Total Phenolic Content, Antioxidant, Antimicobial and Anticancer Activities of Lespedeza Bicolor Turcz (Papilionaceae)

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Anticancer activity against Human lung carcinoma (LU-1) and Human prostrate carcinoma (LnCap) along with antimicrobial and antioxidant activity on DPPH ((1,1)-diphenyl-2-picrylhydrazyl) and Hydrogen peroxide radicals scavenging activity and the contents of total phenolic and flavonoids were assessed in methanol extract of *Lespedeza bicolor*. The highest content of total phenolic content was detected in the arial part of *Lespedeza bicolor* (0.5-1.7 mg gallic acid equiv./g), while the highest content of total flavonoids was found in the aerial part of *Lespedeza bicolor* (0.102-0.148 mg/g D/W). *Lespedeza bicolor* arial parts and root extract showed IC_{50} value of 12.5µg/ml and 50µg/ml against human lung carcinoma (LU-1) whereas, ≤ 12.5 µg/ml and 12µg/ml were calculated against Human prostrate carcinoma (LnCap) cell line. MIC value of 20-35 µg ml^{-1} has been observed gainst *Aspergillus fumigates, Aspergillus niger, Fusarium solani* and *Mucor sp* in comparision with 1-2.5µg/ml of Terbinafine used as a standard fungicide. MIC value of 20 µg/ml and 35 µg ml^{-1} of *Lespedeza bicolor* arial parts and root extract against bacterial pathogen *Klebsiella pneumonia* and 20-50 µg ml^{-1} against *Enterococcus* has been measured. DPPH radical scavenging activity of *Lespedeza bicolor* with IC_{50} values of ≤ 50 µg/ml and ≤ 200 µg ml^{-1} was observed whereas, hydrogen peroxide scavenging activity with IC_{50} values of ≤ 25 µg/ml for arial parts and ≤ 50 µg ml^{-1} for the root extract of *Lespedeza bicolor* has been shown with galllic acid (R2= 0.819) and ascorbic acid (R2= 0.728). These data suggested that the methanolic extract of *Lespedeza bicolor* could be potential candidates for natural antioxidants and anticancer.

Keywords: Lespedeza bicolor, anticancer activity, antioxidant, Antimicrobial

1. Introduction

Lespedeza bicolor Turcz (Papilionaceae) commonly called; bush clover has been collected from natural high saline and arid habitat of District Mardan, Pakistan (34° 05′ to 34° 32′ north latitudes and 71′′ 48′ to 72° 25′ east longitudes. According to [1] Six pterocarpans isolated from the root bark of *Lespedeza bicolor* has exposed significant levels of bacterial neuraminidase inhibitory activity with IC_{50} = 0.09-3.25µM. *Lespedeza bicolor* constituents including flavonoids, alkaloids, terpenes, organic acids, and stigmasterols have been screened for anti-inflammation, reducing blood sugar, antioxidation anti-radiation, anticancer, and anti-tumor by [2]. The work of [3] showed that the total amount of hydrolyzed amino acid was 148.95 mg/100g, free amino acids

were 106.39 mg/100g and that of γ-aminoisobutyric acid was recorded 12.57 mg/100g in *Lespedeza bicolor* stem extract. The contents of neutral lipids, glycolipids, and phospholipids in *Lespedeza bicolor* seed detected by [4] were 71.75%, 23.26% and 4.99% respectively. 12 flavonoids including Quercetin, kaempferol, trifolin, isoquercetin, homoorientin, and orientin has been isolated from *Lespedeza* bicolor by [5]. N, N-dimethyltryptamine isolated from *Lespedeza bicolor* **var. japonica** has uterus contracting action in 1-2 × 10^{-6} dilution. The [6] work revealed that the Leaves, shoots and inflorescences of *Lespedeza bicolor* have been used in the treatment of acute and chronic nephritis, azothemia and dieresis.

2. Materials and Methods

Extraction

Fresh aril parts and root of *Lespedeza bicolor* (300g) were collected, rinsed with distilled water and air dried for 12 days. The leaves were ground into powder, then soaked in 80% methanol and incubated for two weeks at room temperature (25 °C). The mixture was filtered twice, using whatman-41 filter paper. The extracts were dried by removing the methanol using a rotary film evaporator.

Preliminary phytochemical screening

Phytochemical screening of the *Lespedeza bicolor* was performed to detect the presence of different classes of constituents, such as alkaloids, flavonoids, saponins, steroids, terpenes, Coumarins, Anthraquinone, phlobatannins, Cardiac glycosides and tannins [7]. Total phenolic contents of *Lespedeza bicolor* were determined by the Folin-Ciocalteu colorimetric method [8]. Tannin content was determined by using [9] method. Total Flavonoids content was determined according to the standard protocol [10]. The absorbance was measured immediately at 510 nm spectrophotometer. Alkaloid content was determined by [11] method using 10% acetic acid followed by concentrated ammonium hydroxid. Saponin contents were calculated as percentage of the dried fraction using [12] method.

