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Biological and phytochemical investigations of crude extracts of *Astragalus creticus*

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Abstract: This study was carried out to isolate the secondary metabolites and to evaluate the antibacterial, antifungal, antioxidant, phytotoxic, anti-leishmanial and α-glucosidase activities of dichloromethane and methanol extracts of whole plant of *Astragalus creticus.* Preliminary phytochemical screening indicated flavonoids, saponins, tannins and cardiac glycosides in this plant. Phytochemical evaluation of methanol extract resulted in isolation and characterization of Ethyl gallate, 1-triacontanoic acid, quercimeritrin, kaempferol-7-*O*-*β*-D-glucopyranose, myricetin, kaempferol, betulinic acid, stigmasterol and Daucosterol. The structures of the compounds were determined by Mass and NMR spectroscopy. The methanol extract exhibited better activity against *Staphylococcus aureus* (58.75%) while dichloromethane extract was found to be very active against *Bacillus subtilis* (56.30%).The methanol extract demonstrated highly significant phytotoxic (92.68% at 1000μg/ml) and antioxidant (64.55±0.43%) potential while both extracts identified best inhibition of α-glucosidase enzyme. The plant extracts showed non-significant antifungal and anti-leishmanial activities. To our knowledge, it's a first research study on *Astragalus creticus* that indicate a great biological and phytochemical potential in it.

Keywords: *Astragalus creticus*, antibacterial activity, *Lemna minor*, phytotoxic activity, secondary metabolites.

INTRODUCTION

Astragalus creticus is an important plant of genus *Astragalus* and its botanical characteristics are described as a lower shrub with a short blackish procumbent stem, scaly branches, pinnated leaves, woody petioles and broad downy pointed stipules. The plant is native to Greece, mountains of S. Balkans, Crete, Asia and widely distributed in Southern Punjab tribal areas like Sakhi Sarwar and Baluchistan of Pakistan (Ullah *et al*. 2013)**.** In Pakistan, the aerial parts are traditionally used as sedative and tonic. The local name of this plant is Aghazi shatra **(**Amiri *et al*. 2020)**.** The plants of genus, *Astragalus* are commonly used in folk medicine of China, Korea, Japan and Mongolia (Butkutė *et al*. 2018). In Tibetan folk medicine, *Astragalus* plants are recommended for regeneration of injured tissues, strengthening of body and excretion of toxins. In traditional system of West Siberia and Far East, they are used as stimulator of immune system (Mamedova and Isaev 2004). *Astragalus* root from various species is a popular herbal drug in TCM (Traditional Chinese medicines) because of its uses as hepatoprotective, immunostimulant, antiperspirants, antidiabetic and diuretic agent (Bensky *et al.* 2004). Many biological activities are reported in *Astragalus* plants like antibacterial, antifungal, antioxidant, cytotoxic, leishmanicidal, immunomodulatory, antidiabetic and anti-inflammatory activities. Phytochemical investigations indicated at least 200 active constituents mainly of cycloartane and oleanane type saponins, flavonoids in free or glycosidic forms, sterols and polysaccharides (Li *et al*. 2014).

Astragalus creticus is first time evaluated for phytochemical and biological potential due to medicinal importance of plants of genus *Astragalus*.

MATERIALS AND METHODS

Chemicals &Equipments

Chemicals; *n*-hexane, dichloromethane, ethyl acetate, chloroform, methanol, ethanol, sulfuric acid, isopropyl alcohol and vanillin, TLC plates 20x20cm pre-coated with silica gel 60 F_{254} of Merck, Germany, Silica gel; 40-63μm and 63-100μm of Fluka, Germany and Equipments; TLC tank of Desaga, Germany, open glass columns of various strength; CR 60/50, 40/50, 40/30 and 20/30 of Quickfit, England, Buchi Rotavapor R-200, Switzerland, Shimadzu weighing balances, Japan, Oven of Memmert, U.K, Gallenkamp melting point apparatus of Weiss Technik, UK, Shimadzu UV 240 spectrophotometer, Japan, JASCO A-302 IR spectrophotometer, Japan, AVANCE-400 ^{1}H-NMR & AVANCE-100 ^{13}C-NMR Spectrophotometers of BRUKER and Finnigan VARIAN-MAT 312 Mass-spectrometer of SIS, USA were used.

