A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2013; 8: 336-342**
Journal homepage: www.banglajol.info; www.bdjpharmacol.com
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-007X (Print); 1991-0088 (Online); DOI: 10.3329/bjp.v8i3.13888

# Antidiabetic and antimicrobial activities of fractions and compounds isolated from *Berberis brevissima* Jafri and *Berberis parkeriana* Schneid

**Saqib Ali[1], John Igoli[2], Carol Clements[2], Dima Semaan[2], Muhammad Alamzeb[1], Mamoon-Ur-Rashid[1], Syed Qaisar Shah[3], Valerie A. Ferro[2], Alexander I. Gray[2] and M. Rafiullah Khan[1]**

*[1]Phytopharmaceutical and Nutraceutical Research Laboratories (PNRL), Institute of Chemical Sciences, University of Peshawar, Peshawar 25120, Pakistan: [2]Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, 161 Cathedral Street Glasgow, G4 0RE, Scotland, UK; [3]Nuclear and Molecular Research Laboratories, Institute of Chemical Sciences, University of Peshawar, Peshawar 25120, Pakistan.*

## Article Info

Received: 21 February 2013
Accepted: 16 July 2013
Available Online: 1 August 2013

**Keywords:**
Antidiabetic
Antimicrobial
*Berberis brevissima*
*Berberis parkeriana*

Number of Figures: 2
Number of Tables: 2
Number of Refs: 27

**Correspondence:** MRK
e-mail: rafikhan43@hotmail.com

## Abstract

Methanol extracts of the roots of *B. brevissima* and *B. parkeriana* were examined for antidiabetic and antimicrobial activities. Six compounds i.e berberine, 8-oxo-berberine, dehydrocheilanthifoline, columbamine, jatrorrhizine and glutamic acid were isolated from the active fractions of these species for the first time. The antidiabetic activity was determined against Protein Tyrosine Phosphatase 1B (PTP 1B), a negative insulin regulator while the antimicrobial activity was performed against four bacterial strains: *Mycobacterium marinum* ATCC BAA535, sporadic Methicillin Resistant *Staphylococcus aureus* (*SMRSA*), endemic Methicillin Resistant *Staphylococcus aureus* (*EMRSA*) and *Escherichia coli* ATCC.8739 at different concentrations. Amongst the isolated compounds 8-oxo-berberine was found to be the most active antidiabetic (29 % of the positive control) while jatrorrhizine was the most active and selective antimicrobial against *SMRSA* and *EMRSA* (-1.2 % of the DMSO (D) control).

## Introduction

According to the International Diabetes Federation about 366 million people around the world had diabetes in 2011 and the number was predicted to double by the year 2030 (IDF, 2013). The high risk of diabetes and health-related complications has led to research for active antidiabetic compounds from plants (Maqsood et al., 2009).

Berberidaceae is a well-known family for a number of medicinal uses and is incorporated in the British and the Indian pharmacopeias (Meliani et al., 2011; British Pharmacopeia, 2011). Antibacterial, anticancer, anticoagulant, antifatigue, antipyretic, hypoglycemic and hypo-tensive activities have been reported for the crude solvent extracts and isolated compounds of various *Berberis* species (Freile et al., 2003). Root extracts of *Berberis aristata* form a bitter tonic used as a laxative and antiseptic (Singh et al., 2007). Some *Berberis* species are reported to have anticancer activity in human epidermal cells (Nicasio et al., 2011). An ethanolic extract of the roots of *Berberis lycium* has been reported to possess antidiabetic activity (Uniyal and Tewari, 1991). *Berberis* spp. possess berberine as a major constituent along with other principle isoquinoline alkaloids e.g. berbamine, jatrorrhizine and palmatine (Freile et al., 2003).

Berberine, has shown significant antibacterial and antifungal activities against *Staphylococcus aureus* and *Candida* spp (Aydemir and Biloglu, 2003). The root extracts of *B. aristata*, *B. asiatica* and *B. lycium* have also been reported to show good antifungal activity against

*Aspergillus flavus* and *A. terreus* (WHO, 2009).

