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Phytochemical screening and biological activities of *Ranunculus arvensis*

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Abstract

Ranunculus arvensis, belongs to family Ranunculacea, have 2500 species throughout the world. Due to their local importance for various diseases and great biological potentials the plant was selected for further studies. Antioxidant activity was determined by DPPH scavenging method while phytotoxcicity was carried out through autoclaved petriplates growth inhibition. Antibacterial activity analysis was carried out by using microplates alamar blue assay. The Antioxidan, phytotoxic and antimicrobial results showed that this plant need detail phytochemical screening to isolate active chemical constituents which could be of scientific values.

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Introduction

Medicinal plants becoming more important with time due to the presence of potential drug compounds. Due to biologically active compounds like phytochemicals these practically play the role of medicines (Krishnaiah *et al.*, 2009**)**.

Ranunculus arvensis (L), commonly known as corn buttercup, belong to family Ranunculaceae. It is native to Europe, but mostly found in high mountains of the Mediterranean Region, Southeastern, and Eastern parts of Anatolia and also in Pakistan (Kose *et al.,* 2008; Sayhan *et al.,* 2009). Ranunculaceae is a large family comprises approximately 2500 species spread all over the world (Fostok *et al.,* 2009).

The genus *Ranunculus* of this family consists of 50 genera and 2000 species, while in Pakistan, it has22 genera and 114 species. The *Ranunculus* plants are used in folk medicine for the treatment of asthma, gout, arthritis and high fever (Akbulut *et al.,* 2011). The therapeutic effect is mainly due to the presence of phytochemical ingredients present in different parts of plants. Keeping in view the need of these vital constituents, we were intended to explore its biological potential. In this study the whole plant of *R. arvensis* was subjected to phytochemical analysis and antioxidant, phytotoxic and antimicrobial activities were determined. The present study has been undertaken due to their biological medicinal properties like antioxidant, and antimicrobial activities of various crude fractions.

Materials

Locally available whole plant *Ranunculus arvensis*. All chemicals were used of analytical grade and were purchased from Merck and sigma Aldrich.

Methods

Plant collection and identification

The *R.* arvensis was collected from Village Feroz Abad, Hassan Khel Bannu, in KPK province Pakistan, in April 2014 and identified by Dr. Faizan, (Department of Botany) University of Science and Technology, Bannu.

Pretreatment of plant

R. arvensis was first collected and then dried under shade for a month and then it was used for extraction.

Extraction

1 kg plant was extracted with 80% aq. MeOH for three days at room temperature and evaporated under reduced pressure at 40 C^{o} to obtain gummy residue. This residue was initially extracted with n-hexane to remove fatty materials. The defatted MeOH residue was suspended in water, and then fractionated into dichloromethane, ethyl acetate and butanol.

Phytochemical Screening tests

For phytochemical analysis different test was conducted by using standard protocols.

Alkaliods

For determination of alkaloids Methanolic extort was filtered and 2 ml of 1% aqueous HCl was added to it. It was animated for some time with the addition of two drops of dragondroff reagent . The appearance of reddish brown turbidity indicat alkaloids (Harborne, 1998).

Flavonides

For the flavonides determination, 5 ml from methanolic extract was mixed with 5 ml of dilute ammonia solution by adding 2 drops of concentrated H_2SO_4. Afterwards yellow colour appeared which indicated the presence of flavonoids (Sofowora, 1993).

Anthraquinones

*F*rom methanolic extract 1 ml was added to 2 ml of 5% KOH solution and then the solution was filtered. The pink colour appeared which showed the presence of anthraquinones (Harborne, 1998).

Saponins

Similarly for the determination of saponins, 1 gram sample was boiled in 10 ml distilled water in in water bath for thirty minutes and then filtered after cooling. 5 ml of filtrate was mixed with 2.5 ml distilled water and then shaked till the formation of lather like materials which indicated the presence of saponins (Sofowora, 1993).

Steriods

20 grams sample was soaked in ethanol and boiled for 10 mintes. The mixture was filtered and then and ethanolic fraction was separated. The remaining sample was dissoloved in 3 ml chloroform followed by the addition of acetic anhydride and 2 drops of concentrated sulphuric acid. After addition brown ring appearance shows the presence of steroids (Anwar *et al.,* 2016).

Glycosides

100 µl methanolic extract was placed in a test tube and add 400 µl of acetic anhydride in it. Two drops of concentrated sulphuric acid was also added. Blue-Green colour shows that glycosides are present in plant (Sofowora, 1993).

