Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Evaluation of Antibacterial Effect of *Gymnema sylvestre* R.Br. Species Cultivated in Pakistan

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ARTICLE HISTORY (16-089)

Received:	April 09, 2016
Revised:	May 21, 2017
Accepted:	May 23, 2017
Published online:	May 31, 2017

Key words:
Antibacterial
Cytotoxicity
Gymnema sylvestre R.Br.
MIC
MTT Assay
Sequential leaf extracts
Well diffusion Method

ABSTRACT

The present study was designed to evaluate the antibacterial activity and safety of sequential extracts of *Gymnema sylvestre* R.Br. (Gurmar) leaves. Antibacterial activity of chloroformic, hexane, ethanolic and aqueous extracts of *Gymnema sylvestre* leaves was evaluated against five bacterial poultry pathogens i.e. *Staphylococcus aureus, Clostridium perfringens type-A, Escherichia coli, Salmonella enterica, Haemophillus paragallinarum* by using agar well diffusion method. Stock solutions of 0.1gm/1ml of all the extracts were prepared. Chloroform and ethanolic extracts of *Gymnema sylvestre* showed better antibacterial activity against all isolates of selected microorganisms, while hexane extract showed antibacterial activity against *Salmonella enterica, Staphylococcus aureus, Haemophillus paragallinarum* and *Clostridium perfringens type-A,* but no activity against *Escherichia coli.* On the other hand, aqueous extract showed antibacterial activity only against *Clostridium perfringens type-A,* while no activity against remaining four bacteria. MTT Assay was performed to evaluate *in-vitro* cytotoxicity of these extracts by using Vero cell line. Cell Survival Percentage was calculated. Results of MTT assay showed that 2900, 3612.50, 4075 and 1562.50µg/ml concentrations of hexane, chloroform, ethanol and aqueous extracts respectively had no cytotoxic effect. The antibacterial and MTT Assay suggested that sequential extracts of dried leaves of *Gymnema sylvestre* can be used as a safer antibacterial agent against the above bacteria. All results were compared statistically using DMR (Duncan's Multiple Range) Post hoc test at $P \leq 0.05$ which showed that ZOI and MIC values were significantly varied between groups while there was no variation within same group.

To Cite This Article: Tahir M, Rasheed MA, Niaz Q, Ashraf M, Anjum AA and Ahmed MU, 2017. Evaluation of antibacterial effect of *Gymnema sylvestre* R.Br. species cultivated in Pakistan. Pak Vet J, 37(3): 245-250.

INTRODUCTION

A lot of research work has been carried out to discover novel antimicrobial agents from different sources like animals, plants and microorganism. Plant products have been used to treat different ailments all around the world (Hossen *et al*., 2016). Phyto-chemicals obtained from plants normally kill or inhibit growth of bacteria (Singh *et al.*, 2003). In the development of novel drugs many plants have been investigated for their therapeutic properties (Nitta *et al.*, 2002). Instead of engineered/ synthetic drugs, anti-microbials of plant origin show least adverse effects and have an enormous therapeutic potential to cure or heal numerous infectious diseases.

Drug resistance to pathogenic microorganisms has been commonly reported worldwide. The expanding recurrence of microorganisms that are resistant to commonly used antibiotics is increasing nowadays. As compare to developed countries, the rate of resistance to these medications is higher in under developed countries because of extensive and indiscriminate use of antibiotics over last few decades (Akram *et al.*, 2007). Furthermore, individual's capacity to self-cure without proper checkup and consultation from a doctor/physician (Bronson and Barrett, 2001). Indigenous medicinal plants are natural sources for valuable ingredients that can be utilized as a therapeutic agent for different ailments. Plant materials remained a key source for combating ailments, including infectious diseases, and various plants have been investigated as novel drugs for the development of new therapeutic agents. Thus, the emergence of multiple drug resistance of pathogens has necessitated a search for new

antimicrobial substances from other sources including plants (Lin *et al.*, 2005).

Gymnema sylvestre R.Br. has traditional uses in the treatment of asthma, eye complaints and snake bite. It also possesses antimicrobial, anti-hypercholesterolemic and hepatoprotective properties (Praveen *et al.*, 2011).

