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Antibacterial and Antioxidant Activities of Two *Asteracious* Species Growing in Pakistan

DURRE SHAHWAR*, SAIF ULLAH, NAEEM AHMAD,
SAMI ULLAH and MUHAMMAD AKMAL KHAN
Department of Chemistry, Research Lab-II, G.C. University, Lahore-54000, Pakistan
Fax: (92)(429)213341; Tel: (92)(421)11000010
E-mail: drdshahwar@yahoo.com; drdshahwar@gmail.com

Emergence of drug resistant bacteria and increased oxidative stress are the new havocs for humanity. This study was designed to examine *in vitro* antibacterial and antioxidant activities of extracts from *Xanthium strumarium* L. (*Asteraceae*) and *Blumea lacera* DC. (*Asteraceae*). All the plant parts were extracted successively in *n*-hexane, chloroform and methanol using soxhlet-apparatus. Antibacterial activity was assessed by agar-well diffusion method. The extracts were also screened for possible antioxidant activity using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical and total antioxidant activity assay. Methanol extracts were more active than other solvent extracts against test organisms. All the extracts of *X. strumarium* showed high antibacterial activity. The MICs of all the active extracts were found in the range of 62-500 μg mL^{-1}. In the DPPH assay, the methanol extract of *B. lacera* was found to be superior to all (IC_{50} = 0.3 mg mL^{-1}), whereas almost all the extracts particularly the polar ones exhibited antioxidant activity in total antioxidant assay. The correlation studies revealed close relation between antioxidative potential and phenolic content of the extracts. Hence *X. strumarium* and *B. lacera* are a potential source for further research activities including isolation of antibacterial and antioxidant components.

Key Words: *Blumea lacera*, *Xanthium strumarium*, *Asteraceae*, DPPH, Phenolic contents.

INTRODUCTION

Emergence of drug-resistant microorganisms is swiftly reversing advances of the previous 50 years of research[1]. To find antibacterial agents from natural sources against resistant microorganisms is an urgent need for public health. About 119 pure chemical substances from plants are already in use as medicines throughout the world[2].

Xanthium strumarium L. (*Asteraceae*) is well known for the treatment of leucoderma, fever, scrofula, herpes and cancer[3]. Carboxyactractyloside (a highly toxic agent which also possesses hypoglycemic activity) has been isolated from *X. strumarium*[4]. Moreover, the plant also exhibits antitumor[5], antimalarial[6], antitrypanosomal[7] and anthalmintic activities[8]. *Blumea lacera* DC. (*Asteraceae*) is an annual plant

widely distributed in Asia, has strong odour of turpentine and is used as febrifuge, anthelmintic, antipyretic and diuretic in folk medicines[9]. It is also used to cure bronchitis, blood diseases, fever and to aleviate burning sensations[10].

Free radicals are metastable chemical species, which tend to trap electrons from the molecules in the immediate surrounding. These radicals if not scavenged effectively in time, damage crucial biomolecules like lipids, proteins including those present in all membranes, mitochondria, DNA, *etc.* Free radicals are involved in number of diseases like tumor inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cytic fibrosis, neurodegenerative diseases (*e.g.* parkinsonism, Alzheimer's diseases), aids and even early senescence[11]. Human body produces insufficient amount of antioxidants which are essential for preventing oxidative stress[12]. Therefore, this deficiency had to be compensated by making use of natural exogenous antioxidants, such as vitamin C, vitamin E, flavones, β-carotene and natural products in plants[13,14]. Many plants, citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, carotenoids, flavanols and phenolics which possess the ability to scavenge the free radicals in human body. Such plants can be the potential candidates for use in oxidative stress diseases[15].

The aim of present investigation is to evaluate the antioxidant and antibacterial activity of extracts of *Asteracious* species *i.e.*, *Xanthium strumarium* and *Blumea lacera*.

