Pak. J. Bot., 53(1): 197-202, 2021. DOI: http://dx.doi.org/10.30848/PJB2021-1(15)

IN VITRO BIOLOGICAL EVALUATION AND DNA DAMAGE PROTECTION ACTIVITIES OF *COTONEASTER AFGHANICUS* G.KLOTZ AND *TAMARIX ARCEUTHOIDES* BUNGE EXTRACTS

SHAZIA ANWER BUKHARI[1], GHULAM MUSTAFA[1*], SHAHZAD BASHIR[1], MUHAMMAD FARRUKH TAHIR[1] AND FOZIA ANJUM[2]

[1]Department Biochemistry, Government College University, Faisalabad-38060, Pakistan
[2]Department of Chemistry, Government College University, Faisalabad-38060, Pakistan
**Corresponding author's email: gmustafa_uaf@yahoo.com*

Abstract

Two medicinal plants i.e. *Cotoneaster afghanicus* G.Klotz and *Tamarix arceuthoides* Bunge were collected from Quetta and Ziarat valleys of Province Balochistan, Pakistan. These plants were selected because their medicinal and nutraceutical effects were not reported before. n-hexane and chloroform extracts of *C. afghanicus* and *T. arceuthoides* were subjected to find antibacterial, thrombolytic, biofilm inhibition and DNA damage protection activities. Extracts from both plants showed antibacterial activity by forming inhibition zones of different sizes. Strong antibacterial effect was observed against *Micrococcus* spp. (source: poultry farm dust samples) which showed some resistance against antibiotic erythromycin used as standard but n-hexane extract of *T. arceuthoides* showed the highest activity of 27.25 ± 0.37 mm among the extracts. Biofilms are the external matrix of proteins and polysaccharides secreted by bacteria. This matrix provides extra protection to bacteria and therefore, difficult to eradicate. One Gram-positive and one Gram–negative bacterial strain were used to test biofilm inhibition activity. Both n-hexane and chloroform extracts of *C. afghanicus* showed prominent biofilm inhibition activity against *S. aureus* with a % inhibition of 59.72 ± 1.98% and 62.03 ± 1.83%. *C. afghanicus* n-hexane extract exhibited considerable thrombolytic effect of 52.92% clot lysis. Compounds having good antioxidant activities showed DNA damage protection present in higher amounts in n-hexane extract of *T. arceuthoides.*

Key words: Antibacterial, Thrombolytic, Biofilm inhibition, *Tamarix arceuthoides, Cotoneaster afghanicus*.

Introduction

Natural products are a very useful resource to improve health care issues and to treat various diseases in different human communities (Mustafa *et al*., 2016). Plants have become popular because the risk of chronic disorders is lower in people consuming a diet possessing bioactive components (Zhang *et al*., 2015; Osuna-Martínez *et al*., 2014). In Pakistan and other developing countries, there is richness of medicinal plants, vegetables and fruits which have not yet been investigated properly to explore their health promoting benefits (Atta *et al*., 2017). Medicinal values like antimicrobial, antioxidant, thrombolytic, antidiabetic, anticancer, anti-inflammatory and cardiovascular protection of various plants have been explored during the last few decades (Huffman, 2003; Al-Snafi, 2016; Zia-ud-Den *et al*., 2019). Moreover, natural products have also been categorized as preventive agents to treat global health issues including diabetes mellitus (Alam *et al*., 2016) and Alzheimer's disease that is caused by accumulation of amyloid-β plaques and neurofibrillary tangles (Sajjad *et al*., 2018).

Present research work deals with such types of studies on *Cotoneaster afghanicus* and *Tamarix arceuthoides. C. afghanicus* belongs to family Rosaceae (the rose family) a medium sized family and worldwide has 95-125 genera and 2825-3500 species (Lingdi & Alexander, 2003). *C. afghanicus* is widely distributed in Afghanistan, India, Iran, Nepal, Pakistan and Southwestern China and has been used in traditional medicines due to bitter taste (Tamir *et al*., 2001). It is a perennial shrub with clump of long slender stems from the base (Klotz, 1978). Various species of *Cotoneaster* have been used for medicinal purposes such as antiviral, diuretic, cardiotonic and expectorant in several countries (Zengin *et al*., 2014; Bukhari *et al*., 2019). *T. arceuthoides* belongs to family Tamaricaceae a small flowering plant family of about 4 genera and 120 species (Trease & Evans, 2002). These plants are widely distributed in temperate regions of Asia, Africa and Europe and commonly found in sandy places and deserts. Most species of Tamarix are ornamental plants and the plants in this genus are mostly evergreen. Plant body is bushy or tree like with feathery leaves and has pink or white flowers (Mabberley, 2008). No literature on medicinal importance of *T. arceuthoides* has been found. However, preliminary phytochemical studies have been done on other species of *Tamarix*. Devhade, (2015) has reported that *Tamarix ericoides* is known for its medicinal properties as different classes of phytochemicals such as alkaloids, phytosterols, glycosides, saponins and tannins have been revealed in this species. The plant has been used to treat various diseases including diabetes, antidermatosis, paralysis, stomach disorders and painful urination.