Antibacterial and antifungal assays

Antibacterial activity of *Lespedeza bicolor* crude extracts was determined by the agar well diffusion method [13]. The agar tube dilution method was used for determination of antifungal activity of methanolic extracts of *Lespedeza bicolor*.

Antioxidant potential of Lespedeza bicolor

The antioxidant activity of *Lespedeza bicolor* crude extract was assessed in DPPH radical scavenging system using gallic acid and ascorbic acid as a positive control, and the decrease in absorbance was determined at 517 nm [14]. The ability of the extracts to scavenge hydrogen peroxide was determined according to the method of [15].

Anticancer activities

The cytotoxic potential of the total methanolic extract of *Lespedeza bicolor* was determined in the human lung carcinoma (LU-1) and human prostrate carcinoma (LnCaP) cell line at the highest concentration of 20 μg/mL with sulforhodamine B (SRB) method [16].

3. Results and discussion

Several groups of polyphenols (anthocyanins, tannins, flavanones, isoflavones, resveratrol and ellagic acid) are currently used in nutraceuticals industries and functional foods [17]. MIC of *L.bicolor* crude extract against *E. coli* and *B. subtilus,* was found 0.5 mg/ml [18].

Several flavonoids and tannins isolated from medicinal plants have been discovered for their significant role in antibacterial, antifungal and anti-inflammatory activities. It is, therefore, possible that the present activities observed with this extract in the study may be attributable to its total phenolic, total flavonoids and tannins contents.

In table 1 total phenolic content (TPC) was shown in the range of 1.23-1.70 mg/g of the *lespedeza bicolor* extract using a standard curve of gallic acid (R2= 0.783). The total flavonoids are in the range from 0.102-0.148 mg/g D/W shown in Table 1.

Metabolites	*Lespedeza* Arial	*Lespedeza* Root
Tannins (mg/g. D/W)	0.193±0.014	0.064±0.326
Total Flavonoids (mg/g. D/W)	0.148±0.003	0.102±0.001
Alkaloids (mg/g. D/W)	1.8±0.150	1.4±0.255
Saponins (mg/g. D/W)	2.0±0.215	2.2±0.137
Total phenolic content (mg/g. D/W)	1.669±0.06	1.23±0.121

Data are expressed as mean±SEM (n = 3) of three independent experiments
All data expressed as (mg/g. Dry Weight)

Table 1. Tannins, total Flavonoids content (TFC), Alkaloid and Saponins content of methanolic extract of Lespedeza bicolor arial parts and root

The methanolic extracts of *Lespedeza bicolor* arial parts and root were significantly active against the fungal pathogens studied. The arial parts of *Lespedeza bicolor* showed the broadest spectrum of activity against *Aspergillus fumigates, Aspergillus niger, Fusarium solani* and *Mucor sp* with MIC value of 20-35 μg ml^{-1} than the root extract shown in table 2. MIC value of 20 and 35 μg ml^{-1} of *Lespedeza bicolor* arial parts and root extract against bacterial pathogen *Klebsiella pneumonia* and 20-50 μg ml^{-1} against *Enterococcus* has been shown in table 2. Penicillin and Chloramphenicol with MIC value of 1.5-2.5 μg ml^{-1} has been used as a positive control against *Klebsiella* and *Enterococcus* specie.

To better understand the antioxidant potential of *Lespedeza bicolor* extracts of root and arial parts were evaluated for radical scavenging activity against DPPH. Fig.1 illustrated a significant decrease in the concentration of DPPH due to scavenging activity of the extract. DPPH radical scavenging activity of *Lespedeza bicolor* arial parts and root extract with IC_{50} values of ≤ 50 and ≤ 200 μg ml^{-1} respectively with galllic acid (R2= 0.871) and ascorbic acid (R2= 0.780) was shown in table. 3 whereas, hydrogen peroxide scavenging activity with IC_{50} values of ≤ 25 for arial parts and ≤ 50 μg ml^{-1} for the root extract of *Lespedeza bicolor* has been shown in the same table with galllic acid (R2= 0.819) and ascorbic acid (R2= 0.728).

Cytotoxicity results against LU-1 and LnCaP cell lines are summarized in table 3. *Lespedeza bicolor* arial parts and root extract showed IC_{50} value of 12.5 and 50 μg/ml against LU-1 whereas, ≤ 12.5 and 12 μg/ml were calculated against LnCaP cell line. Interestingly, *Lespedeza bicolor* possessed the highest inhibition potential against human lung carcinoma (LU-1) and human prostrate carcinoma (LnCaP) cell lines indicating its ultimate potential for biopharmaceutical uses.