Gymnema sylvestre is an amazing herb belongs to *Asclepiadaceae* family. The plant is commonly known as Periploca of the woods (English); Gurmar (Hindi); Meshashringi, Madhunashini (Sanskrit); Kalikardori (Marathi); Mardashingi (Gujrathi) (Kanetkar *et al.*, 2007).

It is mostly cultivated in Asia including Japan, India, Indonesia, Malaysia, Sri Lanka, China, Australia, Vietnam, tropical regions of Africa (Kanetkar *et al.*, 2007).

The *G. sylvestre* is being used as astringent, anodyne to improve appetite and digestion, as tonic for liver, an emetic, anthelminthics, diuretic, cardiotonic, bowel evacuating agent, an expectorant, antipyretic and uterotonic, antidiabetic, antihyperlipidaemic, weight loss remedy (Pierce, 1999).

Various organic and aqueous extracts of *G. sylvestre* leaves have showed antimicrobial activity against various bacterial pathogens like; *B. pumilis, B. subtilis, P. aeruginosa* and *S. aureus, S. typhimurium, S. paratyphi, Proteus vulgaris, E. coli* and *K. pneumonia* (Pasha *et al.*, 2009; Paul and Jayapriya, 2009; Satdive *et al.*, 2003).

Various microbial organisms are threatening the poultry industry by causing infections. Some commonly occurring poultry infections include; septicemia, infectious arthritis, bumble foot, infectious diarrhea and coliform infections, infectious coryza, necrotic enteritis, food poisoning by Salmonella species (Blackall *et al.*, 1997).

In such bacterial infections antibiotic / antibacterial agents are used either to prevent or cure the bacterial infections. But unluckily in Pakistan most of antibacterial agents are facing the emergence of resistance. So, there is a need to develop new antibacterial agents to which these common bacteria are not resistant. The prime focus while searching and developing a new antibacterial agent is that it should have maximum safety and lower lethality. Keeping in view this factor the natural sources like plants, herbs, shrubs are being investigated for their antibacterial activity as usually they are safer and have wider therapeutic index. So, the current study was aimed to achieve the above-mentioned objectives for the prevention and cure of poultry infections with safety and economy.

MATERIALS AND METHODS

Experimental design: Four sequential extracts of *Gymnema sylvestre* were obtained by extracting dried leaves powder with hexane, chloroform, ethanol and distilled water using Soxhlet Apparatus. The antibacterial activity was investigated against *Staphylococcus aureus, Clostridium perfringens type-A, Salmonella enterica, Escherichia coli* and *Haemophillus paragallinarum* by well-diffusion method. Minimum inhibitory concentrations (MICs) were assessed for the extracts which exhibited antibacterial activity. Cytotoxicity of all extracts was evaluated by using MTT-Assay on Vero cell line. Fresh leaves were obtained, identified and authenticated from Department of Botany GCU, Lahore against Voucher # GC.Herb.Bot.2890. The leaves were ground and 200g was taken for preparation of crude extract. The dried powder was extracted sequentially with hexane, chloroform, ethanol and distilled water by using Soxhlet Apparatus (Jeyaseelan *et al.*, 2012). All extracts were dried in an incubator at 37°C and collected into air-tight dark bottles and weighed to get percentage yield by using the formula as described by (Jabeen *et al.*, 2009).

Assessment of antibacterial effects: Five poultry pathogenic bacteria *Staphylococcus aureus, Clostridium perfringens type-A, Salmonella enterica, Escherichia coli* and *Haemophillus paragallinarum* were obtained from the Department of Microbiology, UVAS, Lahore. All experimental bacteria were isolated on specific nutrient media and subjected to gram-staining and various biochemical tests like Catalase Test, IMViC Test for their confirmation.

The stock solutions of Hexane, Chloroform and Ethanol were freshly prepared by dissolving 0.1gm (100mg) of dried extract in 1ml of DMSO while aqueous extract was prepared by dissolving 0.1gm of dried extract in 1ml of pre-autoclaved PBS in pre-autoclaved Eppindroff's tubes separately.

Antibacterial activity: Sterile nutrient agar petri plates were taken and wells were created. These petri plates were swabbed with sterilized cotton swab dipped in pre-standardized bacterial suspension. Then 100µl of each extract was added in every well while control well received 100µl of DMSO followed by 24 hours incubation and zones of inhibition were measured (Nkere and Iroegbu, 2005).