From both these families a number of plants have been reported to have different medicinal effects like antibacterial, antifungal, antioxidant, thrombolytic, antidiabetic, anti-inflammatory and anticancer (Sharif *et al*., 2017; Bukhari *et al*., 2019; Han *et al*., 2012; Sokkar *et al*., 2013). Different methods have been employed to find compounds as potential drug candidates such as isolation and purification of active secondary metabolites from different natural sources including medicinal plants (Mustafa *et al*., 2017). Therefore, current study was designed to explore medicinal values of n-hexane and chloroform extracts of *C. afghanicus* and *T. arceuthoides*. Various activities including antibacterial, biofilm inhibition, thrombolytic and DNA damage protection assay were determined from *C. afghanicus* and *T. arceuthoides* extracts.

Materials and Methods

Collection of plant material: Plants chosen for research were *C. afghanicus* and *T. arceuthoides* which were collected from sandy rocks of Quetta and Ziarat valleys. These were identified by Dr. R.B. Tareen Professor and Chairman of Department of Botany, University of Baluchistan, Quetta, Pakistan.

Preparation of plants extracts: Whole plants with roots, shoots and leaves were taken, washed with cold water, dried under shade and ground to powdered forms. For extraction, 200 g of each powdered plant was taken in separate flasks along with measured volume of *n*-hexane. Incubated on orbital shaker for 7-8 days and filtered. Filtrate was concentrated at negative pressure evaporator at 45°C and residues were re-suspended to make chloroform extract by the same procedure.

Antibacterial activity: Test microbes: Pure bacterial cultures of *Escherichia coli* (source: water), *Bacillus subtilis* (source: soil sample), *Staphylococcus aureus* (source: skin infection) and *Pseudomonas* spp. (source: wound infection) were collected from Microbiology Department of Government College University Faisalabad (GCUF) Pakistan and pure cultures of *Streptococcus pyogenes* (source: open wound infection from dogs), *Staphylococcus aureus* (source: milk sample from mastitis cases of buffalo), *Escherichia coli* (source: milk sample from mastitis cases of buffalo) and *Micrococcus* spp. (source: poultry farm dust samples) were collected from Microbiology Department of University of Agriculture, Faisalabad (UAF) Pakistan. To ensure the pure cultures, all bacterial strains were streaked to obtain pure colony then each bacterial strain was cultured in nutrient broth (Oxoid, UK) at 37°C for further experiments.

Antibacterial assay by disc diffusion method: To determine the antibacterial activity of extracts, disc diffusion method was used as described by Mustafa *et al*., (2016) with some modifications. To agar plates, nutrient agar (Oxide, UK) 2.8 g/100 mL was dissolved in distilled water, mixed and sterilized by autoclaving at 121°C and 15 psi pressure and poured in Petri dishes homogeneously and removed air bubbles formed during agitation. After solidification of agar plates 100 µL of inoculum of each strain was spread on petri dishes. The dried plant extracts were dissolved in dimethyl sulfoxide (DMSO) and 100 µL of plants extracts were applied to 9 mm filter paper discs and placed by gently pressing on growth medium. The petri plates were then incubated at 37°C for 20-24 hours. DMSO used as negative control and ciprofloxacin as positive control. Inhibited bacterial growth formed clear zones called zones of inhibition which were measured in millimeters (mm).

