Research Article

EVALUATION OF ANTIBACTERIAL, ANTIFUNGAL, ENZYME INHIBITION AND HEMOLYTIC ACTIVITIES OF *CARYOPTERIS ODORATA* FRACTIONSMuhammad Athar Abbasi^{1*}, Aziz-ur-Rehman¹, Viqar Uddin Ahmad², Tauheeda Riaz¹ and Farah Khlaid¹¹Department of Chemistry, Government College University, Lahore, Pakistan²HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

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Article Received on: 10/10/13 Revised on: 07/11/13 Approved for publication: 20/12/13

DOI: 10.7897/2230-8407.041203

ABSTRACT

The purpose of this study was to explore the antibacterial, antifungal, enzyme inhibition and hemolytic activities of various fractions of *Caryopteris odorata* (Ham. Ex Roxb.). The methanolic extract of plant was dissolved in distilled water and partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol sequentially. Antibacterial activity was checked against *Staphylococcus aureus*, *Bacillus subtilis*, *Pasturella multocida* and *Escherichia coli* by the disc diffusion method using streptomycin sulphate, a standard antibiotic, as positive control. Polar fractions showed good antibacterial potential against these strains. These fractions also showed good MIC values. Antifungal activity was studied against four fungi i.e. *Aspergillus niger*, *Aspergillus flavus*, *Ganoderma lucidum* and *Alternaria alternata* by the disc diffusion method using fluconazole, a standard antifungal drug, as positive control. Ethyl acetate fraction was found active against *A. niger* and *G. lucidum* while chloroform and *n*-butanol fractions were active against *A. flavus*. Enzyme inhibition studies were performed against four enzymes i.e. α -glucosidase, butyrylcholinesterase, acetylcholinesterase and lipoxxygenase. Ethyl acetate and *n*-butanol soluble fraction exhibited good activity against α -glucosidase with IC₅₀ values 79.77 ± 0.97 and 82.77 ± 0.88 respectively. Chloroform soluble fraction showed moderate activity against butyrylcholinesterase (IC₅₀ 69.71 ± 0.95) while chloroform and ethyl acetate soluble fraction exhibited good activity against the lipoxxygenase with IC₅₀ values 65.28 ± 0.57 and 78.71 ± 0.69 respectively. Ethyl acetate fraction showed high toxicity i.e. 85.14 ± 0.83 %.

Keywords: *Caryopteris odorata* (Ham. Ex Roxb.), antimicrobial activity, butyrylcholinesterase, α -glucosidase, lipoxxygenase, hemolytic activity.

INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants. One of such resources is folk medicines. Systematic screening of them may result in the discovery of novel effective compounds¹. Interest in a large number of traditional natural products has increased. Plants are the sources of natural pesticides that make excellent leads for new pesticide development². Fungal infections remain a significant cause of morbidity and mortality despite advances in medicine and the emergence of new antifungal agents³. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases¹. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential. Some enzymes are potentially pro-oxidant so these cause the generation of radicals. So it is necessary to inhibit the enzymes to prevent various diseases and plants are the rich sources of flavonoids which effectively inhibit the enzymes⁴. *Caryopteris odorata* (Ham. Ex Roxb.) belongs to the family Verbenaceae which is a family of ca. 100 genera and nearly 2600 species, mostly tropical and subtropical in distribution⁵. In Pakistan it is represented by 17 genera and ca. 35 species⁶. The family is characterized mostly by woody habit (shrubs or trees, zygomorphic flowers, androecium didynamous, 2 carpels, ovary usually 4 loculed with terminal or sub terminal style. Fruit is usually drupe or nutlets. The family is important for teak timber i.e. *Tectona grandis* and some ornamental plants⁷. *Caryopteris odorata* is a shrub distributed in Subtropical or outer Himalayas from Pakistan to Bhutan, India and Bangladesh. It is common in the low hills of Punjab, and has also been reported from Baluchistan.