Sequential extracts of *Gymnema sylvestre*, which showed zones of inhibition, were further subjected to evaluate MIC by using serial dilution method (Gulfraz *et al.*, 2011). MIC was evaluated using 96-well ELISA plates. A 100µl of nutrient broth was added from 1-12th well in each row. Then 100µl of Hexane extract was added in well No.1 and two folds diluted up to 10th well. Then picked 100µl from well No. 10 and discarded. The same procedure was repeated for remaining three sequential extracts. Then 100µl of bacterial suspension was added from 1-11th well. Same procedure was repeated for all bacterial isolates. All Petri plates were covered, wrapped, labeled and placed in incubator at 37ºC for 24 hours. OD values were determined by using ELISA reader at λ=655nm.

Cytotoxicity assay: Vero cell line was obtained from Quality Operational Laboratory, UVAS, Lahore. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] Assay was performed as described by (Mosmann, 1983). MTT dye was dissolved in specific concentrations in each dilution and was tested separately. The experimental group contained media, sequential extracts (i.e. 100µl super-saturated solution of each extract), MTT dye (200µg/ml), whereas negative control have media only while positive control has media and 20% DMSO. The concentrations of supersaturated solutions of extracts (Hexane=5800, Chloroform=14450, Ethanol=16300 and Aqueous=25000µg/ml) to be tested and control were added in separate wells at a rate of 200µg/ml. Each dilution was tested in triplicate wells. Plate was incubated

at 37^0C for 48 hours. Results were presented in terms of cell survival percentage (CSP) by using following formula:

$$CSP = \frac{\text{Mean OD of Test} - \text{Mean OD of Negative Control}}{\text{Mean OD of Positive Control}} \times 100$$

Statistical analysis: The data obtained was statistically analyzed by using SPSS-v.16.0. Results of ZOI and MIC were analyzed by using one-way ANOVA, antibacterial and MTT assay were compared by using DMR (Duncan's Multiple Range) Post-hoc test at $P \leq 0.05$.

RESULTS

In this study, percent yield of hexane, chloroform, ethanolic and aqueous extract was 1.6, 2.03, 14.0 and 11.4%, respectively. The Mean zones of inhibition values (Table 1) of *G. sylvestre* leaf extracts showed that chloroform and ethanolic extracts have more antibacterial activitity against all five microorganisms when compared with hexane and aqueous extracts. Only chloroform and ethanolic extracts showed antibacterial activity against all five microorganisms (*Staphylococcus aureus, Salmonella enterica, Escherichia coli, Haemophillus paragallinarum, Clostridium perfringens type-A*) while hexane extract showed antibacterial activity against *Salmonella enterica, Staphylococcus aureus, Haemophillus paragallinarum, Clostridium perfringens type- A* but no activity against *Escherichia coli.* On the other hand, aqueous extract showed antibacterial activity only against *Clostridium perfringens type-A,* but no activity against remaining four bacteria. While analyzing results based upon minimum inhibitory concentration (MIC) as described in table 2, the chloroform extract has more antibacterial effect when compared with hexane. Hexane extract was more potent than aqueous extract whereas ethanolic extract was the least potent.

When overall antibacterial effects of all the extracts were evaluated against all bacterial strains, it was observed that *Clostridium perfringens type-A* was the bacterium most vulnerable to antibacterial activity of sequential extracts of dried leaves of *G. sylvestre* as it responded to all four sequential extracts and gave maximum zones of inhibition (10-22mm range) while no other bacteria showed such bigger zones. Statistical analysis showed that ZOI and MIC values were significantly different between the groups while within the same group they were non-significant.

On the basis of minimum inhibitory concentration (MIC), it can be assumed that chloroform extract has more antibacterial active components as compared to hexane extract. While hexane extract has more antibacterial active components as compared to ethanolic extract. The activity of aqueous extract is negligible as it showed response against only single bacterium.

