Enzyme inhibitory, Antifungal, Antibacterial and hemolytic potential of various fractions of *Colebrookia oppositifolia*

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Abstract: The purpose of the present investigation was to assess the enzyme inhibition, antifungal, antibacterial and hemolytic activities of various fractions of *Colebrookia oppositifolia* Smith. The MeOH extract of plant was dissolved in dist. water and partitioned with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH sequentially. Enzyme inhibition studies were done against four enzymes i.e. *α*-glucosidase, butyrylcholinesterase, acetyl cholinesterase and lipoxygenase. Ethyl acetate fraction possessed very good activity against *α*-glucosidase (IC_{50} 57.38±1.23µg/mL). $CHCl_3$ fraction displayed good activity against *α*-glucosidase and lipoxygenase while moderate activity against butyryl cholinesterase. EtOAc fraction displayed good activity against lipoxygenase. 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. Aqueous fraction displayed good activity against *G. lucidum* and *A. flavus*. 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. Chloroform, ethyl acetate and aqueous fraction showed good activity against *E. coli*. Chloroform fraction showed good activity against *B. subtilis*. Ethyl acetate fraction showed good activity against the *P. multocida.* All the studied fractions showed very less toxicity i.e. < 7%.

Keywords: *Colebrookia oppositifolia* Smith, enzyme inhibitory potential, antifungal potential, disc diffusion method, *α*-glucosidase, lipoxygenase, hemolytic effects.

INTRODUCTION

Medicinal plants have been using for relief from illness for five millennia as written in the documents of early civilization in India, China, India and Near east, but it is undoubtedly an art as old as the mankind. The pharmacological screening of various compounds of natural origin is source of numerous therapeutic agents. Now-a-days, the drugs isolated from higher plants have significant position in modern medicine (Mahesh and Satish, 2008). Great diversity of substances can be produced by the higher plants, having antimicrobial activity. Also, several plants have been studied for their ability to produce antifungal agents. Such plants have been proven as worthy sources of the botanical pesticides (Suprapta and Khalimi, 2012). It has been discovered after attentive and extensive research in whole the world that natural products from plants have ability to control over-activity of the many enzymes. Such natural products, showing enzyme inhibitory potential, have been used as natural therapeutics for the cure of several diseases (Abbasi *et al*., 2012).

As the plants are found as potential sources for enzyme inhibitors, antifungal and antibacterial agents, a study was done to evaluate the enzyme inhibition, antifungal, antibacterial and hemolytic activities of various fractions of *Colebrookia oppositifolia* Smith. *Colebrookia oppositifolia,* locally known in as Kala Behakar, Pathan or Bhinda, is common in India and Pakistan and belongs to the family *Labiatea*e/*Lamiaceae*. It is widely used ethno-medicinally e.g., its leaves are applied to ulcers and wounds as an antiseptic. The extract of its roots, containing flavones, is used in treatment of epilepsy. Fruits of *Annona squamata*, roots of *Asparagus racemosus*, stem of *F. bengalensis* and shoots (along with leaves and stem) of *C. oppositifolia* are crushed and taken for cure of urinary problems. *C. oppositifolia* contains numerous glycoflavonoids and flavonoids. A pure acteoside was isolated from its aerial parts having good hepatoprotective ability (Riaz *et al*., 2012). It has been found to possess strong antioxidant potential (Riaz *et al*., 2011). Many important flavonoids and terpenoids such as ursolic acid, betulonic acid, negletein, 5,2′,6′-trihydroxy-7-methoxy flavone, quercetin and 5,7,2′-

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trihydroxyflavone-2′-*O*-*β*-D-glucopyranoside have been isolated from this plant (Riaz *et al*., 2012).

To the best of our knowledge, no such detailed studies have been carried out on this plant, therefore, enzyme inhibition studies were done against four enzymes i.e. *α*-glucosidase, butyrylcholinesterase, acetylcholinesterase and lipoxygenase. 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. The fractions were also checked for their toxicity by their hemolytic effects. A very small study on antibacterial activity of this plant has been done against the waterborne pathogens (Ahmad *et al*., 2009), we have extended the work by evaluating the activity against some common disease causing bacteria. 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.