Micro-organisms	Tested materials (MIC µg ml^{-1}) ±SEM		
Fungai	***Lespedeza* Arial**	***Lespedeza* Root**	***Terbinafine (µg/ml)**
Aspergillus fumigatus	20±0.381	40±0.241	1.5±0.075
Aspergillus niger	≤ 35±0.305	≤ 45±0.254	1.5±0.075
Aspergillus flavus	≥ 70±0.672	60±0.167	≥ 2.0±0.124
Fusarium solani	≤ 30±0.380	≤ 40± 0.244	2.0±0.122
Mucor Sp	25±0.355	≤ 40±0.239	≤ 2.0±0.191
Klebsiella pneumonia	20±0.401	≤ 35±0.168	-
Enterococcus	≥ 20±0.372	≥ 50±0.388	-

Data are expressed as mean±SEM (n = 3) of three independent experiments
*Terbinafine 1-2.5µg/ml is used as a standred fungicide, *Penicillin and Chloramphenicol 1-3.5µg/ml is used as a standred antibiotics.

Table 2. Antifungal activities (expressed in MIC) of methanolic extracts of Lespedeza bicolor arial parts and root

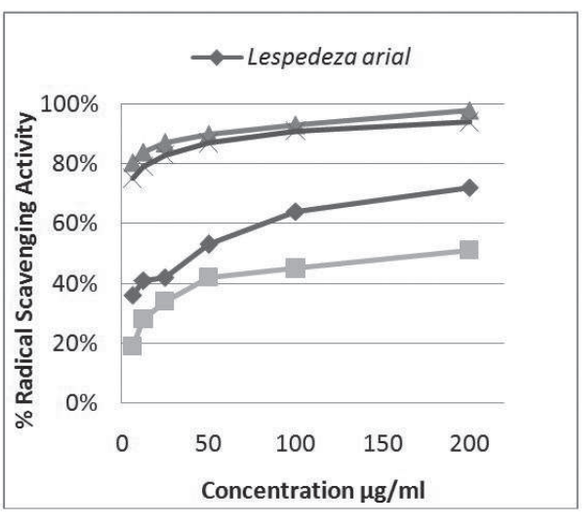

Fig 1. Analysis of DPPH Radical Scavenging activity of methanolic extract of arial parts and root of *Lespedeza bicolor*

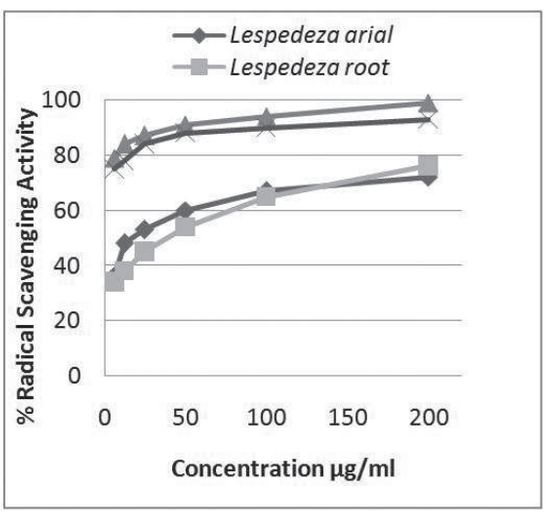

Fig 2. Analysis of Hydrogen Peroxide Radical Scavenging activity of methanolic extracts of arial parts and root of *Lespedeza bicolor bicolor*

Micro-organisms	Tested materials IC_{50} values (µg ml^{-1}) ±SEM	
Anticancer assays	***Lespedeza* Arial**	***Lespedeza* Root**
*[1]LU-1	12.5±0.168	≤50±0.199
*[2] LnCaP	≤ 12.5±0.144	12.5±0.154
Antioxidant assays		
DPPH Radical Scavenging Activity	≤ 50±1.431	≤ 200±0.210
Hydrogen peroxide-scavenging activity	≤ 25± 0.099	≤ 50±0.184

Data are expressed as mean±SEM (n = 3) of three independent experiments
*1Human lung carcinoma
*2Human prostrate carcinoma
Colchicine with IC50 values 0.02±0.002 is used as Standard anticancer drug

Table 3. Cytotoxicity against (LU-1) and (LnCaP) and antioxidant activities of methanolic extracts of *Lespedeza bicolor* arial parts and root expressed as IC_{50} (µg ml^{-1})

4. Conclusion

In conclusion, the high antimicrobial, antioxidant and cytotoxic potential of *Lespedeza bicolor* highlight the need of further investigations to isolate the active principle and their subsequent evaluation. The results also suggest the presence of biologically active principles which may be worth further investigation and elucidation. Further studies are in fact currently under way to isolate and characterize the active principle(s) of the crude extract.

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6. References

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