MTT assay was performed on supersaturated solutions of sequential extracts of *Gymnema sylvestre* leaves. Results revealed that low concentrations of all of four sequential extracts of *Gymnema sylvestre* leaves were not toxic (Table 3, 4, 5 and 6). Cell survival percentages (CSP) were below 50% when given at concentrations of 5800µg/ml (38.76%), 7225µg/ml (43.71%), 8150µg/ml (44.90%) and 3125µg/ml (41.84%) by hexane, chloroform, ethanolic and aqueous extracts respectively. Hexane extract was safer only in case of *Clostridium perfringens type-A* as its MIC value fell in safety zone of CSP, while MIC for remaining bacteria came in cytotoxic range. Chloroform extract was safer for all of sensitive bacterial strains as their MIC values came in the safety zone of CSP. Ethanolic extract was cytotoxic as MIC values for all the experimental bacteria fell in the cytotoxic range of CSP. Aqueous extract showed antibacterial activity only against *Clostridium perfringens type-A* which came in cytotoxic range of CSP (Not in safer range). Finally, on the basis of MIC and CSP for all of four sequential extracts, it is concluded that chloroformic extract is the most active and safe extract against all of five experimental bacteria, while hexane extract is safe against only *Clostridium perfringens type-A* and ethanolic and aqueous extracts are cytotoxic on their MIC values for all the experimental bacteria. MTT assay results indicate that this plant can be used therapeutically after isolating its antibacterial constituents and modifying into proper pharmaceutically stable dosage form with safety.

DISCUSSION

In current study the antibacterial effect and cytotoxicity profile of sequential extracts of *Gymnema sylvestre* R.Br leaves was studied. A study conducted by (David and Sudarsanam, 2013) in which aqueous, methanol, chloroform and hexane extracts (non-sequential extracts) of *Gymnema sylvestre* R.Br. leaves were tested for antimicrobial activity against *Staphylococcus aureus, Bacillus cereus, Klebsiella pneumonia* and *Escherichia coli.* Methanol and aqueous extracts showed significant antibacterial activity against tested bacteria which are in accordance with results of current study where hexane, chloroform, ethanol and aqueous extracts (sequential extracts) showed remarkable antibacterial activity against all of experimental bacteria.

Another study conducted on the phytochemical analysis and antimicrobial activity of *Gymnema sylvestre* showed that the plant is rich in saponins and many other phyto-chemicals with therapeutic value. Chloroform extracts of aerial and root parts of *Gymnema sylvestre* showed higher antimicrobial activity when compared with acetone and diethyl ether. The root extracts of chloroform showed competitive MIC and minimum bactericidal concentration values towards the pathogens. These results

Table 1: Summary of mean ZOI for *S. enterica, S. aureus, H. paragallinarum, C. perfringens type-A* and *E. coli*

Bacteria / Extracts	*Salmonella enterica* (mm)	*Staphylococcus aureus* (mm)	*Haemophilus paragallinarum* (mm)	*Clostridium perfringens type-A* (mm)	*Escherichia coli* (mm)
Hexane	8.33 ± 1.53^b	6.67 ± 0.58^c	7.00 ± 0.00^b	18.33 ± 0.58^a	0.00 ± 0.00^a
Chloroform	7.33 ± 0.58^b	7.67 ± 0.58^b	12.67 ± 2.31^c	18.67 ± 3.06^a	10.00 ± 2.00^b
Ethanol	7.67 ± 0.58^b	11.67 ± 4.04^c	12.00 ± 0.00^c	19.00 ± 2.00^a	10.33 ± 2.52^b
Aqueous	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	13.67 ± 4.04^a	0.00 ± 0.00^a

Mean±SD within a column carrying the different superscripts differ significantly ($P \leq 0.05$).

Table 2: Summary of mean MIC for *S. enterica, S. aureus, H. paragallinarum, C. perfringens type-A* and *E. coli*

Bacteria / Extracts	*Salmonella enterica* (µg/ml)	*Staphylococcus aureus* (µg/ml)	*Haemophilus paragallinarum* (µg/ml)	*Clostridium perfringens type-A* (µg/ml)	*Escherichia coli* (µg/ml)
Hexane	6250.0±0.00^{c}	6250.0±0.00^{ab}	9375.0±5412.66^{b}	3125.0±0.00^{ab}	0.0±0.00^{a}
Chloroform	911.5±596.69^{b}	1562.5±0.00^{a}	520.8±225.52^{a}	781.3±0.00^{a}	1302.1±451.05^{a}
Ethanol	12500.0±0.00^{d}	14583.0±9547.03^{b}	20833.0±7216.88^{c}	8333.3±3608.44^{b}	12500.0±0.00^{c}
Aqueous	00.0±0.00^{a}	0.0±0.00^{a}	0.0±0.00^{a}	5468.8±6200.98^{ab}	0.0±0.00^{a}

Mean±SD within a column carrying the different superscripts differ significantly (P≤0.05).