MATERIAL AND METHODS

Plant material

The plant *Colebrookia oppositifolia* Smith was collected from Kotli, Azad Kashmir in June 2009. It was identified by Dr. Muhammad Ajaib, a taxonomist at Department of Botany, GC University, Lahore. In the Herbarium of the same Department, a voucher specimen (GC. Herb. Bot. 622) has been placed.

Extraction and fractionation of antioxidants

The shade-dried whole plant was ground (17 kg) and exhaustively extracted with MeOH (20L × 5) at room temperature. The extract was evaporated to yield the residue (1026 g). It was dissolved in distilled water (2L) and partitioned with *n*-hexane (1L × 4), $CHCl_3$ (1L × 4), EtOAc (1L × 4) and *n*-BuOH (1L × 4) respectively. The four organic fractions and the remaining aqueous fraction were subjected to rotavapour (Laborta 4000-efficent Heidolph) separately [(*n*-hexane at 35°C, chloroform at 37°C, ethyl acetate at 45°C, *n*-butanol at 50°C and water at 60°C to concentrate them. The yields of *n*-hexane soluble fraction, $CHCl_3$ soluble fraction, EtOAc soluble fraction, *n*-BuOH soluble fraction and remaining aqueous fraction were 201g, 148g 124g, 285g and 268g respectively. The residues of each fraction thus were used to assess their *in vitro* enzyme inhibitory, antifungal, antibacterial and hemolytic potential.

Phytochemical screening

Qualitative phytochemical screening of all five crude extracts, *i.e*., the *n*-hexane soluble fraction, the chloroform soluble fraction, the ethyl acetate soluble fraction, the *n*-butanol soluble fraction and the remaining aqueous fractions, was performed to identify the phytochemical constituents, i.e., alkaloids, terpenoids, saponins, tannins, sugars, phenolics, flavonoids and cardiac glycosides, using standard procedures (Sofowara, 1993; Trease and Evans, 1989; Ayoola *et al*., 2008).

Enzyme inhibition assays

α-Glucosidase assay

The *α*-glucosidase inhibition activity was done as per reported method (Pierre *et al.,* 1978).

Cholinesterases assay

Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE) inhibition activities were performed according to established methods (Ellman *et al.,* 1961).

Lipoxygenase assay

Lipoxygenase (LOX) inhibition activity was checked according to cited method (Baylac and Racine, 2003*;* Evans, 1987; Tappel, *1953*).

Calculation of IC_{50} values

IC_{50} values (concentration that cause 50% inhibition of the enzyme) of samples were measured by using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Antibacterial and antifungal assay

Microbial strains

The samples both irradiated and un-irrdiated were tested separately against two strains of Gram-positive bacteria: *Bacillus subtilis* JS 2004 and *Staphylococcus aureus*, API Staph TAC 6736152 and two strains of Gram-negative bacteria: *Pasteurella multocida* (local isolate) and *Escherichia coli* ATCC 25922 and four fungal strains *Aspergillus niger, Aspergillus flavus, Ganoderma lucidum* and *Alternaria alternata*. Pure bacterial and fungal strains were collected from the Dept. of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. Verification of the identity and purity of the strains was done by the Institute of Microbiology of the same university. In the Nutrient agar (NA, Oxoid), the bacterial strains were cultivated at 37ºC overnight.

Disc diffusion method

Disc diffusion method was used to check antibacterial and antifungal activities of plant extracts. Suspension of the tested microorganisms (100µL), contained 10^7 CFU/ml (colony-forming units) of bacteria cells on the Nutrient agar medium. The 9mm diameter filter discs were impregnated separately with extracts' solution and placed on agar plates that were already inoculated with tested microorganisms. Streptomycin sulphate (Oxoid, UK) (30 µg/dish) was taken as positive reference for bacterial strains to compare the sensitivity of isolate/strain in the

analyzed microbial species. The discs with no samples were taken as negative control. Plates were kept at 4ºC for 2 hours and then incubated for 18 hours at 37ºC for bacterial strains. Evaluation of the antibacterial and antifungal activity for the organisms was done by measurement of diameter of the growth inhibition zones in millimeters and by comparison to that of positive and negative controls (CLSI, 2010).