Keeping in view the activities of the genus *Berberis*, we have selected *Berberis brevissima* Jafri and *Berberis parkeriana* Schneid to investigate their antidiabetic and antimicrobial activities. In the current study we have isolated six compounds and are reporting the activities of their fractions and the isolated compounds.

## Materials and Methods

*General:* Compounds were isolated from fractions of crude extracts using Silica gel 60 (0.063-0.200 mm, Merck, Germany) for column chromatography (CC) and silica gel 60 $PF_{254}$ for preparative thin layer chromatography (TLC). Characterization of compounds was achieved using NMR instruments (Bruker AVANCE 500 and 400 MHz, Germany) using DMSO-$d_6$ as solvent. The mass spectra (EIMS) were recorded on a JEOL MSRoute through direct insertion probe. All the chemicals used were from Sigma International.

*Plant Material: Berberis brevissima* Jafri and *Berberis parkeriana* roots (3 kg each) were collected from the north west of Pakistan, July 2009. The voucher specimens (No. Bot/10701 and 8719 respectively) were deposited in the herbarium of the Botany Department, University of Peshawar.

*Extraction and isolation:* The scheme described in **Figure 1** was used for the extraction and fractionation of the plant material.

*Fraction A (BRA and PRA):* These fractions were recrysta -lized to give berberine **(1)** (2 g), mp 208-209°C (lit 208-210°C) (Suau et al., 1998).

*Fraction C (BRC and PRC):* The BRC fraction was subject -ted to CC using silica gel (210 g) and eluted with $CHCl_3$-MeOH with increasing polarity. The subfractions obtained were further subjected to preparative chromatography (PC) using $CHCl_3$-MeOH-$NH_3$ (80:20:0.5 and 75:25:0.5) to give 8-oxo-berberine **(2)** (9.1 mg) and dehydrocheilanthifoline **(3)** (7.9 mg) respectively. Fraction PRC was subjected to CC on silica gel (200 g) eluted with $CHCl_3$-MeOH (20:80) followed by PC using $CHCl_3$-MeOH-$NH_3$ (10:90:0.5 and 75:25:0.5) to give columbamine **(4)** (11.8 mg) and dehydrocheilanthifoline **(3)** (5.3 mg).

*Fraction D (BRD and PRD):* The BRD fraction (15.0 mg) was subjected to CC on silica gel (210 g) and eluted with $CHCl_3$-MeOH with increasing polarity. The four fractions obtained were further separated on PC which afforded jatrorrhizine **(5)** (9.7 mg) and glutamic acid **(6)** (5.8 mg). Similarly the fraction PRD (15.0 mg) was subjected to CC on silica gel (210 g) and eluted with $CHCl_3$-MeOH, which gave jatrorrhizine **(5)** (2.3 mg).

Antidiabetic assay

*Buffer preparation:* The buffer was composed of: 2.975 g of HEPES (H3375) (25 mM), 1.461 g of sodium chloride (S9625) (50 mM), 0.32 g of dithiothreitol (D5545) (2 mM), 0.365 g of ethylene diamine tetra acetic acid (E1644) (EDTA, 2.5 mM) and 5 mg of bovine serum albumin (A2153) (BSA 0.01 mg/mL) were dissolved in 500 mL of distilled water and the pH was adjusted to 7.2. All chemicals were purchased from Sigma Aldrich, UK.

*Sample preparation:* The concentration of stock solutions for all the samples was 10 mg/mL. Samples were screen -ed at 100 µg/mL by taking 2 µL of each sample and adding it to 98 µL buffer.

*Enzyme preparation:* The enzyme Protein Tyrosine Phosphatase 1B (PTP 1B, purchased from Invitrogen, Life Technologies, UK) was prepared by adding 100 µL to 25 mL of the buffer, then aliquoted into 250 µL lots (sufficient for 1 plate) which were then stored at -80°C until used.

*Substrate preparation:* 6,8-Difluoro-4-methylumbelliferyl phosphate (DiFMUP) (Invitrogen D6567, Molecular Probes, Life Technologies, UK) was used as the substrate, which was dissolved in DMSO. Seven µL of the stock (10 mM) was added to 1.75 mL of buffer and stored at -20°C until used.