Sometimes it is also cultivated as an ornamental⁶. Its powdered leaves and flowers are used in diabetic foot ulcer, tumors and wounds⁸. *Caryopteris odorata* has been found to be a rich source of antioxidants⁹. Many compounds have been isolated from this plant such as 8-O-trans-cinnamoyl caryoptoside, 8-O-trans-cinnamoyl shanzhiside methylester, 8-O-trans-cinnamoyl mussaenoside, 8-O-cafeoyl massenoside, 2-ethyl-*n*-hexyl benzoate, 2-ethyl-*n*-hexyl trans-cinnamate, bis-dodecylbenzene-1, 4-dicarboxylate, trans-cinnamic acid, cis-cinnamic acid, hencicosanoic acid, methyl tricontanoate and octacosanyl tricontanoate^{10,11}. Keeping in view the medicinal importance of *Caryopteris odorata*, we have evaluated various organic and aqueous fractions of this plant for their antibacterial and antifungal potential, enzyme inhibition and hemolytic activity as such type of detailed preliminary work has not been performed yet on this plant. Antibacterial activity was checked against two gram-positive bacteria i.e. *Staphylococcus aureus* and *Bacillus subtilis* and two gram-negative bacteria i.e. *Pasturella multocida* and *Escherichia coli* by the disc diffusion method using streptomycin sulphate, a standard antibiotic, as positive control. Antifungal activity of crude fractions of plant was studied against four fungi i.e. *Aspergillus niger*, *Aspergillus flavus*, *Ganoderma lucidum* and *Alternaria alternata* by the disc diffusion method using fluconazole, a standard antifungal drug, as positive control. Enzyme inhibition studies were done against four enzymes i.e. α -glucosidase, butyrylcholinesterase, acetylcholinesterase and lipoxxygenase. The fractions were also checked for their toxicity by their hemolytic effects.

MATERIALS AND METHODS

Plant Material

The plant *Caryopteris odorata* (Ham. Ex Roxb.) was collected from hills of Azad Kashmir, India in July 2012, and identified by Mr. Muhammad Ajaib (Taxonomist), Department of Botany, GC University, Lahore. A voucher specimen no. (G.C. Herb. Bot. 862) has been deposited in the herbarium of the same university.

Extraction and Fractionation

The shade-dried ground whole plant (6.8 kg) was exhaustively extracted with methanol (12 L × 4) at room temperature. The extract was evaporated to yield the residue (633 g), which was dissolved in distilled water (1.5 L) and partitioned with *n*-hexane (1 L × 4), chloroform (1 L × 4), ethyl acetate (1 L × 4) and *n*-butanol (3 L × 4), respectively. These fractions were concentrated separately on rotary evaporator and the residues thus obtained were used to evaluate their *in vitro* bioactivity studies.

Antibacterial and Antifungal Assay

Microbial Strains

The samples both irradiated and un-irradiated were individually tested against a set of microorganisms, including two Gram-positive bacteria: *Staphylococcus aureus* (*S. aureus*), API Staph TAC 6736152, *Bacillus subtilis* (*B. subtilis*) JS 2004, two Gram-negative bacteria: *Escherichia coli* (*E. coli*) ATCC 25922, and *Pasteurella multocida* (*P. multocida*) (local isolate) and four fungal strains *Aspergillus niger*, *Aspergillus flavus*, *Ganoderma lucidum* and *Alternaria alternata*. The pure bacterial and fungal strains were obtained from Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Purity and identity were verified by the Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan. Bacterial strains were cultured overnight at 37°C in Nutrient agar (NA, Oxoid).

Disc Diffusion Method

The antibacterial and antifungal activities of the plant extracts were determined by disc diffusion method. Briefly, 100 µL of suspension of tested microorganisms, containing 10⁷ colony-forming units (CFU)/ml of bacteria cells on NA medium. The filter discs (9 mm in diameter) were individually impregnated with extracts' solution, placed on the agar plates which had previously been inoculated with the tested microorganisms. Discs without samples were used as a negative control. Streptomycin sulphate (30 µg/dish) (Oxoid, UK) was used as positive reference for bacteria to compare sensitivity of strain/isolate in analyzed microbial species. Plates, after 2 h at 4°C, were incubated at 37°C for 18 h for bacteria strains. Antibacterial and antifungal activity was evaluated by measuring the diameter of the growth inhibition zones (zone reader) in millimeters for the organisms and comparing to the controls¹².

Measurement of MIC

The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the sample capable of inhibiting the complete growth of the bacterium being tested. MIC was determined graphically as an extra plotation of linear relationship to zero value.