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Collection and identification of plant

The plant was collected near Fort Manro (tribal area of southern Punjab) in March, 2014 and identified as *Astragalus creticus* by a taxonomist in Institute of pure and applied Biology, Bahauddin Zakariya University, Multan and voucher number "www.the plantlist.org/tpl1.1/record/ild.32000" was allotted.

Preparation of plant extracts

The whole plant of *Astragalus creticus* was shade dried for 15 days, grinded and finally weighed. 1000gm dried plant was extracted by maceration process in dichloromethane and methanol solvents alternatively. The extracts were concentrated by Rotavapor under reduced pressure and codes; ACWPD (dichloromethane extract) and ACWPM (methanol extract) were allotted.

Secondary metabolites detection

Secondary metabolites in *Astragalus creticus* were detected by applying various tests like Dragendorff's reagent (Alkaloids), dilute HCL test (flavonoids), Borntrager's test (free and bound anthraquinones), Keller Kiliani test (cardiac glycosides), Ferric chloride test (Tannins) and Froth test (saponins) (Evans, 2009). The procedures of tests are given in table 1.

Antibacterial assay (Microplate alamar blue assay)

The extracts were tested against *Escherichia coli* (NCTC 10418), *Bacillus subtilis* (NCTC 8236), *Salmonella typhi, Staphylococcus aureus* (NCTC 6571) and *Pseudomonas aeruginosa* (ATCC 10145). Alamar blue reagent was aliquoted and stored at 80°C. The microtiter plates containing 5×10^5 CFU/mL added in MHIIB media with final volumes up to 100µL and incubated at 37°C. The drug was diluted (twofold) with standard (diluted 4,096 to 64µg/mL) and tested extracts (diluted 64 to 0.5µg/mL). 50µL of drug dilution was added in experimental and control wells after 24hrs and biofilms were exposed to the drugs for 20hrs at 37°C temperature. 5µL of Alamar blue reagent was added in each well with continuous shaking for an hour at 37°C and absorbance was checked at 570nm and 600nm using Perkin-Elmer Wallac Victor microplate reader (Pettit *et al.* 2005). % Reduction of Alamar blue reagent was calculated using underlined formula

$$[(\mathcal{E}_{ox})\lambda_2 A\lambda_1 - (\mathcal{E}_{ox})\lambda_1 A\lambda_2 / (\mathcal{E}_{red})\lambda_1 A'\lambda_2 - (\mathcal{E}_{red})\lambda_2 A'\lambda_1] \times 100$$

Antifungal assay (Agar tube dilution method)

Stock solutions of extracts were dissolved into DMSO. Sabouraud dextrose agar was prepared and dispensed into a screw capped test tubes. Test tubes were autoclaved at 121°C for 15min and cooled at 50°C. The samples were pipetted from the stock solution into sabouraud agar media. The tubes were solidified and then each tube was inoculated with inoculum of 4mm diameter of 7 days old culture of fungus. All tubes were inoculated for growth for 7-10 days at 28-30°C temperature and humidity (40%-50%). The test tubes of no visible growth of microorganisms were taken to represent the MIC (µg/ml) values after incubation period (Brass *et al*. 1979).

Phytotoxicity assay (Lemna minor bioassay)

E-medium was prepared and pH was maintained at 5.5-6.0 by adding KOH pellets. 8 sets of 20 vials each for 500, 50, 5ppm and control were prepared. 1000, 100 and 10µL dilutions of extracts were added to 500, 50 and 5ppm vials respectively. The solvent was placed for overnight to evaporate. 2mL E-medium and a single *Lemna minor* L. plant having a rosette of three fronds were added to each vial, placed in glass dish filled up to 2cm water and sealed with stopcock. Glass dish was placed in growth chamber for 7 days at 26°C under fluorescence and incandescent light. No. of fronds were counted and recorded on 3rd& 7th day (Einhellig *et al*. 1985).