EXPERIMENTAL

Fresh plants of *X. strumarium* and *B. lacera* were collected from different localities of Lahore and identified at Department of Botany G.C. University, Lahore. Specimen vouchers (GCU-Bot-124-07 and GCU-Bot-125-07) were deposited at Herbarium, G.C. University, Lahore.

Preparation of extracts: Aerial parts, fruits and roots of *X. strumarium* and aerial parts of *B. lacera* were air dried and separately extracted successively in *n*-hexane, chloroform and methanol by using soxhlet apparatus, to get the extracts, XHA, XHF, XHR, XCA, XCF, XCR, XMA, XMF, XMR, BH, BC and BM (where X; *X. strumarium*, B; *B. lacera*, H; *n*-hexane, C; chloroform, M; methanol, A; Aerial parts, F; Fruit, R; Roots).

Microorganisms: All the extracts were tested individually against eight microorganisms including *Escherichia coli* ATCC-8789, *Nocardia asteroides* PTCC-NA, *Proteus mirabilis* ATCC-0321P, *Bacillus licheniformis* PTCC-BL, *Bacillus subtilis* PTCC-BS, *Streptococcus thermophilus* PTCC-ST, *Salmonella typhimurium* ATCC-10428 and *Micrococcus luteus* PTCC-ML, which were kindly provided by PCSIR Laboratories Complex, Ferozepur Road, Lahore.

Antibacterial activity: Antibacterial activity of plant extracts (dissolved in 6 % aq. DMSO) was evaluated using a little modified well agar-diffusion method[16]. Eighteen hour aged inocculum suspensions of different bacterial strains in nutrient-broth (2.5 % aq., Fluka) were added to sterilized nutrient agar suspension (≈ 50 ± 5 ºC,

2.8 % aq., Biolab.). To get the final concentration of ≈ 10^5 CFU and 25 mL of mixture was briskly poured in sterilized petri plates. On solidification (≈ 40 min later) 5 mm diameter wells were cut in plates using a sterile cork-borer and 100 µL of extract solutions (20 mg mL^{-1}) were added to the wells. The plates were then incubated for 18 h at 35 °C. Antibacterial activity of the extracts was evaluated by measuring inhibitory zones in mm and compared with that of standard streptomycin solution (500 µg mL^{-1}) and negative control DMSO (6 % aq.). The experiments were run in triplicate and presented as average ± confidence level ($\alpha = 0.05$ and $n = 3$).

Determination of minimum inhibitory concentration (MIC): Minimum inhibitory concentration (MIC) was determined by Muller-Hinton broth (MHB) dilution method[17]. All the extracts of *X. strumarium* and *B. lacera* were diluted to get the concentrations of 500, 250, 125, 62 and 31 µg mL^{-1}. One mL bacterial suspension (1×10^4 cfu mL^{-1}) was incubated with same amount of *X. strumarium* and *B. lacera* extracts in MHB at 37 °C for 24 h. The MICs were taken as the lowest concentration of antibacterial agent that inhibited bacterial growth. Growth inhibition (lack of turbidity) was determined visually. Table-2 shows the MICs of *X. strumarium* and *B. lacera* extracts. Experiments were carried out in triplicate.

Antioxidant activity

DPPH Radical scavenging assay: The hydrogen or electron donation ability of the corresponding extracts was measured from bleaching of purple methanol solution of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical[18]. This spectrophotometric assay uses the stable radical DPPH as a reagent. DPPH absorbs at 517 nm, and as its concentration is reduced by existence of an antioxidant, the absorption gradually disappears with time.