Tannins

1 g sample was added in 10 ml water for boiling then filtered after cooling. Afterwards 1% ferric chloride solution was added drop by drop to the filtrate till Green black precipitate was formed (Trease and Evans, 1989).

Terpeniods

For the identification of terpeniods 5 ml from sample was took and then it was dissolved in 2 ml of chloroform added 2 to 3 ml H_2SO_4 for the formation of layers. The greyish color will be appearing which shows that terpenoids are present (Harborne, 1973).

The fractions were subjected to their biological potential after phytochemical screening at Department of Biotechnology, University of Science and Technology, Bannu and H.E.J. Research Institute of Chemistry, International Centre for Biological and Chemical Sciences, University of Karachi.

Antioxidant assay

Antioxidant activity was measured by using DPPH assay (Gyamfi *et al.,* 1999). The DPPH solution was set by dissolving 3.2 mg in 100 ml of 82% methanol and then 2.8 ml of DPPH solution was added to glass vial followed by the addition of 0.2 ml of test sample solution, in methanol, leading to the final concentration of 1 µg/ml, 5 µg/ml, 10 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml. Both solutions kept in dark at fixed room temperature for 1houre and then measured at 517 nm. 2.8 ml of 82% methanol was used as blank while 0.2 ml of methanol and 2.8 ml of DPPH solution was used as control performed in triplicate with Inhibition formula given below. IC_{50} value was calculated by graph pad prism software. % Scavenging = absorption of control– absorption of fraction × 100 / absorption of control *Phytotoxic assay* McLaughlin and Rogers Protocol were used for phototoxic activity (McLaughlin and Rogers, 1998). The various concentrations 10, 100 and 1000 ppm in respective solvents was made. The seeds of wheat were washed with dilute water and 1% mercuric chloride. 5 ml of each fraction was set in autoclaved plate and five seeds were placed in each plate and note root and shoot inhibition after five days.

Antifungal assay

Antifungal activities of different samples were tested against *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*, and *Candida glaberata* by agar tube dilution protocol. The test samples were prepared by dissolving 400 mg in 1 ml of sterile DMSO for stock solutions while SDA was used for fungus growth in acidic medium with high concentration of glucose to get a concentration of 32.5 g/500 mL in distilled water followed by autoclaving at 121°C for 15 min. The tubes were allowed for cooling to 50°C and non-solidified SDA was loaded with samples from the stock solution to make 400 µg/ml concentrations and then tubes were allowed for solidify at a room temperature at slanting position. After seven days old fungal culture was removed.

Agar surface streak was employed for non-mycelial growth with DMSO with reference antifungal drugs were used as the negative and positive controls, respectively at 27-29°C for 7 days for incubation and then evaluated and measured by the following formula.

Percentage of Inhibition of fungal growth = 100 – linear growth in test sample (mm) × 100 linear growth in control (mm).

The standard drugs used in the assays were miconazole and amphotericin B (Atta-ur-Rahman *et al.,* 2001; Rashid *et al.,* 2009).

Antibacterial assay

The antbacterial activity was performed by using the microplate alamar Blue assay (Pettit *et al.*, 2005; Sarkar *et al.,* 2007). Organisms were grown in Mueller Hinton medium and Inoculums were adjusted to 0.5 McFarland turbidity index. Different Stock solutions were made for different test fractoins were made in DMSO (1:1 concentration) and then give media to all wells performed in triplicate. Add crud fractions in wells, control wells do not contain any test materials. 96 well plate up to 200µl were made. Finally add $5x10^6$ cells in all wells including both control and test.

Plate with parafilm and incubate for 18-20hrs. Alamar Blue Dye was dispensed in each well and shaken at 80 RPM in a shaking incubator for 2- 3hrs covered with foil in shaking incubator. Alamar Blue dye from blue to pink indicated the growth in bacterial strains. Absorbance was recorded at 570 and 600nm by the ELISA reader.

Results and discussion

Phytochemical screening

The phytochemical screening results in (Table 1) showed that alkaloid, steroids, terpeniods, tannins, glycosides, saponins and flavonides are the major constituents of *R. arvensis* due to positive and negative test results.

Table 1. Phytochemical screening of *R. arvensis*.

Alkaloid	Steroids	Terpeniods	Tannins	Glycosides	Saponins	Flavonides	Anthraqunine
+	+	+	+	+	+	+	-

+= present =absence +

Table 2. Comparison of Antioxidant activities of methanolic and ethyle acetate fraction.