Anti-leishmanial bioassay (6-well serial dilution method)

The extracts were dissolved up to 1mg/ml of Phosphate buffered saline solution with pH 7.4 containing 0.5% methanol. 100µL infected macrophage culture (1.5×106 cells/well) were added in wells of 96 well microtiter plate. 10µl extracts were added in the culture. 10µl PBS added as a negative control while amphotericin B and pentamidine were added separately as a positive control. The plates were incubated at 37°C in humidified chamber with 5% CO_2 for 6 days during which organisms multiplied 1.5-3.0 times. The culture was examined in microscope after staining with Giemsa and then IC_{50} value were calculated (Rahman *et al*. 2001).

Enzymatic assay (a-glucosidase assay)

1mg α-glucosidase was mixed in 100mL phosphate buffer of pH 6.8 along 200mg bovine serum albumin. 10µL tested samples of various conc. (0.52 to 33µg/mL) were mixed in 490µL phosphate buffer and 250µL of 5mM *p*-nitrophenyl-α-D-glucopyranoside in each well of 96-well plate. Contents were pre-incubated at 37ºC for 5min. 250µL α-glucosidase (0.15unit/mL) was added and incubated at 37ºC for further 15min. The reaction was terminated after addition of 2000µL Na_2CO_3 (200mM). Absorbance was measured at 400nm using the 96-well plate reader by calculating *p*-nitrophenol released from *p*-NPG. IC_{50} values were calculated using EZ-Fit Enzyme Kinetics Software (Palanisamy *et al*. 2011). % enzyme inhibition was calculated as

%Inhibition = (Abs. of Control - Abs. of Test / Abs. of Control) × 100

Antioxidant assay (DPPH radical scavenging assay)

10µL test solution was mixed with 90µL of 100µM methanol DPPH solution to make final volume of 100µL in 96-wells plate. Mixture was properly mixed then incubated for 30min at 37ºC. Microplate reader was employed to determine reduction in absorbance at 517nm (Rahman *et al*. 2001). % Inhibition was calculated as

% Inhibition = (Abs. of Control - Abs. of Test / Abs. of Control) × 100

Isolation of compounds

20gm ACWPM extract was treated with open column chromatography based on TLC analysis. The stationary phase was silica gel 60 (Size: 0.063-0.100mm) and mobile phases were EtOAc: MeOH: Water with varying ratios; 100: 14: 7→100: 20:10 and MeOH: Water with a ratio; 50:50 via stepwise elution. Nine fractions of ACWPM were collected named; ACWPM-1 to ACWPM-9. 450mg ACWPM-2 was resolved into six sub-fractions (2A to 2F). The 2D fraction (37mg) was eluted with *n*-hexane: EtOAc solvent system with a ratio of 50: 50 that yielded a pure Compound-1 (23mg). 234mg ACWPM-4 was further processed using eluting system; $CHCl_3$: MeOH: Water (75: 25: 3→70: 30: 4) that yielded a pure Compound-2 (10mg). The fractions; ACWPM-6 (436mg) and ACWPM-7 (419mg) were combined due to same resolution of their components on TLC and named as ACWPM-6 (882mg). The ACWPM-6 (882mg) was chromatographed into seven sub-fractions (6A to 6G). 88mg of 6B was forwarded by utilizing silica gel 60 (Size: 0.040-0.063mm) and solvent system; $CHCl_3$: MeOH: Water (75: 25: 3→70: 30: 4) that yielded a pure Compound-3 (11mg). 47mg of 6F yielded another pure Compound-4 (5mg). The ACWPM-8 (3.6g) was resolved into nine sub-fractions (8A to 8I) using silica gel 60 (Size: 0.063-0.100mm) and $CHCl_3$: MeOH: Water (70: 30: 4→65: 35: 5→60: 40: 5). 300mg 8F was further fractionated into 8F1 (60mg), 8F2 (85mg) and 8F3 (65mg) with a mobile phase; EtOAc: MeOH: Water (77: 13: 10). 85mg 8F2 was forwarded further that purified a Compound-5 (10mg). The sub-fraction; 8G (36mg) yielded another Compound-6 (10mg). The fraction; ACWPM-9 (9gm) was subjected to solvent-solvent extraction that resulted in ACWPM-9-I (2.8gm and soluble in EtOAc) and ACWPM-9 (6gm and soluble in $CHCl_3$). ACWPM-9-I (2.8gm) was eluted by $CHCl_3$: MeOH: Water (60: 40: 5→60: 40: 10) and MeOH: Water (50:50) that was resolved into five fractions and one purified Compound-7 (4mg). The fraction; 9C (169mg) was fractionated into 9C-1 (22mg), 9C-2 (40mg) and 9C-3 (52mg). 9C-3 (52mg) was subjected to column chromatography using $CHCl_3$: MeOH: Water (60: 40: 5→60: 40: 10) that purified Compound-8 (9mg) and Compound-9 (6mg).