Measurement of MIC

The MIC (minimum inhibitory concentration) was stated as lowest concentration of sample that has capability to inhibit complete growth of bacterial strain being tested. It was calculated graphically as an extraplotation of linear relationship to zero value.

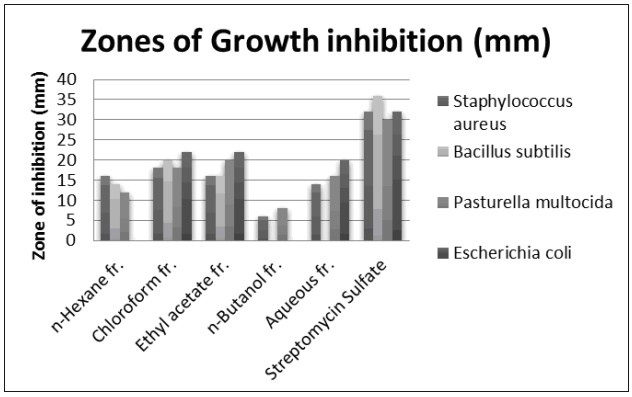

Fig. 1: Zones of inhibition (mm) of various fractions of *Colebrookia oppositifolia* against Gram-positive and Gram-negative bacteria.

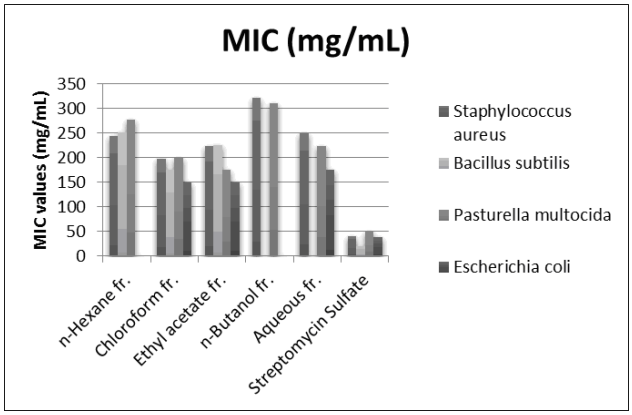

Fig. 2: MIC of various fractions of *Colebrookia oppositifolia* against Gram-positive and Gram-negative bacteria.

Hemolytic activity

Hemolytic activity of the plant extracts was performed according to standard method (Powell *et al*., 2000; Sharma and Sharma, 2001). Blood was collected from various volunteers after their counseling and consent. Heparinized human blood (3mL), freshly obtained, was taken and centrifuged at 1000x*g* for 5 minutes. After discarding plasma, cells were washed with 5mL of chilled (4^oC) sterile isotonic PBS (Phosphate-buffered saline) having pH 7.4, three times. For each assay the concentration of erythrocytes were maintained at 10^8 cells per mL. Then 100µL of each plant extract was mixed separately with human blood (10^8cells/mL). Incubation of samples was done at 37^oC for 35 min and agitation was started after 10 minutes. The samples were placed for 5 minutes on ice immediately after the incubation, then centrifugation was done at 1000x*g* for 5 minutes. From each tube, 100µL of supernatant was taken, and then diluted with chilled (4^oC) PBS 10 times. PBS was used as negative control while triton X-100 (0.1% v/v) was used as positive control and passed through same process. Absorbance of each sample was measured, using µ Quant (Bioteck, USA), at 576 nm. Calculation of % RBCs lysis for each sample was done by following formula.

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

All readings were taken in triplicate and mean values were calculated.