Conc. Units (µg/ml)	Methanol	Ethyl acetate	Ascorbic acid
50	44.34	32.11	72.19
100	49.13	36.56	74.73
150	53.57	42.72	76.98
200	57.95	45.95	78.48
1000	75.25	71.33	87.48

Those tests which shows positive results it means that phytochemicals are present while negative test result means that the target phytochemical is not present.

The DPPH is a stable free radical, used for evalvations in phytomedicine for scavenging activities of different fractions.

Table 3. Phytotoxic activity of methanolic and ethyle acetate fractions.

	Methanol Fraction				Ethyle acetate fraction			
µg/mL	5^{th} day reading (growth in cm)		10^{th} day reading (growth in cm)		5^{th} day reading (growth in cm)		10^{th} day reading (growth in cm)	
	Shoot	Roots	Shoot	Roots	Shoot	Roots	Shoot	Roots
Control	1.12 ± 0.02	1.23 ± 0.03	2.06 ± 0.06	1.79 ± 0.05	1.12 ± 0.02	1.23 ± 0.03	2.06 ± 0.06	1.79 ± 0.05
10	1.06 ± 0.03	1.14 ± 0.02	2.00 ± 0.02	1.54 ± 0.09	1.06 ± 0.03	1.14 ± 0.02	2.00 ± 0.02	1.54 ± 0.09
100	0.88 ± 0.01	0.96 ± 0.03	1.97 ± 0.04	1.34 ± 0.08	0.88 ± 0.01	0.96 ± 0.03	1.97 ± 0.04	1.34 ± 0.08
1000	0.77 ± 0.01	0.83 ± 0.07	1.76 ± 0.05	1.12 ± 0.04	0.77 ± 0.01	0.83 ± 0.07	1.76 ± 0.05	1.12 ± 0.04

The activities of MeOH and EtOAc were determined by using free radicals of 1, 1-diphenyl-1, 2-picryl-hydrazyl (DPPH) (Table 2). The methanolic fraction show good antioxidant activity as compared to ethyle acetate fraction as in (Fig. 1).

The *R. arvensis* was initiated phytotoxic because it inhibition power is greater than control. Fig. 2 shows that MeOH fraction is more phytotoxic result as compared to EtoAct fraction.

Table 5. Extractions activities zone of inhibition of std. drugs (ofloxacine).

Name of Bacteria	(R-M) 1000 µg/ml	R-H) 1000 µg/ml	R-D) 1000 µg/ml	(R-E)1000 µg/ml	Control µg/ml
E. coli	12.81	0	0	8	92.47
B. subtilis	0	23	15.41	0	91
S. aureus	12.06	0	22	5	94
P. aeruginosa	0	0	22.47	5	94
S. typhi	0	7	12.06	0	95

R=Ranunculus, M=Methanol, H=Hexane, D=Dichloromethane, E= Ethyle acetate.

Due to increasing of concentrations, inhibition power was also increased as in Fig. 2.

The fungicidal activities of different fractions were tested against six fungal strains, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani*, and *Candida glaberata*. The results obtained as a whole is not good because the only (R-D) fraction show activity against *Microsporum canis*, *Fusarium solani*, while rest are not.