*Inhibitor preparation:* Bis(4-trifluoromethylsulfonamidophenyl)-1,4-diisopropylbenzine (TFMS, Calbiochem 540211, 10 mg, Millipore, UK) inhibitor was dissolved in DMSO (10 mg in 1.64 mL) and then a displacement curve was drawn for the TFMS (concentration range 0.0003-3 µM).

*Assay:* In a 96-well plate (Costar®, Sigma Aldrich, UK), 10 µL of standard (TFMS) or samples were added to the plate, then 20 µL of PTP1B enzyme was added to each well and incubated for 30 min at 37°C in 5%$CO_2$ in air. After incubation, 10 µL of the substrate was added to the wells followed by incubation for 10 min. The plate was read at 355 nm/460 nm wavelengths using Wallac Victor².

Antimicrobial assay

The extracts and compounds were tested against *Mycobacterium marinum* ATTC.BAA535 an indicator species for potential activity against *Mycobacterium tuberculosis;* 2 antibiotic resistant clinical isolates of *Staphylococcus aureus:* SMRSA 106 and EMRSA 16 and *Escherichia coli* ATTC.8739. The activity of the fractions and compounds was assessed using a well established Alamar blue96™ well microplate assays. Concentrations of 100, 200 and 500 µg/mL of the extracts and pure compounds were assessed.

Ten mg/mL of sample was dissolved in DMSO to prepare the stock solution and then diluted to a concentration of 1 mg/mL using cation adjusted Muller Hinton Broth (MHB). Samples with final test concentrations of 100, 200 and 500 µg/mL were prepared from