Table 3: CSP of hexane extract (sequential extract) of *Gymnema sylvestre* R.Br. Leaves at various concentrations in MTT assay using Vero Cell line

Cytotoxic activity of hexane extract of *Gymnema sylvestre* R.Br. leaves for vero cells			
Wavelength = 570nm			
Sr. No.	Conc. Used(µg/ml)	Mean OD±SD	Cell Survival (%)
1	5800.00	0.2547±0.07446	38.76
2	2900.00	0.3130±0.01453	54.20
3	1450.00	0.3153±0.02511	54.81
4	725.00	0.3393±0.04202	61.16
5	362.50	0.3557±0.05179	65.50
6	181.25	0.3043±0.02438	51.89
7	90.63	0.3397±0.02930	61.27
8	45.31	0.3393±0.02857	61.16
9	22.66	0.3117±0.01185	53.85
10	11.33	0.3560±0.05071	65.58
11	Negative control (cell culture media)	0.1083±0.01328	
12	Positive control (20% DMSO)	0.3777±0.04508	

Table 4: CSP of chloroform extract (sequential extract) of *Gymnema sylvestre* R.Br. leaves at various concentrations in MTT assay using vero cell line

Cytotoxic activity of chloroform extract of *Gymnema sylvestre* R.Br. leaves for vero cells			
Wavelength = 570nm			
Sr. No.	Conc. Used(µg/ml)	Mean OD±SD	Cell Survival (%)
1	14450.00	0.2633±0.02768	44.92
2	7225.00	0.2590±0.05406	43.71
3	3612.50	0.3233±0.01882	61.81
4	1806.25	0.3077±0.06969	57.42
5	903.13	0.3453±0.02150	68.00
6	451.56	0.3420±0.04513	67.07
7	225.78	0.3320±0.05429	64.26
8	112.89	0.3303±0.04215	63.78
9	56.45	0.3307±0.05229	63.89
10	28.22	0.3300±0.04419	63.69
11	Negative control (cell culture media)	0.1037±0.00666	
12	Positive control (20% DMSO)	0.3553±0.02026	

Table 5: CSP of ethanolic extract (Sequential Extract) of *Gymnema sylvestre* R.Br. leaves at various concentrations in MTT assay using vero cell line

Cytotoxic Activity of Ethanolic Extract of *Gymnema sylvestre* R.Br. leaves for Vero Cells			
Wavelength = 570nm			
Sr. No.	Conc. Used(µg/ml)	Mean OD±SD	Cell Survival (%)
1	16300.00	0.217±0.02052	31.40
2	8150.00	0.2643±0.03024	44.90
3	4075.00	0.3260±0.05237	62.52
4	2037.50	0.3267±0.03479	62.72
5	1018.75	0.3060±0.01513	56.81
6	509.38	0.3080±0.02666	57.38
7	254.69	0.3113±0.02178	58.32
8	127.34	0.3193±0.00850	60.61
9	63.67	0.3480±0.02128	68.80
10	31.84	0.3297±0.04759	63.57
11	Negative Control (Cell Culture Media)	0.1070±0.01217	
12	Positive Control (20% DMSO)	0.3503±0.01801	

Table 6: CSP of aqueous extract (sequential extract) of *Gymnema sylvestre* R.Br. leaves at various concentrations in MTT assay using vero cell line