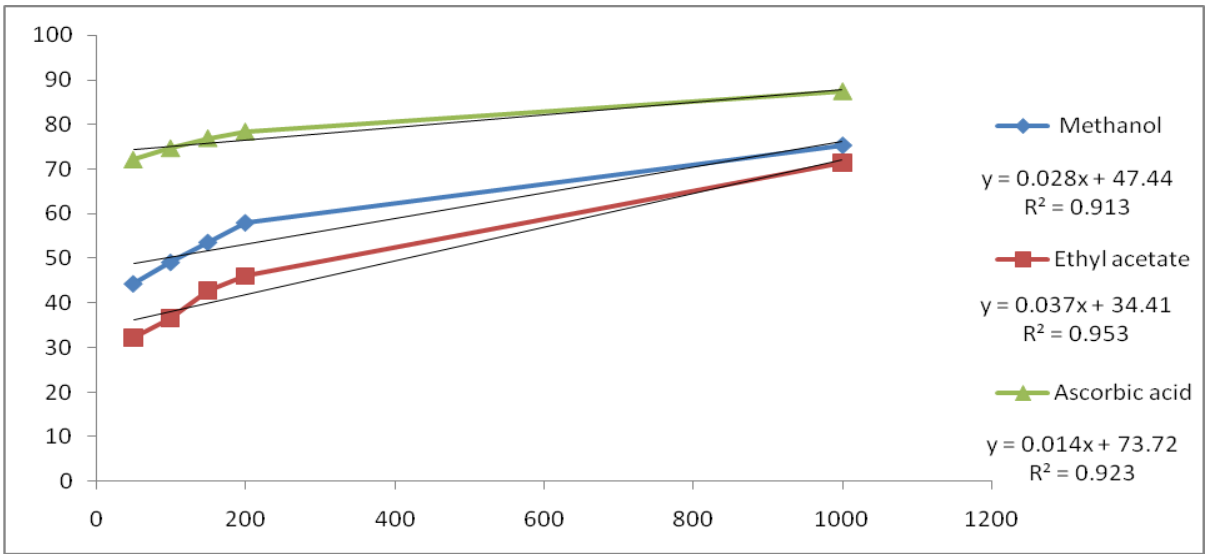

Fig. 1. Comparision of Antioxidant activities of methanolic and ethyle acetate fraction.

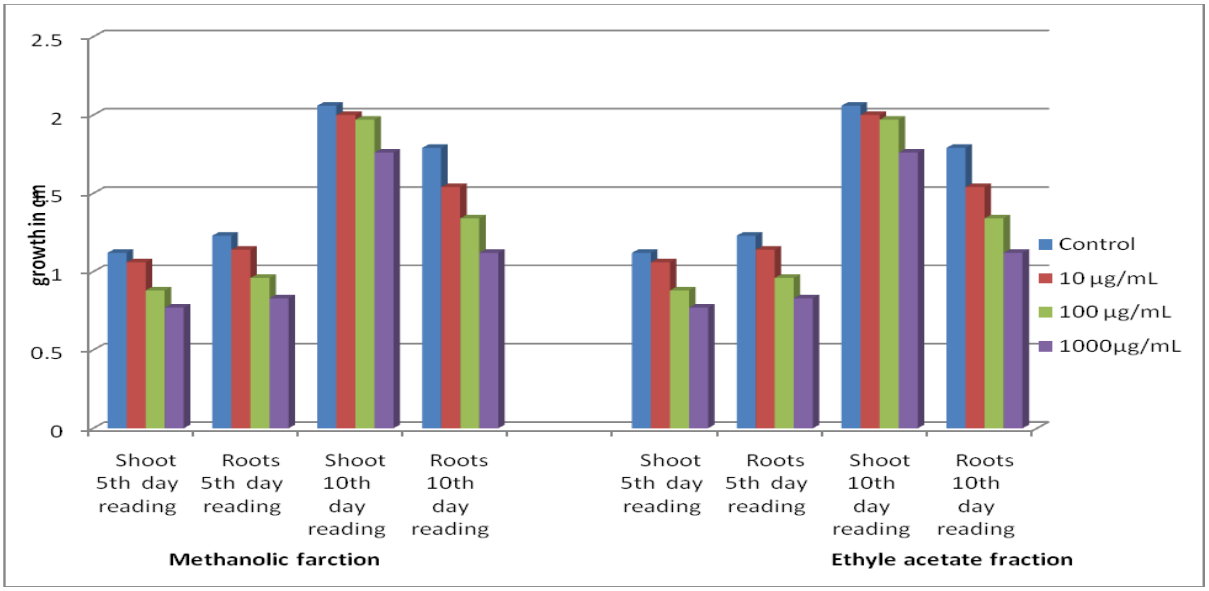

Fig. 2. Comparision of of phytotoxic data of methanol and ethyle acetate fractions.

The antibacterial study was performed against five bacteria *viz.*, *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typh*.

The above table 5 indicated that the (R-D) fraction shows activity as compared with other fractions except (R-H) is only active against *Bacillus subtilis*.