Spectroscopic data of isolated compounds

Compound-1: White Colorless solid; Yield: 23mg; HR-EI-MS: *m/z* 198.0601; ^{1}H- and ^{13}C-NMR data is similiar with those previously reported (Leela and Saraswathy 2013).

Compound-2: White amourphous powder; Yield: 10 mg; HR-EI-MS: *m/z* 452.4588; IR (KBr, ν_{max}, cm^{-1}): 2745 (broad band) (OH), 1715 (C=O), 1473, 1381; ^{1}H- and ^{13}C-NMR data is identical with previous data (Silva *et al*. 2016).

Compound-3: Pale yellow amorphous solid; Yield: 11mg; HR-FAB-MS: *m/z* 465.1175 $[M+H]^+$; UV(CH_3OH, λ_{max}, nm): 261(3.11), 274 (3.71), 371 (4.09) and 315 (4.50); IR (KBr, ν_{max}, cm^{-1}): 3315 (OH), 1661 (C=O) and 1589 (Ar); NMR results are similar with reported data of Compound-3 in DMSO (Guzhva, 2010). NMR assignment for CD_3OD solvent is given as follows. ^{1}H-NMR: (400MHz, CD_3OD) δ (ppm) 6.41 (1H, d, J = 3.1 Hz, H-6), 6.22 (1H, d, J = 3.1 Hz, H-8), 6.91 (1H, d, J = 3.2 Hz, H-2'), 8.01 (1H, d, J = 8.1, Hz, H-5′), 7.60 (1H, dd, J = 8.1, 3.2 H-6′), 13.15 (1H, br s, 5-OH), 5.22 (d, J = 8.1 Hz, H-1″), 4.08 (dd, J = 9.6, 7.8 Hz, H-2″), 4.21 (d, J = 9.6 Hz, H-3″), 4.25 (t, J = 9.6 Hz, H-4″), 3.29 (dt, J = 9.6, 2.6 Hz, H-5″), 4.52 (dd, J = 10.0, 2.6 Hz, H-6″); ^{13}C-NMR (100 MHz, CD_3OD) δ (ppm) 156.5 (C-2) , 141.2(C-3), 181.6 (C-4), 162.4 (C-5), 100.5 (C-6) , 167.0 (C-7) , 95.5 (C-8), 160.4 (C-9), 107.3 (C-10), 121.2 (C-1′), 113.1(C-2′), 148.0 (C-3′), 150.1 (C-4′), 116.2 (C-5′), 121.3 (C-6′), 102.4 (C 1″), 75.5 (C-2"), 79.5 (C-3"), 71.3 (C-4″), 79.4 (C-5″), 63.3 δ (C-6″).

Compound-4, 5, 6, 7, 8 and 9 were identified on the basis of MS, NMR spectra and Co-TLC with authentic samples.

STATISTICAL ANALYSIS

The data was presented as mean ± SD of triplicates experiments, and the IC_{50} values were calculated by means of Graph Pad Prism 6.0 (Graph Pad Software; San Diego, USA).

RESULTS

Secondary Metabolites

The preliminary phytochemical testing is important to gain information about nature of secondary metabolites present in the plants. The tests for detection of secondary metabolites resulted with presence of flavonoids, saponins, tannins and cardiac glycosides in dried plant; *A. creticus* as shown in a table 1.

Identification of compounds 1-9

The comparison of spectral data with already reported material identified the purified compounds as Ethyl gallate (1), 1-Triacontanoic acid (2), Quercimeritrin (3), Kaempferol-7-*O*-*β*-D-glucopyranose (4), Myricetin (5), Kaempferol (6), Betulinic acid (7), Stigmasterol (8) and Daucosterol (9).