Equal volumes (2 mL) of 10^{-4} M DPPH (in MeOH) and extracts (1.0 mg/mL) were mixed and incubated at 30 °C for 15 min in dark. Control solution containing equal volume of DPPH and MeOH was used as blank (A_{blank}). Absorbance of extract solutions at 517 nm was measured to avoid the inaccurate data (excess values) caused by the absorbing materials present in the crude extract without antioxidant activity. Actual absorbance originated by the inhibition of DPPH (A_{sample}) was evaluated by subtracting the absorbance of methanolic extracts (or reference compounds), from absorbance of corresponding DPPH extracts (or reference compound) at 517 nm. Decrease in absorbance indicated the antioxidant activity. Radical scavenging activity was expressed as percentage inhibition of DPPH and estimated by the following formula.

$$\% \text{ Inhibition of DPPH} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Standard antioxidant butylated hydroxy toluene (BHT) was also tested against DPPH and used as reference. The sample solutions having more than 50 % inhibition were diluted in series to get concentrations 0.1-1.0 mg mL^{-1} and % inhibitions were calculated. From the results obtained, a plot of percentage inhibition of DPPH against concentration of extracts was made and IC_{50} determined.

Total antioxidant capacity assay (TAOC): Total antioxidant activity of extracts was evaluated according to the method of Prieto *et al*.[19] as adopted by Khan *et al*.[18]. 0.1 mL of each sample (0.5 mg/mL) was added to 1.9 mL of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate). The blank solution contained 2.0 mL reagent solution only. The mixtures were incubated at 95 ºC for 150 min. After the mixture had cooled to room temperature, absorbance was measured at 695 nm. The antioxidant activity was expressed as the absorbance of the sample. The antioxidant activity of BHT was used assayed for comparison as standard.

Determination of total phenolic content: The amount of total phenolics of the extracts was determined by the Folin-Ciocalteu (Sigma-Aldrich) reagent method[20]. Stock solution of *X. strumarium* and *B. lacera* extracts were prepared in a concentration of 0.5 mg mL^{-1}. This stock solution was further diluted to get five different concentrations (*i.e.* 0.4, 0.3, 0.2, 0.1 and 0.05 mg mL^{-1}). 0.1 mL of each test concentration was mixed with 0.1 mL of Folin-Ciocalteu reagent in test tube and shaken thoroughly. After 1 min, 2.8 mL of sodium bicarbonate solution (10 % $NaHCO_3$) was added and the mixture was allowed to stand for 0.5 h. Absorbance was measured at 725 nm spectrophotometrically. Gallic acid (0.05-0.5 mg mL^{-1}) was used to produce standard calibration curve. The total phenolic content was expressed as mg equivalent of gallic acid (mg GAE) per gram dry weight of the extract by computing with standard calibration curve.

Statistical analysis: Statistics was applied by Microsoft Excel 2003. All the experiments were run as triplicate and presented here as average ± confidence level ($\alpha = 0.05$ and $n = 3$).

RESULTS AND DISCUSSION

Table-1 (inhibition zones) shows the results of antibacterial activity of *X. strumarium* and *B. lacera* extracts against eight bacterial strains. The negative control disk DMSO always showed no inhibition with bacterial lawn growing in contact with well edges. The standard reference streptomycin inhibited all the bacterial strains. Based on larger zones of inhibition, methanol proved to be the best solvent for isolation of bioactive substances from *X. strumarium* and *B. lacera* followed by chloroform. The chloroform extract of arial parts and roots of *X. strumarium* showed good antibacterial activity (MIC value 62 µg mL^{-1}) against *B. subtilis*, *S. typhimurium* and *E. coli*. Methanol extract of *X. strumarium* and *B. lacera* were active against selected bacterial strains namely, *S. thermophilus*, *B. subtilis*, *S. typhimurium*, *N. asteroides*, *E. coli* and *B. licheniformis*. Antibacterial activity was observed as MeOH > $CHCl_3$ > *n*-hexane solvents.