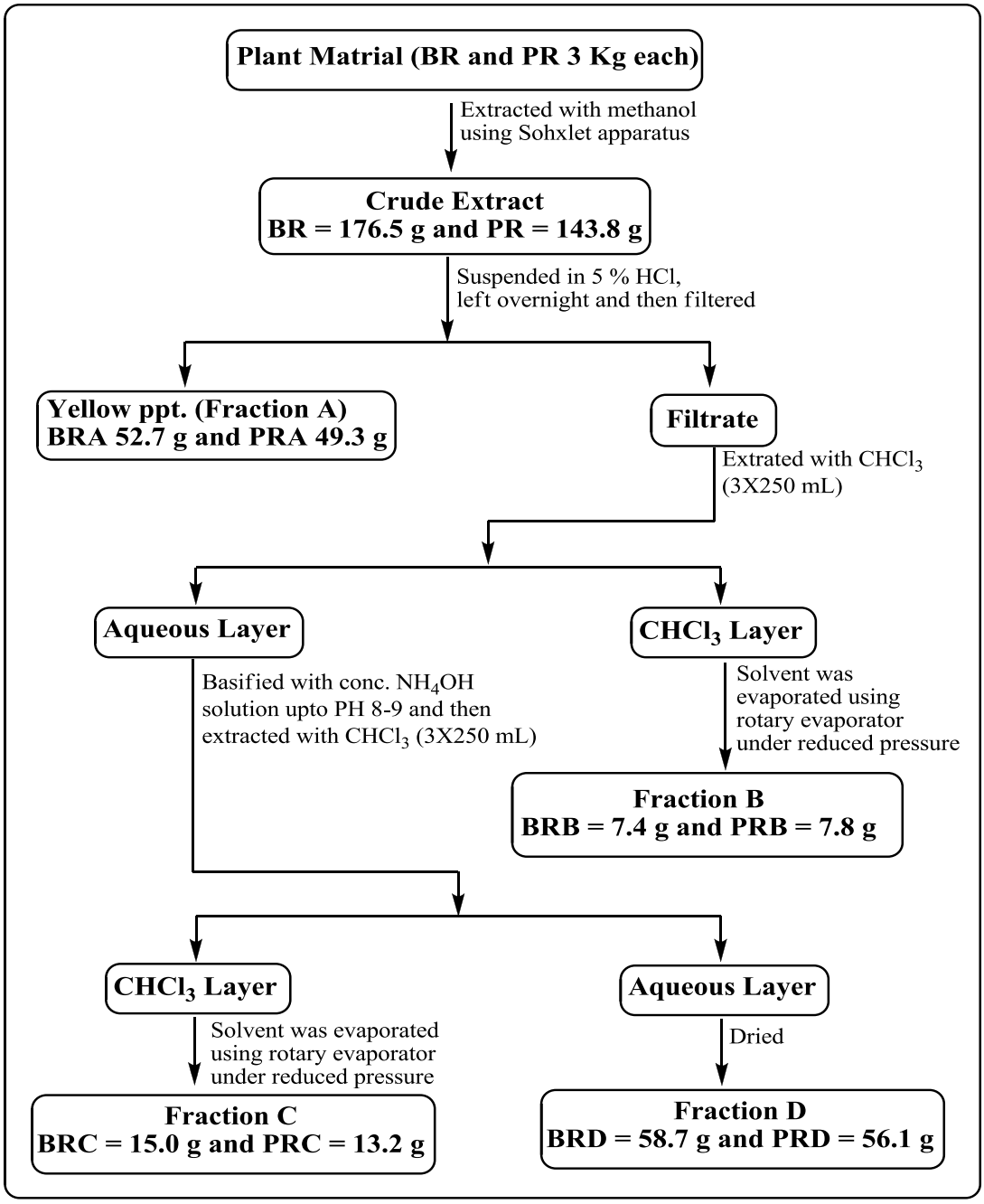


**Figure 1:** Fractionation scheme for *Berberis brevissima* and *Berberis parkeriana*
BR = *B. brevissima* roots, PR = *B. parkeriana* roots, BRA = *B. brevissima* roots fraction A, PRA = *B. parkeriana* roots fraction A, BRB = *B. brevissima* roots fraction B, PRB = *B. parkeriana* roots fraction B, BRC = *B. brevissima* roots fraction C, PRC = *B. parkeriana* roots fraction C, BRD = *B. brevissima* roots fraction D, PRD = *B. parkeriana* roots fraction D

the stock solutions of test samples.

The plate was arranged with DMSO as a negative control in column 1, samples in columns 2-11 and a positive control (Gentamicin) in column 12. The microbes used were diluted up to $3x10^4$ microbes/mL. The bacterial suspensions were adjusted to the same optical density as a 0.5 McFarland standard, then diluted 1 in 10 for *M. marinum* and 1 in 100 for EMRSA, SMRSA and *E. coli*. A 100 µL suspension of the diluted microbes was added to all wells of the 96 well microtitre plate with the exception of well 1 the sterility control. Alamar blue was added to all wells of the MRSA, EMRSA and *E. coli* plates to give a final concentration of 10% (v/v) and a total well volume of 200 µL. The microplates were sealed with parafilm and incubated at 37°C for 24 h.

Thereafter the levels of flouresence were detected using a Perkin Elmer Victor 2 microplate reader in fluorescence mode (excitation 560 nm, emission 590 nm). The