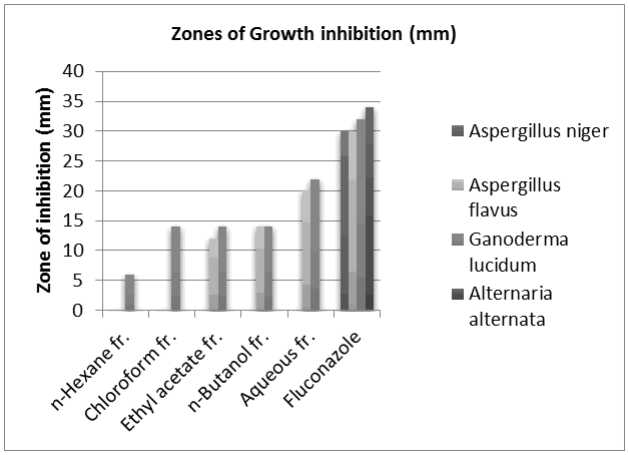

Fig. 3: Zones of inhibition (mm) of various fractions of *Colebrookia oppositifolia* against fungi.

RESULTS

The phytochemical screening was done on all the studied fractions. Tests were performed for the detection of alkaloids, terpenoids, saponins, tannins, sugars, phenolics, flavonoids and cardiac glycosides and the results have been shown in table 1. Enzyme inhibition activities of the studied fractions were checked against four enzymes i.e. *α*-glucosidase, butyrylcholinesterase, acetyl cholinesterase and lipoxygenase and the results have been summarized in the table 2. Antibacterial activities of the studied fractions were 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. Zones of growth inhibition were measured in mm. The results have been summarized in fig. 1. MIC was also calculated and results have been summarized in

fig. 2. Antifungal activity of all the studied fractions of *Colebrookia oppositifolia* Smith was checked against *A. niger, A. flavus, G. lucidum* and *A. alternata* and the results have been illustrated in fig. 3. MIC values of the antifungal assay have been summarized in fig. 4.All the studied fractions were checked for their toxicity by their hemolytic effects and the results are given in fig. 5.

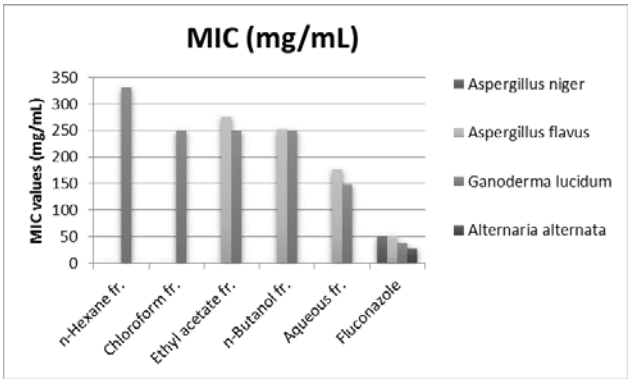

Fig. 4: MIC of various fractions of *Colebrookia oppositifolia* against fungi.

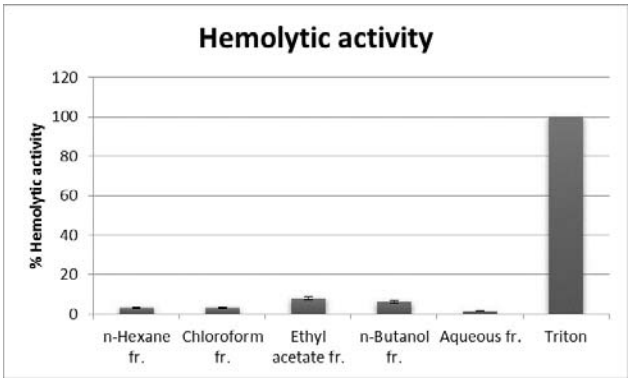

Fig 5: Hemolytic activities of various fractions of *Colebrookia oppositifolia*.

DISCUSSION

Phytochemical screening

The phytochemical screening results of crude fractions of *Colebrookia oppositifolia* (table 1) showed that *n*-BuOH, EtOAc and $CHCl_3$ fraction contain flavonoids, phenolics and alkaloids. Sugars and tannins were found in good amount in aqueous fraction. Cardiac glycosides were present in all fractions but in greater amount in *n*-BuOH, EtOAc and $CHCl_3$ fraction. Terpenes were detected in all the fractions. Except *n*-hexane fraction, tannins and sugars were detected in all fractions. Saponins were detected in aqueous, *n*-BuOH and EtOAc fraction.