Methanolic extracts of *X. strumarium* and *B. lacera* exhibited the maximum antibacterial activity with minimum inhibitory concentration values in the range of 62-125 µg mL^{-1} against *S. thermophilus*, *B. subtilis*, *S. typhimurium*, *N. asteroides*, *E. coli* and *B. licheniformis*. The results of minimum inhibitory concentration are

TABLE-1
ZONES OF INHIBITION FOR *X. strumarium* AND *B. lacera* EXTRACTS

Parts of plant	Extracts (20 mg mL^{-1})	Diameter of inhibition zone (mm) excluding well diameter							
		Str. t	*M.l*	*B.s*	*S.t*	*P.m*	*N.a*	*E.c*	*B. l*
Aerial parts	XHA	7.7±1.1	4.6 ±1.1	13.6± 1.2	–	–	–	–	6.5±1.5
	XCA	15.3±1.6	2.3±1.6	20.6±1.1	20.2±1.2	13.4±1.2	10.2±1.0	18.1±1.8	5.3±0.7
	XMA	18.1±1.2	7.0±0.6	22.2±0.9	11.3±2.4	15.6±0.9	13.0±1.9	18.4±1.2	12.5±1.4
Fruit	XHF	–	2.0±0.1	5.7±0.9	–	–	–	–	2.5±0.3
	XCF	–	–	10.1±0.7	3.7±0.1	1.4±0.6	1.1±0.1	–	–
	XMF	20.1±0.1	–	20.5±3.4	17.5±0.9	–	2.2±0.3	19.4±0.1	12.5±0.3
Root	XHR	–	–	6.1±0.1	10.0±1.68	–	–	–	2.8±0.2
	XCR	15.4±0.9	0.9±0.1	16.3±1.4	20.1±0.1	13.5±0.9	5.5±0.3	1.7±1.2	–
	XMR	4.3±0.6	17.3±1.2	22.2±0.2	–	12.2±0.2	14.5±0.9	18.4±1.5	15.6±1.1
Aerial parts	BH	–	4.4±0.5	2.2±0.1	–	3.9±0.1	1.9±0.1	–	–
	BC	10.2±0.3	2.2±1.0	–	–	–	7.2±0.2	–	–
	BM	12.3±1.2	5.2±1.1	9.7±0.1	18.1±0.1	10.6±1.0	12.4±0.2	12.6±0.3	2.0±0.1
Standard streptomycin (0.5 g mL^{-1})		2.0±0.7	12.2±0.9	20.1±1.0	16.4±1.0	20.3±1.1	15.4±0.4	18.4±0.4	16.7±0.2

Str.t: *Streptococcus thermophilus*, *M.l*: *Micrococcus leuteus, B.s*: *Bacillus subtilis*, *S.t*: *Salmonella typhimurium*, *P.m: Proteus mirabilis*, *N.a: Nocardia asteroides*, *E.c: Escherichia coli*, *B.l: Bacillus licheniformis,* X*: Xanthium strumarium*, H: *n*-hexane, C: chloroform, M: methanol, A: Arial parts, F: Fruit, R: Roots, B: *B. lacera,* –: not active, mean±confidence level, $\alpha = 0.05$, n = 3, (negative control DMSO did not show any inhibition of microorganisms).

depicted in Table-2. Methanolic extract of *X. strumarium* showed lowest MIC value (62 µg mL^{-1}) against *B. subtilis*, *B. licheniformis*, *N. asteroides* and *E. coli* while methanolic extract of *B. lacera* was most active against *S. typhi-murium* with MIC 125 µg mL^{-1}. Among all the tested strains, *E. coli* was the most susceptible strain to almost all the extracts, while *S. typhimurium* was the most resistant followed by *S. thermophilus* and *N. asteroides*. Phytochemical study of the extracts revealed the presence of alkaloids, triterpenoids, flavonoides and polyphenoles which is in accordance with the reported work[10,21-23].

Free radical scavenging activity of the extracts was measured by DPPH$^{\bullet}$ assay. The results of DPPH radical scavenging and total antioxidant activities of different extracts of *X. strumarium* and *B. lacera* are summarized in Table-3. IC_{50} values ranged from 0.3-0.9 mg mL^{-1}. The highest DPPH radical scaven-ging activity was found in methanolic extract of *B. lacera* (IC_{50} = 0.3 mg mL^{-1}) followed by methanolic extract of *X. strumarium* roots (IC_{50} = 0.6). Generally the polar extracts were found stronger free radical scavengers than non-polar extracts.