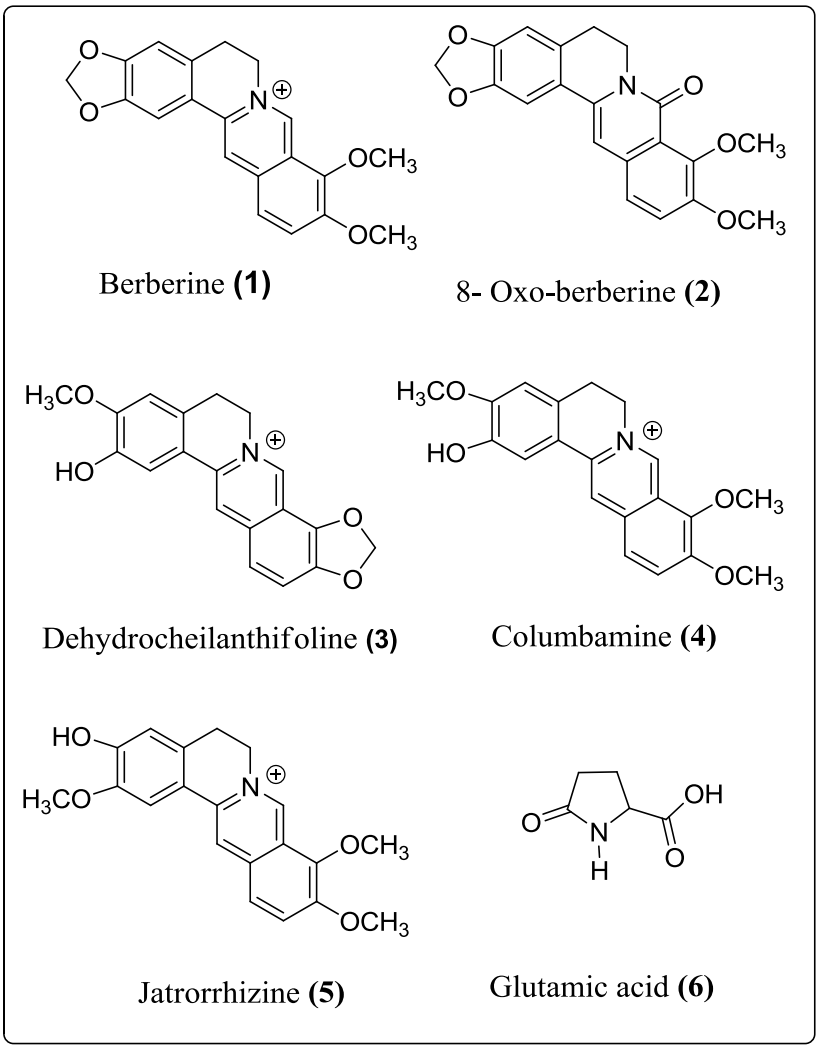


**Figure 2:** Structures of compounds **1-6** isolated from the fractions of *B. brevissima* and *B. parkeriana*

*M. marinum* plates were incubated at 31°C for 5 days prior to addition of Alamar blue to give a final concentration of 10% (v/v). After a further 2 h incubation the fluorescence was determined as previously described. The percentage of the DMSO control values were recorded as % D control.

## Results and Discussion

A total of six compounds were isolated for the first time from the different fractions of *B. brevissima* and *B. parkeriana*. The structures of the compounds were established using spectroscopic techniques (UV, FT-IR, $^{1}$H NMR, $^{13}$C NMR, COSY, HMBC, HSQC, NOESY and mass spectrometry). Comparison with data reported in the literature confirmed the compounds to be berberine **(1)** (Hsieh et al., 2004), 8-oxo-berberine **(2)** (Fajardo et al., 1982), dehydrocheilanthifoline **(3)** (Cooper et al., 1972), columbamine **(4)** (Hsieh et al., 2004), jatrorrhizine **(5)** (Hsieh et al., 2004) and glutamic acid **(6)** (Tanaka et al., 2007). Structures **1-6** are given in **Figure 2**.

*Antidiabetic activities of fractions obtained from B. brevissima and B. parkeriana:* The fractions obtained were tested against PTP 1B (a negative insulin regulator). Samples that showed 40% of the control (i.e. 60% inhibition) were considered to be potentially active. Preliminary screening showed that fraction B (BRB = 66% and PRB = 59%) and D (BRD = 48% and PRD = 51%) were inactive against PTP 1B. The BRC fraction showed 32% activity of the control while PRC showed 37% of the control (100%).

*Antidiabetic activities of pure compounds isolated from the two Berberis spp.:* These compounds were tested for antidiabetic activity against PTP 1B. Amongst the compounds 8-oxo-berberine (**2**) was significantly active and showed 29% of the control. The other isolated compounds i.e berberine (**1**) (35%), dehydrocheilanthifoline (**3**) (38%), columbamine (**4**) (33%) and jatrorrhizine (**5**)

**Table I: Antimicrobial activities of fractions from *B. brevissima* and *B. parkeriana***

| Fractions | M. marinum % of D control 100 µg/mL | SMRSA % of D control 200 µg/mL | EMRSA % of D control 200 µg/mL | E. coli % of D control 500 µg/mL |
|---|---|---|---|---|
| BRB | 33.5 | 61.7 | 88.6 | 62.0 |
| BRC | 2.5 | 0.2 | 0.2 | 45.9 |
| BRD | 2.4 | 82.6 | 81.6 | 64.9 |
| PRB | 48.0 | 56.0 | 92.3 | 62.9 |
| PRC | 12.7 | -1.1 | -1.1 | 43.3 |
| PRD | 17.6 | 1.8 | -0.9 | 68.3 |