Enzyme inhibitory potential

Enzyme inhibition activities of the studied fractions were checked against four enzymes i.e. *α*-glucosidase, butyrylcholinesterase, acetyl cholinesterase and lipoxygenase and the results have been summarized in the table 2. *α*-Glucosidase inhibitors are molecules which are used as oral anti-diabetic drugs for the type-2 diabetic mellitus patients. Postprandial hyperglycemia take vital role in developing of type-2 diabetes as well as other complications related with diseases e.g. macroangipathy, microangiopathy, neuropathy and nephropathy (Baron, 1998). Inhibitors of this enzyme can retard liberation of the *D*-glucose of disaccharides and oligosaccharides from the dietary complex carbohydrates, so, delay the absorption of glucose, hence result in reduced postprandial hyperglycemia (Harold and Lebovitz, 1997). So, inhibition of the *α*-glucosidase is reflected as essential to manage the type-2 diabetes.

The main function of the AChE is that it catalyzes the hydrolysis of acetylcholine, which is the neurotransmitter, as a result causing the nerve impulse termination at cholinergic synapses (Quinn, 1987). BChE has been observed to be present in considerably higher amounts in Alzheimer's plaques. So, it is significant to search novel cholinesterase inhibitors for introduction of novel drugs to treat Alzheimer's disease and such type of other diseases (Bertaccini, and Substance, 1982).

The cholinesterase inhibitors have capability of reversibility and irreversibility, through which enhance the quantity of acetylcholine available for neuromuscular and neuronal transmission (Gauthier, 2001). Many neuromuscular and neurological disorders involve the reduction of cholinergic activity. Most effective compounds are the ligands which have ability to inhibit breakdown of the acetylcholine. Lipoxygenases produce unsaturated fatty acid hydro peroxides by catalyzing addition of molecular oxygen to fatty acids which contain *cis*-1,4-pentadiene system (Clapp *et al*., 1985; Kamal *et al*., 1987). LOX products have been found to play a key role in various disorders e.g., tumor angiogenesis (Nie and Honn, 2002), inflammation and bronchial asthma (Steinhilber, 1999).

It was observed from the results (table 2) that ethyl acetate fraction possessed very good activity against *α*-glucosidase. It showed 73.98±1.32% inhibition of enzyme at concentration of 0.1mg/mL. Its IC_{50} value was calculated as 57.38±1.23µg/mL as compared to acarbose which showed IC_{50} value 38.62±0.04µg/mL. Chloroform fraction also showed good activity having IC_{50} values 99.38±1.96µg/mL. Chloroform fraction showed moderate activity against butyryl cholinesterase having IC_{50} values 27.18±1.27µg/mL, respectively. None of the fractions showed activity against acetyl cholinesterase. Ethyl acetate and chloroform fractions showed good activity against lipoxygenase having IC_{50} values 42.56±1.18 and 61.39±1.75µg/mL, respectively as compared to baicalein, a reference standard, which showed IC_{50} value 22.4±1.3 µg/mL. Good activity shown by ethyl acetate fraction can be attributed to presence of many flavonoids in this

Table 1: Phytochemical constituents of various fractions of *Colebrookia oppositifolia* smith

Test	*n*-hexane fraction	$CHCl_3$ fraction	EtOAc fraction	*n*-BuOH fraction	Aqueous fraction
Alkaloids	−	+	+	+++	−
Terpenoids	+++	+++	++	+++	+
Saponins	−	−	++	++	++
Tannins	−	+	++	+	++
Sugars	−	+	++	+++	++++
Phenolics	−	+++	+++	++	+
Flavonoids	−	+++	+++	++	+
Cardiac Glycosides	+	++	++	++	+

(+ indicates presence, – indicates absence).