Biofilm inhibition assay: *Staphylococcus aureus* and *Escherichia coli* were used to test the biofilm inhibition activity. A sterile plastic tissue culture 96-well plate with flat bottom was filled with 100 µL of nutrient broth (Oxoid, UK) and 100 µL each of n-hexane and chloroform extracts of both plants were inoculated with bacterial suspension of 20 µL into the wells separately. Bacteria and its nutrient broth without extracts in wells acted as control for both strains (10 µg/20 µL). The 96-well plate was covered and incubated at 37°C for 24 hours in a temperature controlled incubator in aerobic conditions. Ciprofloxacin was used as standard to compare plant extracts results. After incubation, each well was washed thrice with sterile phosphate buffer (220 µL to each well). To remove non-adherent bacteria from wells, the plate was shaken well. After washing, to fix the attached bacteria 99% methanol (220 µL per well) was added and left for 15 minutes. After that, methanol was discarded and left to dry. To stain, 220 µL of 2% crystal violet was added to each well for 5 minutes. To remove excess stain, plate was rinsed under running tap water and air dried at room temperature. The dye bound to adhered cells was re-solubilized with 220 µL of 33% (v/v) glacial acetic acid in each well. Using a micro-plate reader (BioTek, USA) optical density was measured for each well at 630 nm. All the test steps were repeated thrice. Bacterial growth inhibition percentage (INH%) was calculated by following formula:

$$\text{INH (\%)} = 1 - \frac{\text{OD}_{630\,(\text{sample})}}{\text{OD}_{630\,(\text{control})}} \times 100\%$$

Thrombolytic activity: Thrombolytic activity was assessed by method as described by Prasad *et al*., (2006). Briefly, 10 mL of blood taken from a male healthy volunteer (not having anticoagulant medicines from at least 3 months) and divided equally into six separate pre-weighted (W_1) eppendorf tubes and centrifuged for 5 minutes at 2500 rpm and incubated at 37°C for 45 minutes. Blood clotted on incubation and serum was removed carefully. Eppendorf tubes were weighted (W_2) again to gain clot weight (W_C). W_1 was subtracted from W_2. Then 100 µL of each extract and streptokinase with DMSO was added to the clot containing tubes. The tube containing streptokinase was marked as standard. All the test tubes were incubated at 37°C for 90 minutes and after removal of clot lysed, tubes were weighed again and weight loss (W_L) was calculated after clot lyses (thrombolysis). Average weight loss was calculated in percentage by formula of % of clot lysis:

$$*W_C = W_2 - W_1$$

$$\%\ \text{of clot lysis} = \frac{\text{wt. after clot lysis}}{\text{wt. before clot lycises}} \times 100\%$$

or

$$\%\ \text{of clot lysis} = \frac{\text{Wc} - \text{WL}}{\text{Wc}} \times 100\%$$

DNA damage protection assay: The method of Kalpana *et al*., (2009) was used with some modifications to evaluate DNA damage protection activity of extracts. In this method Calf Thymus DNA (CT DNA) was used. The CT DNA was diluted up to three-folds using 50 mM sodium phosphate buffer with pH of 7.4. About 3 µL of the diluted CT DNA was treated with 5 µL of test sample. Then 4 µL of the 30% H_2O_2 was added in the presence of plants extracts and made volume up to 15 µL by adding sodium phosphate buffer (pH 7.4). A solution of 3 µL of

CT DNA was added with 11 µL of sodium phosphate buffer (pH 7.4) used as negative control and 3 µL of CT DNA treated with 11 µL of the 30% H_2O_2 used as positive control. The relative differences in migration between the native and oxidized DNA was then examined on 1% agarose gel by horizontal DNA gel electrophoresis using a Bio-Rad wide mini system (Tech view, Singapore). The gel was documented by a Syngene model Gene Genius unit (Syngene, Cambridge, UK).

Results and Discussion

Antibacterial activity: To test the antibacterial activity for n-hexane and chloroform extracts of *C. afghanicus* and *T. arceuthoides,* disc diffusion method was executed against bacterial strains collected from two different institutes. Strains collected from GCUF were sensitive against erythromycin that's why ciprofloxacin was used instead of erythromycin. Zones of inhibition were measured in mm including disc size (9 mm). All samples could form inhibition zones of different sizes on agar plates, detail of inhibition zones formed by extracts and standard drugs used have been shown in Tables 1 and 2.