Enzyme Inhibition Assays

α-Glucosidase assay

The α-glucosidase inhibition activity was performed according to the slightly modified method of Pierre *et al.*¹³. Total volume of the reaction mixture of 100 µL contained 70 µL 50 mM phosphate buffer saline, pH 6.8, 10 µL (0.5 mM) test compound, followed by the addition of 10 µL (0.057 units) enzyme. The contents were mixed, pre incubated for 10 minutes at 37°C and pre-read at 400 nm. The reaction was initiated by the addition of 10 µL of 0.5 mM substrate (p-nitrophenyl glucopyranoside). Acarbose was used as positive control. After 30 minutes of incubation at 37°C, absorbance was measured at 400 nm using Synergy HT micro plate reader. All experiments were carried out in triplicates. The percent inhibition was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

IC₅₀ values (concentration at which there is 50 % in enzyme catalyzed reaction) compounds were calculated using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Butyrylcholinesterase assay

Butyrylcholinesterase (BChE) inhibition activity was performed according to standard method¹⁴ with slight modifications. Total volume of the reaction mixture was 100 µL containing 60 µL, Na₂HPO₄ buffer, 50 mM and pH 7.7. Ten µL test compound 0.5 mM well⁻¹, followed by the addition of 10 µL (0.5 unit well⁻¹) BChE. The contents were mixed and pre-read at 405 nm and then pre-incubated for 10 minutes at 37°C. The reaction was initiated by the addition of 10 µL of 0.5 mM well⁻¹ substrate (butyrylthiocholine bromide) followed by the addition of 10 µL DTNB, 0.5 mM well⁻¹. After 30 minutes of incubation at 37°C, absorbance was measured at 405 nm. Synergy HT (Bio Tek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM well⁻¹) was used as positive control. The percent inhibition was calculated by the help of following equation:

$$\text{Inhibition (\%)} = \frac{1 - \text{Abs. of test compound}}{\text{Abs. of Control}} \times 100$$

IC₅₀ values (concentration at which there is 50% enzyme inhibition) of compounds were calculated using EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Acetylcholinesterase assay

Acetylcholinesterase (AChE) inhibition activity was performed according to standard method¹⁴ with slight modifications. Total volume of the reaction mixture was 100 µL. It contained 60 µL Na₂HPO₄ buffer with concentration of 50 mM and pH 7.7. Ten µL test compound (0.5 mM well⁻¹) was added, followed by the addition of 10 µL (0.005 unit well⁻¹) enzyme. The contents were mixed and pre-read at 405 nm. Then contents were pre-incubated for 10 minutes at 37°C. The reaction was initiated by the addition of 10 µL of 0.5 mM well⁻¹ substrate (acetylthiocholine iodide), followed by the addition of 10 µL DTNB (0.5 mM well⁻¹). After 30 minutes of incubation at 37°C, absorbance was measured at 405 nm. Synergy HT (Bio Tek, USA) 96-well plate reader was used in all experiments. All experiments were carried out with their respective controls in triplicate. Eserine (0.5 mM

well¹) was used as a positive control. The percent inhibition was calculated by the help of following equation:

$$\text{Inhibition (\%)} = \frac{1 - \text{Abs. of test compound}}{\text{Abs. of Control}} \times 100$$

IC₅₀ values (concentration at which there is 50 % enzyme inhibition) of compounds were calculated using EZ-Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

Lipoxygenase assay

Lipoxygenase (LOX) activity was assayed according to the reported method¹⁵⁻¹⁷ but with slight modifications. A total volume of 200 µL assay mixture contained 140 µL sodium phosphate buffer (100 mM, pH 8.0), 20 µL test compound and 15 µL (600 U) purified lipoxygenase enzyme (Sigma, USA). The contents were mixed and pre-read at 234 nm and pre incubated for 10 minutes at 25°C. The reaction was initiated by addition of 25 µL substrate solution. The change in absorbance was observed after 6 minutes at 234 nm. Synergy HT (Bio Tek, USA) 96-well plate reader was used in all experiments. All reactions were performed in triplicates. The positive and negative controls were included in the assay. Baicalein (0.5 mM well⁻¹) was used as a positive control. The percentage inhibition was calculated by formula given below.

$$\text{Inhibition (\%)} = \frac{1 - \text{Abs. of test compound}}{\text{Abs. of Control}} \times 100$$

IC₅₀ values (concentration at which there is 50 % enzyme inhibition) of compounds were calculated using EZ-Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA).

