yield of phytochemicals possessing good antioxidant potential while the solvents of medium polarity may

be suitable for the extraction of flavonoids and tannins from the studied parts of *A. rosea*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Nawaz H, Akram H, Ishaq QHM, Khalid A, Zainab B, Mazhar A. Polarity-dependent Response of Phytochemical Extraction and Antioxidant Potential of Different Parts of *Alcea rosea*. *Free Radicals and Antioxidants*. 2022;12(2):49-54.