BRB = *B. brevissima* roots fraction B, BRC = *B. brevissima* roots fraction C, BRD = *B. brevissima* roots fraction D, PRB = *B. parkeriana* roots fraction B, PRC = *B. parkeriana* roots fraction C, PRD = *B. parkeriana* roots fraction D

**Table II: Antimicrobial activities of compounds isolated from *B. brevissima* and *B. parkeriana***

| Compounds | M. marinum % of D control 100 µg/mL | SMRSA % of D control 200 µg/mL | EMRSA % of D control 200 µg/mL | E. coli % of D control 500 µg/mL |
|---|---|---|---|---|
| Berberine (1) | 47.4 | 1.1 | 0.5 | 57.8 |
| 8-Oxo-berberine (2) | 1.6 | 18.9 | 32.6 | 56.3 |
| Dehydrocheilanthifoline (3) | 26.4 | 0.1 | -0.8 | 44.1 |
| Columbamine (4) | 45.9 | -0.6 | -0.9 | 51.7 |
| Jatrorrhizine (5) | 18.8 | -1.2 | -1.2 | 39.4 |
| Glutamic acid (6) | 0.9 | 35.7 | 47.8 | 49.3 |

(36%) also showed good activity. Glutamic acid (**6**) (78%) was inactive.

*Antimicrobial activity of B. brevissima and B. parkeriana fractions:* The fractions of the two species were also tested against four microbial strains i.e *M. marinum*, *MRSA*, *EMRSA* and *E. coli*. Various concentrations were used against different strains. The concentration tested against *M. marinum* was 100 µg/mL, *SMRSA* and *EMRSA* was 200 µg/mL and *E. coli* was 500 µg/mL. Fraction A consisted of mainly berberine (**1**), a well-known isoquinoline alkaloid, therefore we did not test it against these strains. In preliminary screening, the fractions whose %D control was less than 5 were considered to be active. Fraction B (BRB and PRB) was not active against all the used strains. The BRC fraction showed good activity (2.4% of D control) against *M. marinum*, *SMRSA* (0.2% of D control) and *EMRSA* (0.2 of %D control) while against *E. coli* it was inactive (45.9% of D control). The PRC fraction showed selectivity as well as good activity against *SMRSA* (-1.1% of D control) and *EMRSA* (-1.1% of D control) while it showed no activity against the other two strains. The BRD fraction was more selective in activity and was only active against *M. marinum* (2.4% of D control). The PRD fraction was also active against *SMRSA* (1.8% of D control), *EMRSA* (-0.9% of D control) and *M. marinum* (17.6% of D control) while it was not active against *E. coli* (68.3% of D control). None of the fraction showed activity against *E. coli* **(Table I)**.

*Antimicrobial activity of pure compounds isolated from the tested fractions of B. brevissima and B. parkeriana:* The isolated compounds were also tested against the four strains of bacteria which were tested against the fractions. Berberine (**1**) showed good selectivity and activity against *SMRSA* and *EMRSA* (1.1 and 0.5% of D control). The isoquinoline alkaloid, 8-oxo-berberine (**2**) showed high selectivity and good activity (1.6% of D control) against *M. marinum* and was inactive against the other bacterial strains. Dehydrocheilanthifoline (**3**), columbamine (**4**) and jatrorrhizine (**5**) were active against *SMRSA* (0.1, -0.6 and -1.2% of D control respectively) and *EMRSA* (-0.8, -0.9 and -1.2% of D control respectively) while against *M. marinum* and *E. coli* these compounds were inactive. Like 8-oxo-berberine, glutamic acid established good activity and high selectivity against *M. marinum* but was inactive against the other tested strains (**Table II**).