Hemolytic Activity

Hemolytic activity of the plant extracts was studied by the method used by Sharma and Sharma (2001) and Powell *et al.* (2000)^{18,19}. Three ml freshly obtained heparinized human blood was collected from volunteers after consent and counseling. Blood was centrifuged for 5 minutes at 1000 x g plasma was discarded and cells were washed with three times with 5 ml of chilled (4°C) sterile isotonic Phosphate-buffered saline (PBS) pH 7.4. Erythrocytes were maintained 10⁸ cells per mL for each assay. Hundred µL of each extract was mixed with human (10⁸ cells/ml) separately. Samples were incubated for 35 minutes at 37°C and agitated after 10 minute. Immediately after incubation the samples were placed on ice for 5 minutes then centrifuged for 5 minutes at 1000 x g. Supernatant 100 µL were taken from each tube and diluted 10 time with chilled (4°C) PBS. Triton X-100 (0.1 % v/v) was taken as positive control and phosphate buffer saline (PBS) was taken as negative control and pass through the same process. The absorbance was noted at 576 nm using µ Quant (Biotech, USA). The % RBCs lysis for each samples were calculated.

$$\text{Percentage hemolysis} = \frac{\text{Abs. of sample} - \text{Abs. of blank}}{\text{Abs. of positive Control}} \times 100$$

Each sample was assayed in triplicate and mean values were calculated.

RESULTS AND DISCUSSION

Antibacterial Activity

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first

step towards this goal is the *in vitro* antibacterial activity assay²⁰. Many low molecular weight metabolites are present in higher plants which provide them protection from the various microbial infections. A number of barriers provide disease resistance in the plants including physical aspersoria, lignifications and defensive proteins. These metabolites inhibit the spore germination of microbes⁴. The results for antibacterial potential of studied plant fractions showed (Figure 1) that remaining aqueous fraction have good inhibitory potential against *Staphylococcus aureus* and *Pasturella multocida* having zone of inhibition 22 mm. *n*-Butanol fraction exhibited good inhibitory potential against *Bacillus subtilis* and *Escherichia coli* showing zone of inhibition 23 mm and 22 mm respectively. Chloroform fraction showed good activity against *Bacillus subtilis* (20 mm) while ethyl acetate fraction showed good activity against *Pasturella multocida* (21 mm). All the other fractions showed very less or no activity. The results were compared with rifampicin, a reference antibiotic drug. These observations have been made on the basis of measurements of zones of inhibition in mm. Minimum inhibitory concentration (MIC) was also calculated and results have been showed in Figure 2. Lower the MIC value higher will be the inhibitory potential of fraction. *n*-Butanol soluble fraction showed lowest MIC value for *Escherichia coli* i.e. 152.5. Remaining aqueous fraction has good MIC value against *Pasturella multocida* 152. The MIC value of ethyl acetate fraction was found to be 222.5 mg/ml against *Staphylococcus aureus*, *Bacillus subtilis* and *Pasturella multocida*. MIC value of ethyl acetate fraction was calculated as 245 mg/ml against all the studied bacterial strains.

Antifungal Activity

Antifungal activity of all the studied fractions of *C. odorata* was checked against *A. niger*, *A. flavus*, *G. lucidum* and *A. alternata*. It was observed from the results (Figure 3) that *n*-hexane fraction showed very less activity. Ethyl acetate fraction exhibited good activity against *A. niger* and *G. lucidum* i.e. 22 and 20 mm respectively. Chloroform and *n*-butanol fraction showed good activity only against *A. flavus* i.e. 21 and 22 mm respectively. All the other fractions showed very less or no activity. The results were compared with fluconazole, a reference antifungal drug. These observations have been made on the basis of measurements of zones of inhibition in mm. MIC was also calculated and results have been summarized in Figure 4. Ethyl acetate fraction showed good MIC values against *A. niger* and *G. lucidum* i.e. 140 and 158 mg/ml respectively. Chloroform and *n*-butanol fraction showed good MIC value against *A. flavus* i.e. 154 and 160 mg/ml respectively.

Enzyme Inhibitory Potential

Many flavonoids as well as hydroxycinnamic acids are the very effective inhibitors of the various enzymes. Some enzymes such as xanthine oxidase, cyclo-oxygenases, lipoxygenases and cytochrome P450 isoforms are potentially pro-oxidant so these cause the generation of radicals. So it is necessary to inhibit the enzymes to prevent various diseases and plants are the rich sources of flavonoids which effectively inhibit the enzymes⁴. Enzyme inhibition activities of the studied fractions were checked against four enzymes i.e. α -glucosidase, butyrylcholinesterase, acetylcholinesterase and lipoxygenase and the results have been summarized in the Table 1.