Antibacterial activity

ACWPM exhibited antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* with 58.75% and 30.20% inhibition respectively. ACWPD was found

Table 1: List of secondary metabolites and their identification

Secondary metabolites	Procedure	Observation	Result
Alkaloids	3g drug dissolved in 1% HCl, heated for 2-3 min, filtered and treated with dragendorff's reagent	No Orange red ppt.	Negative
Flavonoids	2g drug boiled in 20ml water, filtered, added drops of dil. HCl and alkaline with NaOH	Yellow color	Positive
Anthraquinones	2g drug extracted with hot water, filtered, extracted with CCL_4 and then 5ml dil. NH_3 solution added to CCL_4 layer.	No Cherry red ppt. in NH_3 solution layer	Negative
Cardiac glycosides	3g drug in 10ml of 70% alcohol, boiled, filtered, treated with 1ml lead subacetate, filtered, extracted with chloroform, evaporated chloroform layer, added 3.5% $FeCL_3$ and H_2SO_4	Brown color at junction of two layers (deoxy sugar) and pale green at above layer	Positive
Tannins	3g drug in 30ml water for 10min, filtered and added 2ml $FeCL_3$	Black ppt.	Positive
Saponins	2g drug in 10ml water	Persistent froth after shaking	Positive

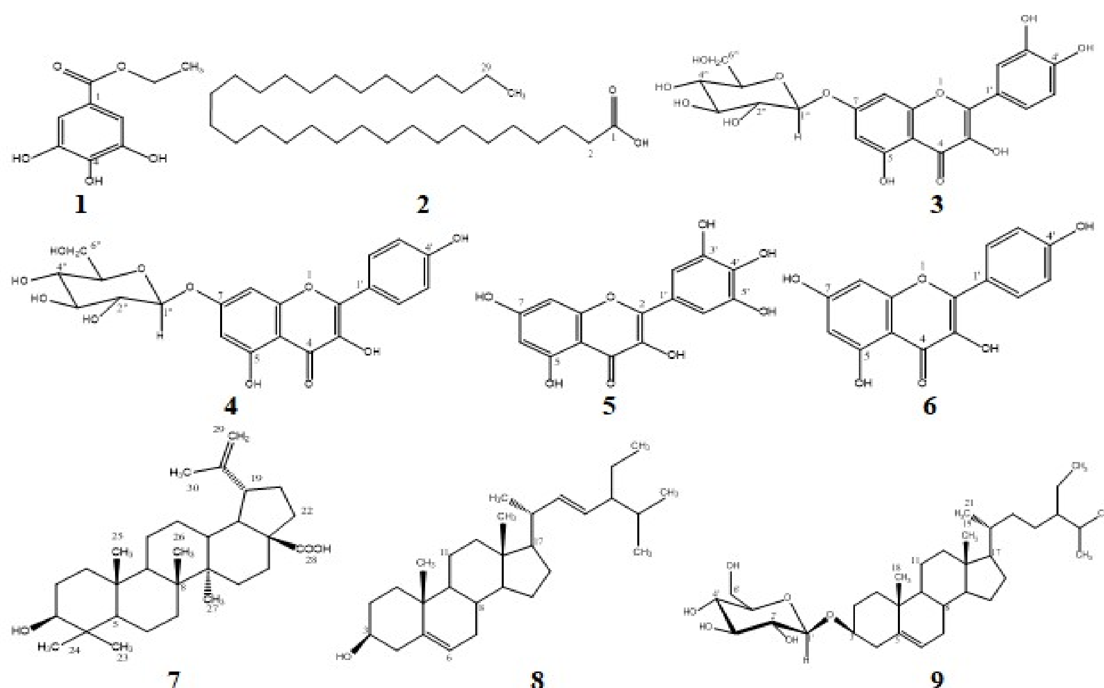

Fig. 1: Structures of the isolated compounds (**1-9**)

to be active against *Bacillus subtilis* and *Staphylococcus aureus* with 56.30% and 29.50% inhibitions respectively. Both extracts were inactive against *Salmonella typhi* and *Pseudomonas aeruginosa* as given in a table 2.

Antifungal activity

The extract; ACWPD exhibited non-significant activity against *Candida albicans* only with 11.66% inhibition while both extracts were found to be inactive against all other fungal strains and expressed in table 3.

Phytotoxic activity

Lemna minor bioassay is a quick and sophisticated technique to measure the phytotoxic potential of plant extracts. The extracts; ACWPD and ACWPM showed significant activities against *Lemna minor* at concentration of 1000µg/mL with 48.78% and 92.68% Growth regulation respectively as expressed in table 4.