In the past, diabetes was treated orally with medicinal plants or their crude extracts based on folk medicine (Kumar et al., 2010). More than 150 plant extracts have been used to treat *diabetes mellitus* (Eddouks et al., 2005; Choie et al., 1991), but there is still a need to identify insulin substitutes from medicinal plants. In the present

study, the effect of various fractions (BRB, BRC, BRD, PRB, PRC and PRD) and their isolated compounds were examined against PTP 1B. The antidiabetic study showed that fraction C (BRC and PRC) is very active against PTP 1B. Fraction C (BRC and PRC) and D (BRD and PRD) were further analyzed to isolate active constituents. Out of the six isolated compounds, 8-oxo-berberine (**2**) showed good activity against PTP 1B. Other isolated alkaloids were also good in activity since the literature has shown that many alkaloids have antidiabetic activities (Maqsood et al., 2009), while gluta -mic acid (**6**) was inactive.

The crude methanolic extract of *B. aristata* DC, has shown significant hypoglycemic activity in streptozo-tocin-induced diabetic rats (Upwar et al., 2011). Animals treated with a *Berberis* root extract and berberine have also shown considerable decrease in blood glucose levels as well as glycosylated hemoglobin levels in diabetic animals (Gulfraz et al., 2008). Berberine enhances the intracellular calcium which increases degranulation and release of insulin from β-cells (Xu et al., 1997).

A major therapeutic intervention in modern medicine, used in surgery, chemotherapy and other diseases, involves antibiotics (Brag et al., 2005). However resis-tance to antibiotics of many harmful bacteria is develop -ing daily (Schito et al., 2006). The need for better drugs to combat the problem of resistance can not be over emphasized (Wagh and Vidhale, 2010). In the present study, we have tested *B. brevissima* and *B. parkeriana* fractions and the six isolated compounds against four strains of bacteria. Among the tested fractions; fraction C (BRC and PRC) and D (BRD and PRD) showed good activity against three strains while all of the fractions were inactive against *E. coli* ATTC.8739. The two frac-tions were further studied for their active constituents responsible for their antimicrobial activity. The isolated compounds showed good selectivity and activity against three bacterial strains while they were all inactive against *E. coli*.

A literature survey showed that although *Berberis* species are medicinally important, their antibacterial activities are limited (Dutta and Panse, 1962). *B. aristata* extracts have significant antibacterial and antifungal potential against clinical and other reference strains (Shahid et al., 2009), while the isoquinoline alkaloids have significant in vitro antimicrobial activities (Zhang et al., 2012).

In conclusion, the present study suggests that *B. brevissima* and *B. parkeriana* have potential activity for treating diabetes and could be useful against some antibiotic resistant microbes. This provides some guidance towards further *in vitro* and *in vivo* testing of compounds to further assess their antidiabetic and antimicrobial properties.

## References


Aydemir N, Bilaloglu R. Genotoxicity of two anticancer drugs, gemcitabine and topotican, in mouse bone marrow *in vivo*. Mutat Res. 2003; 537: 43-51.

British Pharmacopoeia. Appendix 1A, A30, The Stationary Office, London, 2011, p 5.

Brag LC, Leite AA, Xavier KG, Takahashi JA, Bemquerer MP, Chartone-Souza E, Nascimento AM. Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*. Can J Microbiol. 2005; 51: 541-47.

Choi JS, Yokozawa T, Oura H. Improvements of hyperglycaemia and hyperlipemia in streptozotocin diabetic rats by methanolic extract of *Prunus dagidana* stems and its main components pruning. Planta Med. 1991; 57: 208 -11.

Cooper SF, Mockle JA, Santavy F. Revision of the structures of the alkaloids A and B isolated from *Coptis groenlandica*. Planta Med. 1972; 11: 313–21.

Dutta NK, Panse MV. Usefulness of berberine (an alkaloid from *Berberis aristata*) in the treatment of cholera. Indian J Med Res. 1962; 50: 732-35.

Eddouks M, Maghrani M, Michel JB, Hypoglycemic effect of *Triticum ripens* P. Beauv in normal and diabetic rat. J Ethanopharmacol. 2005; 102: 228-32.

Fajardo V, Elango V, Cassels BK, Shamma M. Chilenine: An Isoindolobenzazepine Alkaloid. Tetrahedron Lett.1982; 23: 39-42.