It was observed that *Staphylococcus aureus* (collected from GCUF) and *Micrococcus* spp. (collected from UAF) exhibited some resistance against ciprofloxacin and erythromycin, respectively. Against *S. aureus,* ciprofloxacin was able to form inhibition zone of 13.0 ± 0.29 mm whereas *C. afghanicus* extract of n-hexane showed inhibition zone of 15.75 ± 0.41 mm and 16.75 ± 0.33 mm for chloroform extract. n-hexane and chloroform extracts of *T. arceuthoides* showed zones of inhibition of 15.25 ± 0.32 mm and 14.25 ± 0.29 mm, respectively. Both plants extracts showed large inhibition zones than standard for *S. aureus* exhibiting that the phytochemicals present in these extracts were more active than that of ciprofloxacin (antibiotic used). Same trend of inhibition zones formation was observed against *Micrococcus* spp. but in this case only n-hexane extract (27.25 ± 0.37 mm) of *T. arceuthoides* and chloroform extract (26.5 ± 0.28 mm) of *C. afghanicus* showed good inhibition zones as compared to erythromycin which formed inhibition zone of 25.35 ± 0.26 mm. Abdelgawad, (2017) also found a significant antibacterial activity from butanol extract of *Tamarix nilotica*. The pure compounds isolated from butanol extract of *T. nilotica* such as flavone and dihydroflavonol showed significant antibacterial activity. Similarly, the methanolic extract of *Cotoneaster nummularioides* also exhibited a strong antibacterial activity against *Bacillus cereus* and showed inhibition zones of 12 mm for 400 mg/mL concentration (Sani & Yaghooti, 2016). It was proved by several studies that against bacterial pathogens, antibacterial effect of plant extracts is due to the presence of phenolic contents (Baydar *et al*., 2004; Vaquero *et al*., 2007). Plant extracts exhibited the antibacterial activity owing to the presence of phenolic contents. These phenolic contents are very important in nutraceuticals and processed foods as natural antiseptic agents to prolong the shelf life. Recent epidemiological studies have revealed that incidence of chronic diseases such as cancer and cardiovascular diseases can be reduced by polyphenols obtained from natural resources (Sharif *et al*., 2018).

Biofilm inhibition activity: Bacterial biofilm inhibition ability of different extracts of *T. arceuthoides* and *C. afghanicus* was evaluated and findings are reported in (Fig. 1.) Ciprofloxacin was used as standard having biofilm inhibition activity of 77.57 ± 2.37% and 82.65 ± 2.74% against *S. aureus* and *E. coli* respectively. Against *S. aureus*, the highest biofilm inhibition activity (62.03 ± 1.98% and 59.72 ± 1.83%) was observed by chloroform and n-hexane extracts of *C. afghanicus* whereas chloroform extract of *T. arceuthoides* showed inhibition activity of 45.53 ± 1.45% and n-hexane extract of *C. afghanicus* exhibited inhibition activity of 44.56 ± 1.35% against *E. coli* that was highest among all extracts. All extracts used were in crude form and showed distinguishable biofilm inhibition against *S. aureus* and *E. coli.* Overall results are shown in figure 1. Previously, it was found that the ethanol extract of *T. arceuthoides* showed maximum biofilm inhibition activity (i.e. 65.38 ± 0.26%) that was close to rifampicin used as standard (87.36 ± 0.24%). Fixed oil and essential oil showed 40.28 ± 0.23% and 37.74 ± 0.21% inhibition, respectively (Bukhari *et al*., 2016).

Table 1. Inhibition zones (mm) for strains collected from Department of Microbiology, GCUF.

Strains	*Tamarix arceuthoides* Inhibition zones (mm)		*Cotoneaster afghanicus* inhibition zones (mm)		Standard ciprofloxacin
	n-hexane	Chloroform	*n*-hexane	Chloroform	
B. subtilis	15.75 ± 0.37	16.50 ± 0.39	18.0 ± 0.29	15.25 ± 0.37	41.25 ± 0.38
S. aureus	15.25 ± 0.32	14.25 ± 0.29	15.75 ± 0.41	16.75 ± 0.33	13.0 ± 0.29
E. coli	19.0 ± 0.31	20.5 ± 0.35	18.0 ± 0.27	16.25 ± 0.34	35.75 ± 0.43
Pseudomonas spp.	15.65 ± 0.22	14.65 ± 0.38	17.0 ± 0.27	16.35 ± 0.39	35.50 ± 0.38

Mean ± Standard deviation

Table 2. Inhibition zones (mm) for strains collected from Institute of Microbiology, UAF.