Figure 1: Zones of inhibition (mm) of various fractions of *Caryopteris odorata* against Gram-positive and Gram-negative bacteria

Figure 2: MIC of various fractions of *Caryopteris odorata* against Gram-positive and Gram-negative bacteria

Figure 3: Zones of inhibition (mm) of various fractions of *Caryopteris odorata* against fungi

Figure 4: MIC of various fractions of *Caryopteris odorata* against fungiTable 1: Enzyme inhibition activities of various fractions of *Caryopteris odorata* against α -glucosidase, butyrylcholinesterase, acetylcholinesterase and lipoxigenase

Samples	α -Glucosidase activity		BChE activity		AChE activity		LOX activity	
	% Inhibition (0.1 mg/ml)	(IC ₅₀) μ g/ml	% Inhibition (0.1 mg/ml)	(IC ₅₀) μ g/ml	% Inhibition (0.1 mg/ml)	(IC ₅₀) μ g/ml	% Inhibition (0.1 mg/ml)	(IC ₅₀) μ g/ml
n-Hexane soluble fraction	0.92 $\pm$ 0.03	NIL	1.07 $\pm$ 0.11	NIL	1.33 $\pm$ 0.14	NIL	1.62 $\pm$ 0.24	NIL
CHCl ₃ soluble fraction	26.91 $\pm$ 0.54	NIL	81.78 $\pm$ 0.69	69.71 $\pm$ 0.95	14.30 $\pm$ 0.84	NIL	72.56 $\pm$ 0.47	65.28 $\pm$ 0.57
EtOAc soluble fraction	65.48 $\pm$ 0.32	79.77 $\pm$ 0.97	25.64 $\pm$ 0.58	NIL	5.19 $\pm$ 0.33	NIL	66.06 $\pm$ 0.92	78.71 $\pm$ 0.69
n-BuOH soluble fraction	61.76 $\pm$ 0.85	82.77 $\pm$ 0.88	12.49 $\pm$ 0.29	NIL	18.44 $\pm$ 0.27	NIL	27.09 $\pm$ 0.52	NIL
Remaining aqueous fraction	34.16 $\pm$ 0.55	NIL	27.40 $\pm$ 0.39	NIL	28.91 $\pm$ 0.75	NIL	32.64 $\pm$ 0.46	NIL
Acarbose ^a	90.25 $\pm$ 0.25	38.62 $\pm$ 0.04	Eserine ^a	0.85 $\pm$ 0.001	Eserine ^a	0.04 $\pm$ 0.001	Baicalein ^a	22.4 $\pm$ 1.3

All results are presented as mean $\pm$ standard mean error of three assays.

a) Standard reference drugs

Figure 5: Hemolytic activities of various fractions of *Caryopteris odorata*

The major function of α -glucosidase is to hydrolyze the 1, 4 glycosidic linkage from the non-reducing end of the α -glucosides, α -linked oligosaccharide, and α -glucans substrates to produce α -D-glucose²¹. The inhibitors of enzyme can retard the liberation of D-glucose of oligosaccharides and disaccharides from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial hyperglycemia²². Therefore, inhibition of α -glucosidase is considered important in managing type-2

diabetes. Acetyl and butyryl cholinesterases are responsible for the termination of acetylcholine at cholinergic synapses²³. The major function of AChE is to catalyze the hydrolysis of the neurotransmitter acetylcholine and termination of the nerve impulse in cholinergic synapses²⁴. It has been found that BChE is present in significantly higher quantities in Alzheimer's plaques than in the normal age related non dementia of brains. Hence, the search for new cholinesterase inhibitors is considered to be an important and ongoing