Freile ML, Giannini F, Pucci G, Sturniolo A, Rodero L, Pucci O, Balzareti V, Enriz RD. Antimicrobial activity of aqueous extracts and of berberine isolated from *Berberis heterophylla*. Fitoterapia 2003; 74: 702-05.

Gulfraz M, Mehmood S, Ahmad A, Fatima N, Praveen Z, Williamson EM. Comparison of the antidiabetic activity of *Berberis lyceum* root extract and berberine in alloxan induced diabetic rats. Phytother Res. 2008; 22: 1208-12.

Hsieh TJ, Chia YC, Wu YC, Chen CY, Chemical constituents from the stems of *Mahonia japonica*. J Chin Chem Soc. 2004; 51: 443-46.

IDF Diabetes Atlas. 5th ed. 166 Chaussee da la Hulpe B-1170 Brussels, Belgium (Accessed May 5, 2013, at www.idf.org/diabetesatlas/5e/the-global-burden).

Kumar S, Kumar V, Prakash OM, Antidiabetic and Antilipemic effects of *Cassia siamea* leaves extract in streptozotocin induced diabetic rats. Asian Pac J Trop Med. 2010; 3: 871-73.

Maqsood A, Alamgeer, Tanveer S. A potential adjunct to insulin: *Berberis lycium* Royle. Diabetologia Croatica 2009; 38: 13-18.

Meliani N, Amine ME, Dib, Allali H, Tabti B. Hypoglycaemic effect of *Berberis vulgaris* L. in normal amd streptozocin induced diabetic rats. Assian Pac J Trop Biomed. 2011; 1: 468 -71.

Nicasio P., Santamaria AL, Aranda E, Ortiz S, Gonzalez M. Cytotoxic isoflavones from *Erythrina indica*. Phytochemistry 2011; 58: 1113-20.

Schito GC. The importance of the development of antibiotic

resistance in *Staphylococcus aureus*. Clin Microbiol Infect. 2006; 12: 3-8.

Shahid M, Rahim T, Shahzad A, Tajuddin, Latif A, Fatma T, Rashid M, Raza A, Mustafa S. Ethnobotanical studies on *Berberis aristata* DC. root extracts. Afr J Biotechnol. 2009; 8: 556-63.

Singh M, Srivastava S, Rawat AKS. Antimicrobial activities of Indian *Berberis* species. Fitoterapia 2007; 78: 574-76.

Suau R, Rico R, Romero JML, Najera F, Cuevas A. Isoquinoline alkaloids from *Berberis vulgaris* Subsp. *australis*. Phytochemistry 1998; 49: 2545-49.

Tanaka R. New polar constituents of the pupae of the silkworm *Bombyx mori* L. Isolation and identification of Methionine Sulfoxide, Methionine Sulfone and γ-cyclic di-L-Glutamate. Biosci Biotechnol Biochem. 2007; 71; 3055-62.

Uniyal MR, Tewari LC. Anticancer drugs from U. P. Himalaya. Anc Sci Life 1991; 11: 50-55.

Upwar N, Patel R, Waseem N, Mahobia NK. Hypoglycemic effect of methanolic extract of *Berberis aristata* DC stem on normal and streptozotocin induced diabetic rats. Int J Pharm Pharmaceuti Sci. 2011; 3: 222-24.

Wagh S, Vidhale NN. Antimicrobial activity of *Berberis aristata* against some human pathogenic bacteria and fungi. Biosci Biotechnol Res Comm. 2010; 3: 38-42.

World Health Organization (WHO), 2009. Prevalence data of diabetes worldwide http://www.who.int/mediacentre/factssheets/fs312/en/index.html.

Xu SZ, Zhang Y, Ren JY. Effects of berberine of L- and T-type calcium channels in guinea pig ventricular myocytes. Zhongguo Yao Li Xue Bao 1997; 18: 515-18.

Zhang W, Hu JF, Lv WW, Zhao QC, Shi GB. Antibacterial, antifungal and cytotoxic isoquinoline alkaloids from *Litsea cubeba*. Molecules 2012; 17: 12950-60.