Strains	*Tamarix arceuthoides*		*Cotoneaster afghanicus*		Standard erythromycin
	n-hexane	Chloroform	*n*-hexane	Chloroform	
Streptococcus pyogenes	14.75 ± 0.24	15.25 ± 0.21	15.25 ± 0.19	14.5 ± 0.23	22.75 ± 0.28
Staphylococcus aureus	14.75 ± 0.28	19.5 ± 0.22	20.25 ± 0.26	19.0 ± 0.24	30.25 ± 0.231
Escherichia coli	14.25 ± 0.24	12.75 ± 0.21	21.5 ± 0.35	12.25 ± 0.25	34.75 ± 0.34
Micrococcus spp.	27.25 ± 0.37	11.5 ± 0.16	19.5 ± 0.21	26.5 ± 0.28	25.35 ± 0.26

Mean ± Standard deviation

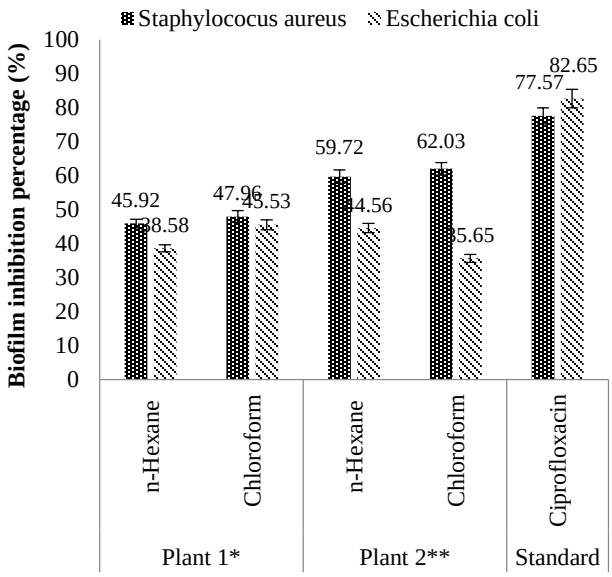

Fig. 1. Biofilm inhibition assay. Plant 1*: *Tamarix arceuthoides*, Plant 2**: *Cotoneaster afghanicus.*