Table 2: Enzyme inhibition activities of various fractions of *Colebrookia oppositifolia* against *α*-glucosidase, butyrylcholinesterase, acetyl cholinesterase, and lipoxygenase

Samples	*α*-Glucosidase activity		BchE activity		AchE activity		LOX activity	
	%Inhibition (0.1mg/ml)	(IC_{50}) µg/mL	%Inhibition (0.1mg/ml)	(IC_{50}) µg/mL	%Inhibition (0.1mg/ml)	(IC_{50}) µg/mL	%Inhibition (0.1mg/ml)	(IC_{50}) µg/mL
n-Hexane soluble fraction	1.02±0.04	NIL	1.37±0.11	NIL	0.87±0.04	NIL	1.58±0.19	NIL
$CHCl_3$ fraction soluble	58.59±0.30	99.38 ±1.96	64.37 ±0.45	27.18 ±1.27	28.28±0.56	NIL	56.11±0.14	61.39 ±1.75
EtOAc soluble fraction	73.98±1.32	57.38 ±1.23	29.80±0.34	NIL	10.96±0.18	NIL	61.18±0.65	42.56 ±1.18
n-BuOH soluble fraction	20.08±0.19	NIL	33.88±0.55	NIL	27.52±0.22	NIL	28.02±0.71	NIL
Remaining-aqueous fraction	15.56±0.74	NIL	27.24±0.69	NIL	29.04±0.91	NIL	37.16±0.25	NIL
Acarbose[a]	90.25±0.25	38.62 ±0.04	Eserine[a]	0.85 ±0.001	Eserine[a]	0.04 ±0.001	Baicalein[a]	22.4 ±1.3

All results are presented as mean ± standard mean error of three assays. (a) Standard reference drugs

fraction i.e. negletein, 5,2′,6′-trihydroxy-7-methoxy flavone, quercetin and 5,7,2′-trihydroxyflavone-2′-*O*-*β*-D-glucopyranoside etc. (Riaz *et al*., 2012).

Antibacterial activity

Several medically important disease-causing organisms have developed resistance to the antibiotics (Palombo and Semple, 2001). So there is constant and distinct need to produce more efficient and safe therapeutic agent. A solution to this problem is application of antibiotic resistance inhibitors of plant origin which are able to retard antibiotic resistance and adversarial effects on the host (Nostro *et al*., 2000; Kim *et al*., 1995). 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. Zones of growth inhibition were measured in mm. The results have been summarized in fig. 1. It was observed that $CHCl_3$ fraction showed good activity against *Bacillus subtilis* (20 mm) and *Escherichia coli* (22 mm). EtOAc fraction showed good activity against the *P. multocida* (20mm) and *E. coli* (22mm). Remaining-aqueous fraction showed good activity only against the *E. coli* (20 mm). Only these values were found significant as compared to blank ($p<0.05$). All the other results were found non-significant ($p>0.05$). These observations have been made on the basis of measurements of zones of growth inhibition in mm.

Statistical analysis of variance (ANOVA) and the Duncan *t*-test supported the experimental results obtained. MIC was also calculated and results have been summarized in fig. 2. MIC values shown by chloroform, ethyl acetate and aqueous fraction against *E. Coli* were 149, 150 and 176 mg/mL, respectively. MIC value of chloroform fraction against *B. Subtilis* was found to be 175mg/mL while that of ethyl acetate fraction against *P. Multocida* was 176 mg/mL. The results mentioned as good were found significant ($p<0.05$). As found in literature, the -OH group present in phenolics, steroids, coumarins and flavonoids, specially at C-6 and C-8 position, was invariably very effective against spectrum of the tested bacteria (Kayser and Kolodziej, 1999). In addition hydroxyl substituted aldehydes and ketones were also found very active (Friedman *et al*., 2003). Secondary metabolites e.g. phenolics, flavones, flavonoids, terpenes, alkaloids,

phenolic glycosides, tannins, sugars, organic acids, quinones etc. have anticeptic action. These disintegrate cytoplasmic membrane and destabilize electron flow system. Terpenes disturb cell structure of micro-organisms. Flavonoids and terpenes inactivate enzymes which synthesize structural components of bacteria (Silva and Fernandes, 2010). The phytochemical investigation (table 1) showed that this plants is rich in such active compounds. Riaz *et al*. (2012) investigated on $CHCl_3$ and EtOAc soluble fractions of this plant which led to isolation of 1-pentacosanol, stigmast-7-ene-3*β*-ol, triacontane and triacontanol from the chloroform soluble fraction while *p*-hydroxybenzoic acid ursolic acid, betulonic acid, negletein, 5,2′,6′-trihydroxy-7-methoxy flavone, quercetin and 5,7,2′-trihydroxyflavone-2′-*O*-*β*-D-glucopyranoside from the ethyl acetate soluble fraction..