Total phenolic content of all the extracts was determined by Folin-Ciocalteu reagent as mg GAE/g dry weight of extract and the results are given in Table-3. The amount of total phenolics varied in different extracts and ranged from 35.6-676.48 mg GAE/g of dry extract.

Co-relationship between total phenolic content and antioxidant activity: The antioxidant activity and total phenolic content of *X. strumarium* and *B. lacera* extracts were positively correlated. The correlation coefficients R^2 were 0.7419

TABLE-2
MIC VALUES OF EXTRACTS OF *X. strumarium* AND *B. lacera* AGAINST EIGHT BACTERIAL STRAINS

Microorganism	Minimum inhibitory concentration (μg mL^{-1})*					
	XMA	XCA	XMF	XMR	XCR	BM
S. thermophilus	125	500	155	NA	500	> 500
M. luteus	NA	NA	NA	125	NA	NA
B. subtilis	62	62	62	62	500	> 500
S. typhimurium	> 500	125	125	NA	62	125
P. mirabilis	500	> 500	NA	NA	> 500	> 500
N. asteroides	500	> 500	NA	62	NA	> 500
E. coli	125	62	500	62	NA	> 500
B. licheniformis	500	NA	500	62	NA	NA

*Values are the mean of three replicates using 1×10^4 CFU of each culture. X: *Xanthium strumarium*, H: *n*-hexane, C: chloroform, M: methanol, A: Aerial parts, F: Fruit, R: Roots, B: *B. lacera,* NA: not active.

TABLE-3
DPPH RADICAL SCAVENGING, TOTAL ANTIOXIDANT CAPACITY AND TOTAL PHENOLICS OF *X. strumarium* AND *B. lacera* EXTRACTS

Sample code	DPPH scavenging assay		Total antioxidant activity (absorbance at 695 nm)	Total phenolics (mg GAE/g)
	% Inhibition (at 1.0 mg mL^{-1})	IC_{50} (mg mL^{-1})		
XHA	4.8 ± 4.61	–	0.529 ± 0.02	224.48 ± 39.97
XHF	21.5 ± 2.67	–	0.379 ± 0.01	103.59 ± 29.71
XHR	13.9± 4.69	–	0.713 ± 0.00	76.04 ± 7.12
XCA	51.8 ± 2.61	0.9 ± 2.93	0.569 ± 0.05	72.93 ± 6.03
XCF	56.5 ± 3.30	0.7 ± 1.55	1.335 ± 0.12	420.48 ± 25.69
XCR	52.4 ± 1.10	0.8 ± 5.39	0.456 ± 0.03	181.37 ± 17.67
XMA	28.7 ± 5.23	–	1.085 ± 0.05	112.92 ± 18.28
XMF	21.1 ± 6.30	–	0.453 ± 0.04	76.93 ± 29.67
XMR	64.4 ± 2.78	0.6 ± 5.02	0.820 ± 0.01	533.82 ± 25.65
BH	14.5 ± 5.94	–	0.412 ± 0.01	36.93 ± 15.08
BC	21.1 ± 0.66	–	1.746 ± 0.01	35.6 ± 13.58
BM	88.3 ± 2.23	0.3 ± 10.82	1.416 ± 0.01	676.48 ± 28.07
BHT	90.4 ± 0.19	0.01 ± 0.55	1.123 ± 0.19	–
Blank	0.00	–	0.262 ± 0.00	–

Results are given as average ± confidence level of three different experiments, (X: *Xanthium strumarium*, H: *n*-hexane, C: chloroform, M: methanol, A: Aerial parts, F: Fruit, R: Roots, B: *B. lacera,* –: not determined).

and 0.5022, respectively of DPPH and total antioxidant methods. The results suggested that the phenolic compounds contributed to some extent to total antioxidant capacity of *X. strumarium* and *B. lacera* extracts.