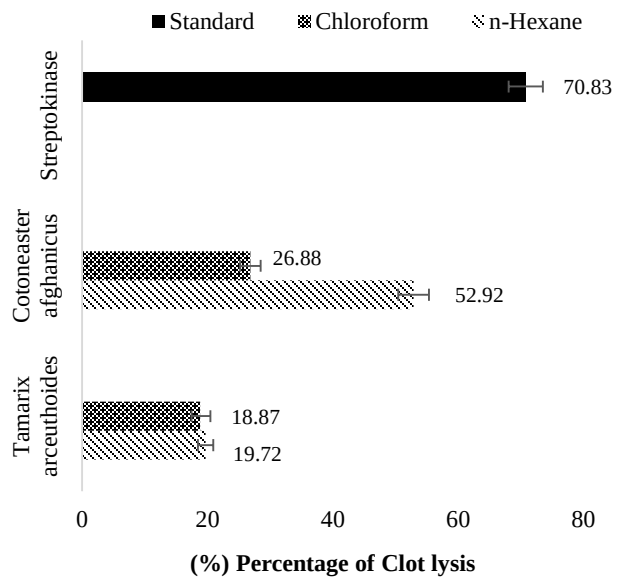

Fig. 2. Thrombolytic activity of *C. afghanicus* and *T. arceuthoides.*

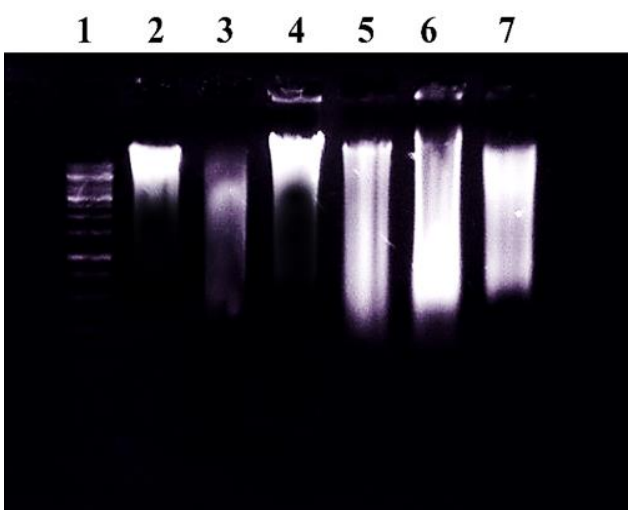

Fig. 3. H_2O_2 induced Calf-Thymus DNA damage protection caused by extracts of *C. afghanicus* and *T. arceuthoides*.
Lane 1: 1 kb DNA ladder; Lane 2: CT-DNA without treatment; Lane 3: CT-DNA treated with H_2O_2 (damaged); Lane 4: CT-DNA treated with *T. arceuthoides* n-hexane extract + H_2O_2; Lane 5: CT-DNA treated with *T. arceuthoides* chloroform extract + H_2O_2; Lane 6: CT-DNA treated with *C. afghanicus* n-hexane extract + H_2O_2; Lane 7: CT DNA treated with *C. afghanicus* chloroform extract + H_2O_2.

In the current panorama of antibiotics, bacteria are becoming resistant so it is the need of time to find new and effective compounds. In future, the compounds present in these crude extracts would be purified and used as antibacterial agents. Bacteria can enclose themselves by secreting a slimy matrix of proteins and polysaccharides known as biofilm. Biofilms are formed by the adhesion of bacteria to damaged tissues or other surfaces. Antibiotic resistance in bacterial biofilms makes difficult to remove them. Resistance could be achieved by different mechanisms and transferred to individual cells (Høiby *et al*., 2010; Awolola, 2014).

Thrombolytic activity: Results for clot lysis by plants extracts are shown in (Fig. 2.) Streptokinase showed the highest clot lysis (70.81 ± 0.84%) that was used as a positive control. Maximum clot lysis among the extracts was observed for *n*-hexane extract of *C. afghanicus* (52.92%) and minimum observed for chloroform extract of *T. arceuthoides* (18.87%). Keeping in view the weights of the clot before and after lysis were considered as average to calculate the clot lysis percentage. In this study, the slight thrombolytic activity of chloroform extract of *T. arceuthoides* is an important finding that could be a significant implication in cardiovascular health (Hussain *et al*., 2014). The formation of blood clot has been reported as critical event because of damaged spans of endothelial cell surface or blockage of blood vessels due to deposition of fibrin, platelets or tissue factor (Ghosh *et al*., 2015). Previously, the phytochemicals from fixed oil and essential oil of *T. arceuthoides* showed more thrombolytic activities as compared to ethanol extract. Fixed oil showed 31.45% and essential oil showed 37.73% clot lysis whereas ethanol extract showed 5.48% only (Bukhari *et al*., 2016). The finding of this study indicates the possibility that novel thrombolytic agents could be developed from extracts of studied medicinal plants.

DNA damage protection assay: Results for DNA damage protection assay are illustrated in (Fig. 3.) The figure showing the DNA protection activities against H_2O_2 induced damaged CT-DNA of n-hexane and chloroform extracts of *T. arceuthoides* and *C. afghanicus*. The damaged DNA showed a smear shape as shown in lanes 3, 5, 6 and 7. In contrast, the protected or undamaged DNA showed a band formation exhibited in lane 2 and 4. CT-DNA from lane 2 was protected because it was not treated with H_2O_2 acted as positive control and CT-DNA from lane 3 was damaged by oxidative stress of H_2O_2 acted as negative control. n-hexane extract of *T. arceuthoides* from lane 4 showed significant DNA damage protection against oxidative stress of H_2O_2. Other extracts of both plants

showed some protection when compared with lane 3 but they were not able to show notable DNA damage protection corresponding to the presence of antioxidant agents. Bukhari *et al*., (2016) reported that essential oil from *T. arceuthoides* exhibited almost complete protection to Calf Thymus DNA against H_2O_2 damage while fixed oil and ethanol extract showed less protection. The presence of flavonoids and phenolic compounds such as trycin and quercetin present in plant extracts have been attributed to DNA protection. These compounds could be involved in preventing the production of reactive oxygen species (ROS) through complexing cations including iron and copper that take part in the formation of hydroxyl radicals (Golla & Bhimathati, 2014).

In conclusion, this is the first study in which antibacterial activity, thrombolytic activity, biofilm inhibition activity and DNA damage protection have been explored from n-hexane and chloroform extracts of *Cotoneaster afghanicus* G.Klotz and *Tamarix arceuthoides* Bunge. The extracts of both medicinal plants exhibited antioxidant potentials and showed significant antibacterial, thrombolytic and biofilm inhibition activities. Only *T. arceuthoides* n-hexane extract among all the extracts exhibited DNA damage protection against H_2O_2 oxidative stress. Bioactive agents from both plants extracts could be extracted and used as medicinal and preservative agents in future.