Cytotoxic activity of ethanolic extract of *Gymnema sylvestre* R.Br. leaves for vero cells			
Wavelength = 570nm			
Sr. No.	Conc. Used(µg/ml)	Mean OD±SD	Cell Survival (%)
1	25000.00	0.0957±0.00757	-1.54
2	12500.00	0.1573±0.01724	14.25
3	6250.00	0.1853±0.02294	21.42
4	3125.00	0.2650±0.02506	41.84
5	1562.50	0.3457±0.02318	62.52
6	781.25	0.3327±0.01274	59.19
7	390.63	0.3513±0.04163	63.95
8	195.31	0.3303±0.04045	58.57
9	97.66	0.3210±0.05724	56.19
10	48.83	0.3170±0.03381	55.16
11	Negative control (cell culture media)	0.1017±0.01901	
12	Positive control (20% DMSO)	0.3903±0.01159	

are supporting the results of current study as chloroform extract showed antibacterial activity against all of five experimental bacteria (Chodisetti *et al.,* 2013). *Gymnema sylvestre* was also evaluated for its antibacterial activity using disc-agar diffusion procedure and found that it has maximum antibacterial activity against *Serratia marcescens* MTCC 86 (Thalikunnil *et al.,* 2011). It also confirmed the antibacterial activity evaluated in current studies. A similar study was conducted on essential oils extracted from *Gymnema sylvestre* R.Br. leaves and found that it inhibits the growth of *Pseudomonas asplenii, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli* (Naik *et al.,* 2011). The results are in accordance with this study as *Escherichia coli* (common pathogen in both studies) were vulnerable to chloroformic and ethanolic extracts of *G. sylvestre* leaves.

The aqueous and methanolic extracts of *Gymnema sylvestre* R.Br. leaves likewise indicated moderate action against the three pathogenic Salmonella species (*S. typhi, S. typhimuriumand, S. paratyphi*). Out of the two extracts utilized, aqueous extract showed higher movement against the *Salmonella* species (Pasha *et al.,* 2009). This study supports the presence of antibacterial activity in aqueous extract (G. sylvestre).

The ethanolic extract of *G. sylvestre* leaves demonstrated wonderful antimicrobial activity against *B. pumilis, B. subtilis, P. aeruginosa* and *S. aureus* while have no activity against *Proteus vulgaris* and *Escherichia coli* (Satdive *et al*., 2003). While in current study *S. aureus* was vulnerable to hexane, chloroform and ethanolic extract while gave no response to aqueous extract. Whereas *E. coli* responded to chloroform and ethanolic extracts which contradicts the results conducted by (Satdive *et al.,* 2003). The aqueous and methanolic extracts of *G. sylvestre* leaves likewise indicated moderate action against the three pathogenic salmonella species (*Salmonella typhi, S. typhimurium* and *S. paratyphi*). Out

of these two extracts used, aqueous extract showed higher activity against the salmonella species (Pasha *et al.,* 2009).

The extracts prepared using successive solvent extraction techniques were evaluated for antimicrobial activity by Agar well diffusion method against *Streptococcus mitis, Streptococcus mutans, Staphylococcus aureus* and *Candida albicans* by using the doses of 25, 50 and 100 mg/ml. The methanol extract showed strong antimicrobial activity with the ZOI ranging from 12-23mm at 25mg/ml (Devi and Ramasubramaniaraja, 2010). The results of this study also confirm the presence of chemical constituents with antibacterial activity in *G. sylvestre* as *Staphylococcus aureus* was responsive in both the cases.

Two medicinal plants namely *Gymnema sylvestre* and *Morinda pubescens* var. pubescens were screened for potential antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*. The antibacterial activity was determined in petroleum ether, chloroform, acetone, methanol and aqueous extracts using disc diffusion method. The chloroform and methanol extract of leaf of *Gymnema sylvestre* showed highest inhibition against *Escherichia coli* and *Klebsiella pneumoniae* respectively. The results showed the most valuable information regarding antibacterial activity of *Gymnema sylvestre* leaves and also support the use of this plant in traditional medicinal system (Murugan *et al.,* 2012). Although Murugan and his coworkers used disc diffusion method instead of well diffusion method they obtained the results which are in accordance with current studies as bacteria were more vulnerable to chloroform extract while least to aqueous extract of *G. sylvestre*.

The antibacterial characteristics of *Gymnema sylvestre* leaf were investigated against five Gram negative (*E. coli, V. cholerae, P. aeruginosa, S. dysenteriae* and *S. flexneri*) three Gram positive (*Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus*) bacteria by using various solvents namely chloroform, petroleum ether and ethanol. The results showed that all the solvent extracts exhibited considerable activity against the bacteria under investigation. The antibacterial activity increased as the concentration of the extract was increased. No antibacterial activity was noted at 10 $mgml^{-1}$, $20mgml^{-1}$ concentrations (Sinha *et al.,* 2010). The results of research conducted by Sinha and co-researchers were in accordance with results of current studies as chloroform and ethanolic extracts (common solvents in both studies) showed considerable antibacterial activity against various gram positive and gram-negative microbes.