strategy to introduce new drug candidates for the treatment of Alzheimer's disease and other related diseases²⁵. Lipxygenases catalyze the addition of molecular oxygen to fatty acids containing a cis-1, 4-pentadiene system to give unsaturated fatty acid hydro peroxide^{26,27}. It has been found that these LOX products play a key role in variety of disorders such as bronchial asthma, inflammation²⁸ and tumor angiogenesis²⁹. From the results it was revealed that ethyl acetate and *n*-butanol soluble fraction exhibited good activity against α -Glucosidase showing % inhibition as 65.48 ± 0.32 % and 61.76 ± 0.85 % respectively. Their IC₅₀ values were calculated as 79.77 ± 0.97 and 82.77 ± 0.88 respectively as compared to acarbose, a reference standard, having IC₅₀ value 38.62 ± 0.04 . All the other fractions showed no activity against this enzyme. Against the enzyme butyrylcholinesterase only chloroform soluble fraction showed moderate activity. Its % inhibition was found to be 81.78 ± 0.69 % while its IC₅₀ value was calculated as 69.71 ± 0.95 comparative to eserine, a reference standard, having IC₅₀ value 0.85 ± 0.001 . None of the fractions exhibited activity against acetylcholinesterase enzyme. Chloroform and ethyl acetate soluble fraction exhibited good activity against the lipxygenase enzyme having % inhibition values 72.56 ± 0.47 % and 65.28 ± 0.57 % respectively. Their IC₅₀ values were found to be 65.28 ± 0.57 and 78.71 ± 0.69 respectively as compared to baicalein, a reference standard, having IC₅₀ value 22.4 ± 1.3 .

Hemolytic activity

The *in vitro* hemolytic activities are now-a-days becoming new area of research in the drug lead discoveries³⁰. In the exploration of the action of the plant extracts on the human blood, it is essential to determine hemolytic activity because this is the indicator of cytotoxicity and bioactivity. *In vitro* hemolysis tests have been employed by many researchers for the toxicological evaluation of the various plants³¹. The studied fractions were examined for their toxicity by their hemolytic effects and the results have been given in Figure 5. The hemolytic activities were compared with the triton, taken as +ve control, having 100 % toxicity and phosphate buffered saline (PBS), taken as -ve control, having 0 % toxicity. It was revealed from the results that ethyl acetate fraction showed high toxicity i.e. 85.14 ± 0.83 %. *n*-Butanol fraction showed moderate toxicity value (33.01 ± 0.59 %) while *n*-hexane, chloroform and remaining aqueous fraction showed very less toxicity i.e. 7.86 ± 0.95 %, 8.27 ± 0.51 % and 5.32 ± 0.12 % respectively. So, the ethyl acetate fraction might be used in pharmacological preparations as anti-tumor drug.

CONCLUSION

It was concluded from the present study that polar fractions of *C. odorata* exhibited good antibacterial potential against some strains (20-22 mm) as well as also showed good MIC values. Ethyl acetate, chloroform and *n*-butanol fraction showed good antifungal potential. Ethyl acetate fraction was found active against *A. niger* and *G. lucidum* while chloroform and *n*-butanol fractions were active against *A. flavus*. Ethyl acetate and *n*-butanol soluble fraction exhibited good activity against α -Glucosidase with IC₅₀ values 79.77 ± 0.97 and 82.77 ± 0.88 respectively. Chloroform soluble fraction showed moderate activity against butyrylcholinesterase (IC₅₀ 69.71 ± 0.95) while chloroform and ethyl acetate soluble fraction exhibited good activity against the lipxygenase with IC₅₀ values 65.28 ± 0.57 and 78.71 ± 0.69 respectively. However, the ethyl acetate soluble

fraction exhibited high toxicity i.e. 85.14 ± 0.83 %. So, these fractions which showed good antimicrobial potential and enzyme inhibition activities might be very useful in pharmacological preparations as antibiotics and enzyme inhibitors while ethyl acetate fraction can be used also in pharmacological preparations in the formulation of anti-tumor drugs.

ACKNOWLEDGMENT

The authors are thankful to Higher Education Commission of Pakistan for financial grant. The authors are also thankful to Dr. Muhammad Shahid and Dr. Muhammad Ashraf for biological activities.

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Cite this article as:

Muhammad Athar Abbasi, Aziz-ur-Rehman, Viqar Uddin Ahmad, Tauheeda Riaz and Farah Khilaid. Evaluation of antibacterial, antifungal, enzyme inhibition and hemolytic activities of *Caryopteris odorata* fractions. *Int. Res. J. Pharm.* 2013; 4(12):9-15 <http://dx.doi.org/10.7897/2230-8407.041203>

Source of support: Higher Education Commission of Pakistan, Conflict of interest: None Declared