Acknowledgement

Authors are very gratified to Dr. Rasool Bukhsh Tareen, Dr. Muhammad Hidayat Rasool and Dr. Sajjad-ur-Rahman for providing plant sample and bacterial strains.

References

Abdelgawad, A.A. 2017. *Tamarix nilotica* (Ehrenb) Bunge: a review of phytochemistry and pharmacology. *J. Microb. Biochem. Technol*., 1: 544-553.

Alam, F., M.A. Islam, M.A. Kamal and S.H. Gan. 2016. Updates on managing type 2 diabetes mellitus with natural products: towards antidiabetic drug development. *Curr. Med. Chem*., 23: 1-37.

Al-Snafi, A.E. 2016. Chemical constituents and pharmacological effects of *Citrullus colocynthis*-A review. *IOSR J. Pharm*., 6: 57-67.

Atta, A., G. Mustafa, M.A. Sheikh, M. Shahid and H. Xiao. 2017. The biochemical significances of the proximate, mineral and phytochemical composition of selected vegetables from Pakistan. *Mat. Sci. Pharm*., 1: 6-9.

Awolola, G.V., N.A. Koorbanally, H. Chenia, F.O. Shode and H. Baijnath. 2014. Antibacterial and anti-biofilm activity of flavonoids and triterpenes isolated from the extracts of *Ficus sansibarica* Warb. subsp. *sansibarica* (Moraceae) extracts. *Afr. J. Tradit. Complement Altern. Med*., 11: 124-131.

Baydar, H., O. Sağdiç, G. Özkan and T. Karadoğan. 2004. Antibacterial activity and composition of essential oils from *Origanum, Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Control*., 15: 169-172.

Bukhari, S.A, M. Qasim, M.S. Masoud, Mahmood-ur-Rahman, H. Anwar, A. Waqas and G. Mustafa. 2019. Evaluation of medicinally important constituents of *Cotoneaster afghanicus* G.Klotz collected from Baluchistan region of Pakistan. *Ind. J. Pharm. Sci*., 81: 259-265.

Bukhari, S.A., A. Waqas, N. Rasool, A.I. Hussain, R.B Tareen, M. Zia-Ul-Haq, M. Moga, L. Dima and D.G. Festila. 2016. Biological studies and GC/MS analysis of *Tamarix arceuthoides*. *Oxid. Commun*., 39: 2999-3011.

Bukhari, S.A., N. Farah, G. Mustafa, S. Mahmood and S.A.R. Naqvi. 2019. Magneto-priming improved nutraceutical potential and antimicrobial activity of *Momordica charantia* L. without affecting nutritive value. *Appl. Biochem. Biotechnol*., 188: 878-892.

Devhade, J.B. 2015. Preliminary phytochemical investigations and medicinal properties of *Tamarix ericoides* Rottl. *Pharm. Innov. J*., 4: 24-26.

Ghosh, A., S. Banik and M.A. Islam. 2015. *In vitro* thrombolytic, anthelmintic, anti-oxidant and cytotoxic activity with phytochemical screening of methanolic extract of *Xanthium indicum* leaves. *Bangladesh J. Pharmacol*., 10: 854-859.

Golla, U. and S.S.R. Bhimathati. 2014. Evaluation of antioxidant and DNA damage protection activity of the hydroalcoholic extract of *Desmostachya bipinnata* L. Stapf. *Sci. World J*., Article ID 215084.

Han, H., Y. Gu, C. Ye, Y. Cao, Z. Liu and J. Yin. 2012. Antithrombotic activity of fractions and components obtained from raspberry leaves (*Rubus chingii*). *Food Chem*., 132: 181-185.

Høiby, N., T. Bjarnsholt, M. Givskov, S. Molin and O. Ciofu. 2010. Antibiotic resistance of bacterial biofilms. *Int. J. Antimicrob. Agents*., 35: 322-332.

Huffman, M.A. 2003. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *Proc. Nutr. Soc*., 62: 371-381.

Hussain, F., M.A. Islam, L. Bulbul, M.M.R. Moghal and M.S. Hossain. 2014. *In vitro* thrombolytic potential of root extracts of four medicinal plants available in Bangladesh. *Anc. Sci. Life*., 33: 162-171.