Natural products are considered as important source of new antibacterial and antioxidant agents. Drugs which are semi-synthetically obtained from natural sources or derived from unmodified natural products corresponded to 78 % of the new

drugs approved by FDA between 1983 and 1994[24]. The present study demonstrated the existence of antibacterial and antioxidant agents in the extracts of *X. strumarium* and *B. lacera* which are effective against bacterial strains *E. coli*, *N. asteroides*, *P. mirabilis*, *B. licheniformis*, *B. subtilis*, *S. thermophilus*, *S. typhimurium* and *M. luteus*. The results presented here are consistent with some previous investigations on the genera of *Xanthium* and *Blumea*. Cerdeiras *et al.*[25] found *X. cavanillesii* effective against *Bacillus subtilis* which was also sensitive in this study. Present findings of *B. subtilis* sensitivity agrees with Kar[26], who reported *Blumea laciniata* as being effective against *B. subtilis* and many other bacterial strains[26]. Many *Asteracious* species such as *Xanthium sirbicum*, *Tagetes lucida*, *Achillea clavennae*, *A. holosericea*, *A. lingulata*, *A. millefolium*[27-29] exhibited moderate to appreciable antibacterial activities against many gram positive and gram negative bacteria. It has already been reported that many medicinal plants were less active against gram negative bacteria than gram positive bacteria[30]. *Escherichia coli* is recognized as human pathogen which causes different human diseases, such as diarrhea, haemorrhagic colitis and occasionally complications such as heamolytic-uremic syndrome and thrombocytopenic purpura[30]. *S. typhimurium* is a leading cause of human gastroenteritis and is used as a mouse model of human typhoid fever[31]. It also causes infection of arthritis which is well recognized complication of rheumatoid arthritis particularly after joint replacement[32]. Since antibacterial activity of the extracts XCF, XMR and BM is comparable with the standard antibiotic (streptomycin), therefore, these extracts can be used in arthritis, diarrhea and haemorrhagic colitis like diseases which are caused by these two gram negative strains. Antimicrobial activity of methanol extracts from leaves of *X. strumarium* and *B. lacera* against *E. coli* has already been reported in the literature[33,34]. To the best of our knowledge, this is the first report of strong antibacterial activity of methanolic extract from roots of *X. strumarium* against *E. coli*. Similarly antibacterial activity of chloroform extract from leaves of *X. strumarium* and methanol extract of *B. lacera* against *S. typhimurium* has not been yet reported in the literature.

The results of DPPH radical scavenging activity analyses demonstrated that the most active radical scavengers were found in the methanol extract of *B. lacera* (BM) followed by the methanolic extracts of roots of *X. strumarium* (XMR). The reaction involved in the free radical scavenging activity followed a concentration dependent pattern. Free radical inhibition of the extracts is correlated with its concentration because it reaches at higher value in the presence of highest extract concentration. The highest total phenolic levels have been detected in BM, XMR and XCF, while the lowest total phenolic level was found in chloroform extract of *B. lacera* (BC). The observed high antioxidant activity could be attributed somewhat to the presence of phenolic substances. But a weak correlation is found between total phenolics and DPPH radical scavenging activity; $R^2 = 0.7419$. A lower correlation between total phenolics and DPPH assay could also be explained by the presence of some other sort of compounds which would react with Folin-Ciocalteu

reagent. Published analyses have revealed that *X. strumarium* and *B. lacera* possess alkaloids, triterpenoids, *etc.*[10,21]. It is also known that phenolic level does not necessarily correspond to the similar antioxidant responses. The response of phenolics in the Folin-Ciocalteu assay also depends on the chemical structure of phenols[35]. This means that antioxidant activity of a sample can not be predicted on the basis of its phenolics. Antioxidant activity may be the result of combined activity of wide range of compounds[36]. This preliminary screening is an interesting evaluation of the antibacterial and antioxidant potential of *B. lacera* and *X. strumarium* and suggests further investigation of the potential of these species as a source of herbal medicines.

Conclusion

The results of this study showed that *X. strumarium* and *B. lacera* extracts possess compounds with significant antibacterial and antioxidant activities which can be purified from the respective extracts.

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