The antimicrobial activity of *Gymnema sylvestre* plant was investigated by using its methanolic extract. The gram positive and gram-negative organisms used in the study, showed susceptibility towards the extracts, with the root extracts at acidic pH, showing higher activity. *E. coli* and *E. cloacae* were found to be the most sensitive and *Pseudomonas aeruginosa,* the resistant type of microorganisms, based on the results obtained from the ZOI. The results support the broad spectrum activity of *Gymnema sylvestre* can be used in the development of new antimicrobial drugs (Bhuvaneswari *et al.,* 2011). Although solvents used in current study differ from this study but common factor is that in both bacteria were vulnerable to *G. sylvestre* extracts which confirm the presence of antibacterial activity in this plant.

Ethanolic, chloroform and ethyl acidic extracts of the aerial parts of *G. sylvestre* similarly showed antibacterial effects against *Proteus vulgaris, Escherichia coli, Pseudomonas aeroginosa, Klebsella pneumoniae* and *Staphylococcus aureus* (Paul and Jayapriya, 2009). The results of current study are in accordance with work done by Paul and Jayapriya as *Escherichia coli* and *Staphylococcus aureus* are responsive to antibacterial action of *G. sylvestre* in both the studies.

It is evident from the previous study, that ethanolic extract obtained from the leaves of *G. sylvestre* has strong antimicrobial activity against *S. aureus* and no activity against *E. coli* (Satdive *et al.,* 2003). By comparing with a study conducted by Naidu *et al.* (2013) it was seen that there was smaller zone of inhibition for *S. aureus* and *E. coli* which may be due to variations in chemical components which act as antibacterial agent.

Generally, antibiotic agents from plant sources appear to be more inhibitory to Gram-positive than Gram-negative micro-organisms. The antibacterial activity of *G. sylvestre* R.Br. leaf extracts could be due to the presence of alkaloids, flavonoids, steroids, phenols, tannins, saponins, and triterpenoids. These pharmacological active ingredients may either act alone or in combination to inhibit bacterial growth and appear to have the strong antibacterial activity.

Present study showed that strongest antibacterial activity was exhibited by chloroformic and ethanolic extracts followed by the hexane and least by aqueous extract. Chloroform and ethanol proved to be most effective solvents for the extraction of broad spectrum antimicrobial compounds from *G. sylvestre* R.Br.

The current study showed that liaison of zones of inhibition (ZOI) and minimum inhibitory concentration (MIC) values of crude extracts of *G. sylvestre* R.Br. leaves vary against different experimental bacteria. This liaison between inhibition zones and minimum inhibitory concentration value may or may not be related in crude extracts. The possible reason behind is that crude extracts have blend of phyto-constituents which may affect the diffusion power of active constituents but the direct liaison of size of zone of inhibition and minimum inhibitory concentration value is expected with pure compounds not with crude extracts.

Conclusions: Finally, it can be concluded that the *Gymnema sylvestre* R.Br. (leaves) cultivated in Pakistan has considerable antibacterial activity and safe to use *in vitro* conditions. *In vivo* evaluation of antibacterial activity of *Gymnema sylvestre* R.Br. is further required. Further the chemical constituents of *Gymnema sylvestre* plant must be characterized and purified; so that, these active constituents may be converted to a proper pharmaceutically stable dosage form and this miracle plant may be used therapeutically to prevent and cure various bacterial infections especially poultry infections.

Acknowledgments: We acknowledge the cooperation and financial support from Higher Education Commission of Pakistan under the Research Project Titled “Replacement

of low level feeding of antibiotics with plant extracts in poultry".

Authors contribution: MAR planned the study and supervised the research work, MT collected identified, authenticated the plant leaves and performed laboratory procedures, AAA provided microbes under study and supervised research work, QN helped in statistics, MA was principal investigator of research project funded by HEC, Pakistan, through which this research work was financially supported. MUA helped in sequential extraction. All authors wrote, revised and approved the manuscript.

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