Anti-leishmanial activity

ACWPM was evaluated for antileishmanial activity against tested organism; *Leishmania donovani.* ACWPM showed lowest activity with IC_{50} value of 95.0±0.47µg/mL after the comparison with standard drugs; Amphotericin B (IC_{50}: 0.49±0.3µg/mL) and Pentamidine (IC_{50}: 5.22±0.7µg/mL) as given in a table 5.

Table 2: Results of antibacterial activity of plant extracts

Extract/standard	Antibacterial activity (% Inhibition at concentration of 3000μg/mL)				
	Salmonella typhi	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
ACWPM	0	0	30.20	58.75	0
ACWPD	0	56.30	0	29.50	0
Imipenem	96.20	95.60	86.20	93.70	95.50

Table 3: Results of antifungal activity of plant extracts

Extract/standard	Antifungal activity (% Inhibition at concentration of 400μg/mL)				
	Candida albicans	*Aspergillus niger*	*Microsporum canis*	*Fusarium Lini*	*Trichophyton rubrum*
ACWPD	11.66%	0	0	0	0
ACWPM	0	0	0	0	0
Amphotericin B	−	100% (20.70μg)	−	−	−
Miconazole	100% (97.80μg)	−	100% (98.10μg)	100% (73.50μg)	100% (113.50μg)

Table 4: Results of phytotoxic activity of plant extracts

Extract/standard	Concentration (μg/ml)	No. of Fronds		% Growth Regulation
		Taken	Survived	
ACWPD	10	41	41	0
	100	41	41	0
	1000	41	21	48.78
ACWPM	10	41	41	0
	100	41	41	0
	1000	41	03	92.68
Control	−	41	41	0
Paraquat	0.015	41	0	100

n=3, ACWPM: *Astragalus creticus* whole plant methanol, ACWPD: *Astragalus creticus* whole plant dichloromethane

Enzymatic activity

The α-glucosidase inhibition assay was done by utilizing standard; Acarbose that showed 65.73±1.93% inhibition with IC_{50} value of 375.82±1.76μmol. The tested samples; ACWPD and ACWPM exhibited 91.28±0.85% and 86.32±0.74% enzyme inhibition with IC_{50} values; 12.42±0.72 and 96.11±0.57μmol respectively as described in a table 6.

Table 5: Results of antileishmanial activity of plant extracts.

Extract/standard	IC_{50}(μg/mL)
ACWPM	95.0±0.47
Amphotericin B	0.49±0.3
Pentamidine	5.22±0.7

n=3, ACWPM: *Astragalus creticus* whole plant methanol, ACWPD: *Astragalus creticus* whole plant dichloromethane

Antioxidant activity

Antioxidant activity was tested by using standard; BHT that showed 82.73±0.95% inhibition with IC_{50} value of 17.82±3.56μg/mL. The extracts; ACWPM and ACWPD exhibited 64.55±0.43% and 55.74±1.43% activity against DPPH with their IC_{50} values; 43.63±4.73 and 52.18±5.57μg/mL respectively. The results are given in the table 7.

DISCUSSION

Plants are rich source of secondary metabolites which are related to their therapeutic potential. Secondary metabolites like flavonoids, glycosides, saponins and tannins protect the plants from animals and microbial growth. These phytochemicals could be beneficial if used for therapeutic purpose in treatment of various diseases after isolation and purification from natural sources (Petrovska, 2012). The phytochemical investigations on *Astragalus creticus* lead to the isolation of a tannin, one fatty acid, one terpene, four flavonoids and two sterols. The purified compounds were reported previously from various plants like Ethyl gallate from *Acacia leucophloea* and *Rhynchosia phaseoloides* (Calderon *et al*. 2006; Leela and Saraswathy 2013), 1-Triacontanoic acid from *Desmodium sambuense* and *Senna trachypus* (Li *et al*. 2010; Silva *et al*. 2016), Kaemferol-7-O-β-D-glucopyranoside from *Cassia renigera* and *Securigera securidaca* (Behbahani *et al*. 2014; Singh *et al*. 2011) of family Fabaceae. The rest of the compounds were already