The results for antibacterial activity showed that $CHCl_3$ and EtOAc soluble fractions displayed good results. $CHCl_3$ soluble fraction might showed good activity due to presence of phenolics, flavonoids and steroids (stigmast-7-ene-3*β*-ol). EtOAc soluble fraction might showed good activity due to presence of negletein, 5,2′,6′-trihydroxy-7-methoxy flavone, quercetin and 5,7,2′-trihydroxyflavone-2′-*O*-*β*-D-glucopyranoside in it.

Antifungal activity

Antifungal activity of all the studied fractions of *Colebrookia oppositifoilia* was checked against *A. niger, A. flavus, G. lucidum* and *A. alternata*. It was observed from the results, that *n*-hexane fraction displayed no activity. Chloroform, *n*-butanol and ethyl acetate fraction displayed moderate activity only against *G. lucidum* and *A. flavus*. Aqueous fraction displayed good activity against *G. lucidum* (20mm) and *A. flavus* (22mm). 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 (fig. 3). Statistical analysis of variance (ANOVA) and the Duncan *t*-test supported the experimental results obtained. The results mentioned as good were found significant as compared to blank ($p<0.05$).

MIC was also calculated and results have been summarized in fig. 4. Aqueous fraction showed MIC values 176 and 149mg/mL against *G. lucidum* and *A. flavus,* respectively. Statistical analysis of variance (ANOVA) and the Duncan *t*-test supported the experimental results obtained. The results mentioned as good were found significant ($p<0.05$). Thus good antifungal activity shown by aqueous fraction may be attributable to presence of tannins and sugars in this fraction as indicated by phytochemical tests.

Hemolytic activity

Natural compounds derived from plants have gained considerable attention due because of their ability to act as chemopreventive and cytotoxic agents. Hemolytic assay is performed to determine whether the particular drug possessing antimicrobial, antioxidant and other bioactivities, could be used in pharmacological applications or not. Now-a-days, *in vitro* hemolytic activities are becoming the new area of research to discover novel drugs (Mukherjee and Rajasekaran, 2010). To explore action of the extracts of plants on the human blood, it is indispensable to find out hemolytic activities, as it is indicator of cytotoxicity and bioactivity. Many researchers have employed *in vitro* hemolytic tests for toxicological evaluation of many plants (Mukherjee and Rajasekaran, 2010).

All the studied fractions were checked for their toxicity by their hemolytic effects and the results are given in fig. 5. The hemolytic activities of the plants' extracts 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 all the studied fractions of *Colebrookia oppositifolia* showed very less toxicity. The *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fraction showed toxicity values 3.08±0.17%, 3.15±0.26%, 7.85±0.86%, 5.96±0.58% and 1.54±0.25%, respectively, so, the fractions which showed good antimicrobial potential and enzyme inhibition activities might be very useful in pharmacological preparations.

CONCLUSION

This study concluded that $CHCl_3$, EtOAc and aqueous fraction of plant have good enzyme inhibitory and antimicrobial effects. Remaining-aqueous fraction possessed very good activity against *α*-glucosidase having IC_{50} value 57.38±1.23µg/mL as compared to acarbose whose IC_{50} value was found to be 38.62±0.04µg/mL. $CHCl_3$ fraction showed good activity against α-glucosidase and lipoxygenase while moderate activity against butyrylcholinesterase. EtOAc fraction showed good activity against lipoxygenase. Aqueous fraction displayed good activity against *G. lucidum* and *A. flavus*. $CHCl_3$, EtOAc and aqueous fraction showed good activity against *E. coli*. $CHCl_3$ fraction showed good activity against *B. subtilis*. EtOAc fraction showed good activity against the *P. multocida.* All the studied fractions showed very less toxicity i.e. <7%. So, these fractions are potentially valuable sources of natural antimicrobials, enzyme inhibitors and bioactive materials and can be used in pharmacological preparations to produce safe, potent and non-toxic drugs.

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