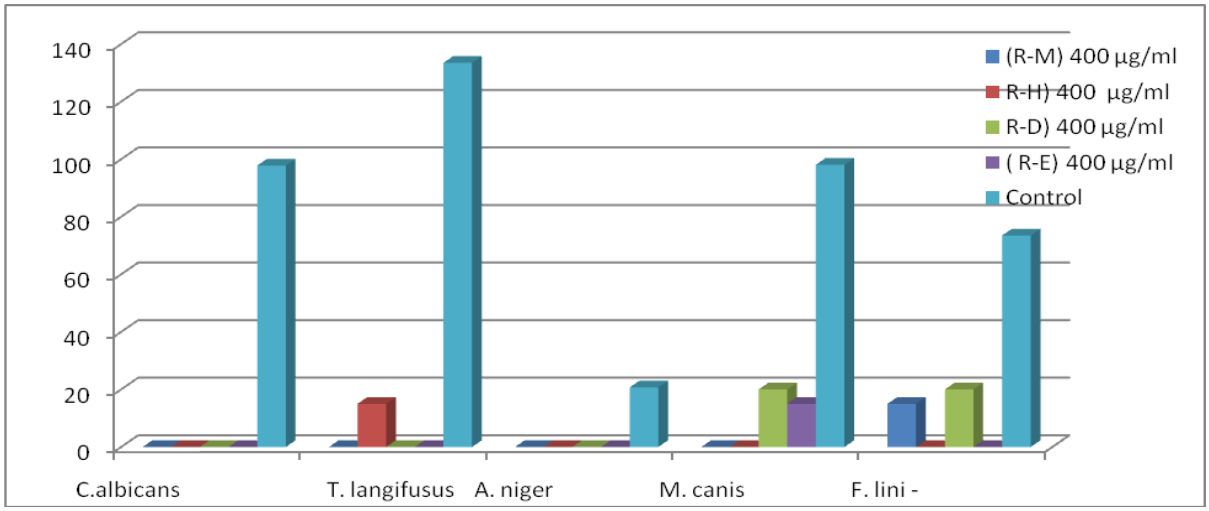

Fig. 3. Shows antifungal activities of various fractions.

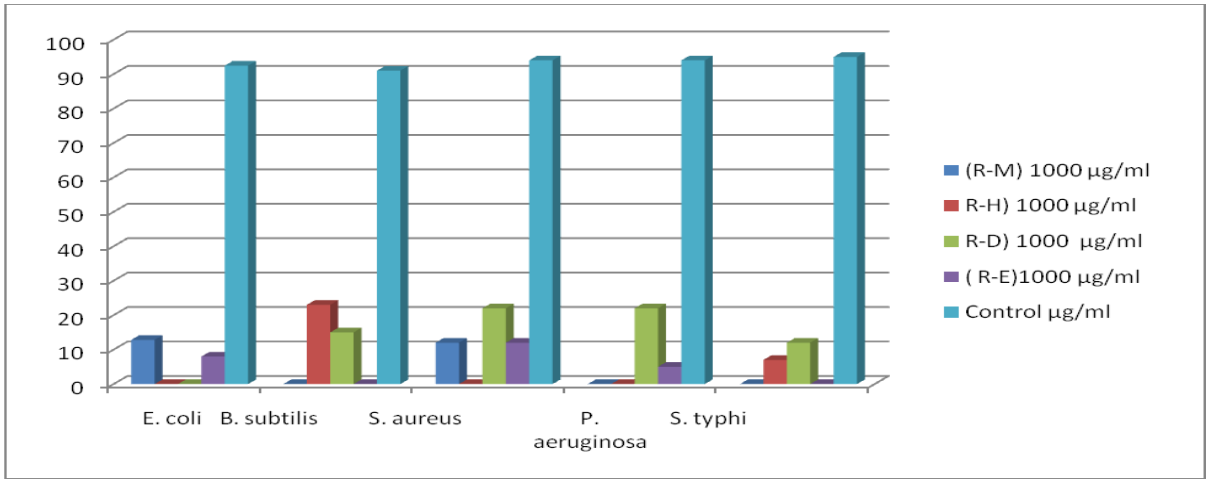

Fig. 4. Shows antibacterial data of different fractions.

Conclusion

It was concluded that the selected plant was a rich source of secondary metabolites. In conclusion, the phytochemical screening of *R. arvensis* showed that alkaloid, steroids, terpeniods, tannins, glycosides, saponins and flavonides are the major constituents as well as show high anti-oxidant, phytotoxic and antimicrobials activities.

Similarly it was noted that medicinal plants are used for the discovering and screening of phytochemical constituents which are the major precursor for the synthesis of new drugs.

Research institutes and pharmaceutical industries have keen interest in medicinal plants for the synthesis of new drugs for the treatment of various diseases.

Similarly from the results it is clear that *Ranunculus arvensis* can also be used in various pharmaceutical products as it contains different bioactive compounds. Further investigations are necessary to identify the compounds.

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