Table 6: Results of α-glucosidase activity of plant extracts

Extract/standard	Concentration (mM)	%Inhibition	IC_{50} (μmol.)
ACWPD	0.5	91.28±0.85	12.42±0.72
ACWPM	0.5	86.32±0.74	96.11±0.57
Acarbose	0.5	65.73±1.93	375.82±1.76

n=3, ACWPM: *Astragalus creticus* whole plant methanol, ACWPD: *Astragalus creticus* whole plant dichloromethane

Table 7: Results of anti-oxidant activity of plant extracts

Extract/standard	Dose (μg/mL)	%DPPH scavenging	IC_{50}(μg/mL)
ACWPM	75	64.55±0.43	43.63±4.73
ACWPD	75	55.74±1.43	52.18±5.57
BHT	75	82.73±0.95	17.82±3.56

n=3, ACWPM: *Astragalus creticus* whole plant methanol, ACWPD: *Astragalus creticus* whole plant dichloromethane, BHT: Butylated hydroxytoluene (Standard).

purified from various species of genus *Astragalus* (Li *et al.* 2014). But they are first time indicated in *Astragalus creticus*.

Antimicrobial resistance is increasing day by day (Mayers *et al.* 2017). The plant derived drugs are getting much attention to the researchers because these are cheaper and better acceptance to general public than purely synthetic medicines (Nagesh and Shanthamma 2009). Antibacterial study on *Astragalus creticus* clearly depicts that ACWPM and ACWPD have potential to inhibit both gram positive bacterial strains like *S. aureus*, *B. subtilis* and gram negative bacteria such as *E. coli*. Among the isolated compounds; ethylgallate, kaempferol-7-*O*-*β*-D-glucopyranose, quercimeritrin and myricetin also reported the antibacterial potential in previous studies against variety of pathogens (Li *et al.* 2014). So, the current antibacterial activity on *Astragalus creticus* may be attributed to these purified phytochemicals.

The plant showed non-significant antifungal and anti-leishmanial activities. This may be due to decrease amount of antifungal and anti-leishmanial agents in this plant or change of solvent of different polarity may result in better properties.

Low agricultural productivity is resulted due to interference of weeds that leads to huge economic losses in the world. Pakistan is an agricultural country in which 70% population rely on agriculture. So, need to explore the phytotoxic agents is a major requirement in Pakistan to reduce low agricultural productivity (Zia-Ul-Haq *et al.* 2012). The results of plant extracts described a great phytotoxic potential in *Astragalus creticus.*

Many traditional plants like *Camellia sinensis, Cleistocalyx operculatus*, *Enydra fluctuans, Horsfieldia amygdaliana, Ipomoea batatas, Nelumbo nucifera* and *Syzygium zeylanicum* used for treatment of diabetes in Vietnam, also showed the inhibition of *α*-glucosidase enzyme (Mai *et al.* 2007). *α*-glucosidase inhibits the release of D-glucose and also cause reduction of the postprandial hyperglycemic excursions (Bhavsar and Talele 2013). That's why α-glucosidase inhibition is important to discover new therapies to control diabetes mellitus. The plant extracts identified the enzyme activity even greater than standard drug. So, it must say that this plant has anti-diabetic potential. In Chinese traditional medicine system, the decoction of root of *A. membranaceus* is used for treatment for diabetes and also scientifically validated for anti-diabetic potential (Agyemang *et al*. 2013). Further studies are required to screen out the anti-diabetic agents from this plant. In recent decades, phenolic and flavonoid-rich natural diets with antioxidant potential have fostered interest in nutrition and food science. The plant polyphenolics like flavonoids, saponins and tannins are key players of antioxidant because they can suppress free radicals production in various ways like scavenging, inhibition of chain at initiation step, chain termination, peroxides decomposition and reducing capacity (Pham-Huy *et al.* 2008). The plant methanol extract showed significant DPPH activity and successively screened for the secondary metabolites like flavonoids which are key markers of antioxidant property.

CONCLUSION

In conclusion, *Astragalus creticus* showed remarkable antibacterial, phytotoxic, antioxidant and *α*-glucosidase activities which possibly occurred due to its isolated phytochemical constituents and also encourage its use as potent antibacterial, phytotoxic, antioxidant and anti-diabetic agent.

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