Kalpana, K., M. Srinivasan and V.P. Menon. 2009. Evaluation of antioxidant activity of hesperidin and its protective effect on H_2O_2 induced oxidative damage on pBR322 DNA and RBC cellular membrane. *Mol. Cell Biochem*., 323: 21-29.

Klotz, G. 1978. Neue oder kritische Cotoneaster-Arten: 8. Wissenschaft. Zeitschr. Friedrich-Schiller-Univ., *Math.-Naturwiss.* 27: 19-26.

Lingdi, L. and C. Alexander. 2003. Spiraea. *Flora of China.*, 9: 47-73.

Mabberley, D.J. 2008. Mabberley's plant-book: A portable dictionary of plants, their classifications, and uses: Cambridge University Press.

Mustafa, G., R. Arif, A. Atta, S. Sharif and A. Jamil. 2017. Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan. *Mat. Sci. Pharm*., 1: 17-26.

Mustafa, G., S. Ahmed, N. Ahmed and A. Jamil. 2016. Phytochemical and antibacterial activity of some unexplored medicinal plants of Cholistan desert. *Pak. J. Bot*., 48: 2057-2062.

Osuna-Martínez, U., J. Reyes-Esparza and L. Rodríguez-Fragoso. 2014. Cactus (*Opuntia ficus-indica*): a review on its antioxidants properties and potential pharmacological use in chronic diseases. *Nat. Prod. Chem. Res*., 2: 1.

Prasad, S., R.S. Kashyap, J.Y. Deopujari, H.J. Purohit, G.M. Taori and H.F. Daginawala. 2006. Development of an *in vitro* model to study clot lysis activity of thrombolytic drugs. *Thromb. J*., 4: 14.

Sajjad, R., R. Arif, A.A. Shah, I. Manzoor and G. Mustafa. 2018. Pathogenesis of Alzheimer's disease: role of amyloid-β and hyperphosphorylated Tau protein. *Indian J. Pharm. Sci*., 80: 581-591.

Sani, M.A. and F. Yaghooti. 2016. Antibacterial effects and chemical composition of essential oil from *Cotoneaster nummularioides* leaves extract on typical food-borne pathogens. *J. Essent. Oil Bear. Pl*., 19: 290-296.

Sharif, S., M. Shahid, A. Atta, M. Abbas and G. Mustafa. 2017. Comparative evaluation of antioxidant potentials of selected medicinal plants native to Pakistan. *Oxid. Commun*., 40: 657-674.

Sharif, S., S. Rashid, A. Atta, A. Irshad, M. Riaz, M. Shahid and G. Mustafa. 2018. Phenolics, tocopherols and fatty acid profiling of wild and commercial mushrooms from Pakistan. *J. Biol. Regul. Homeost. Agents*., 32: 863-867.

Sokkar, N., O. El-Gindi, S. Sayed, S. Mohamed, Z. Ali and I. Alfishawy. 2013. Antioxidant, anticancer and hepatoprotective activities of *Cotoneaster horizontalis* Decne extract as well as α-tocopherol and amygdalin production from in vitro culture. *Acta Physiol. Plant*., 35: 2421-2428.

Tamir, S., M. Eizenberg, D. Somjen, S. Izrael and J. Vaya. 2001. Estrogen-like activity of glabrene and other constituents isolated from licorice root. *J. Steroid. Biochem. Mol. Biol*., 78: 291-298.

Trease, G. and W. Evans. 2002. Pharmacognosy, Saunders. Elsevier, Amsterdam, The Netherlands. 36: 51.

Vaquero, M.R., M. Alberto and M.M. de Nadra. 2007. Antibacterial effect of phenolic compounds from different wines. *Food Control*., 18: 93-101.

Zengin, G., A. Uysal, E. Gunes and A. Aktumsek. 2014. Survey of phytochemical composition and biological effects of three extracts from a wild plant (*Cotoneaster nummularia* Fisch. et Mey.): a potential source for functional food ingredients and drug formulations. *PLoS One*., 9: p.e113527.

Zhang, Y.J., R.Y. Gan, S. Li, Y. Zhou, A.N. Li, D.P. Xu and H.B. Li. 2015. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*., 20: 21138-21156.

Zia-ud-Den, N., G. Mustafa, S. A. Bukhari, F. Anjum, M. Qasim and M. Shahid. 2019. Enhancement of nutraceutical and antioxidant potential of sunflower hybrid seed varieties through chemical priming. *Pak. J. Pharm. Sci*., 32: 1901-1907.

(Received for publication 17 January 2019)