In vitro assessment of antibacterial, antifungal, enzyme inhibition and hemolytic activities of various fractions of *Rhynchosia pseudo-cajan*

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Abstract: The aims of the present investigation were to assess the antibacterial, antifungal, enzyme inhibition and hemolytic activities of various fractions of *Rhynchosia pseudo-cajan* Cambess. The methanolic extract of the plant was dissolved in the water (distilled) and then partitioned with the *n*-hexane, chloroform, EtOAc and *n*-BuOH sequentially. Antibacterial activity was checked against *Escherichia coli*, *Pasturella multocida*, *Bacillus subtilis* and *Staphylococcus aureus* by the disc diffusion method using streptomycin sulphate, a standard antibiotic, as positive control. Chloroform and ethyl acetate soluble fractions showed good activity against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. These fractions also showed good MIC values. The *n*-butanol soluble and remaining aqueous fraction also showed good activity against some strains. 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. Chloroform, *n*-butanol and ethyl acetate soluble fraction showed good activity only against *G. lucidum*. Enzyme inhibition studies were done against four enzymes i.e. *α*-glucosidase, butyrylcholinesterase, acetyl cholinesterase and lipoxygenase. Aqueous fraction possessed very good activity against α-glucosidase, even greater than acarbose, a reference standard drug. Its IC_{50} value was found as 29.81±0.12 µg/ml as compared to acarbose having IC_{50} 38.62±0.04 µg/ml. Chlroform and ethyl acetate soluble fractions also showed good activity against α-glucosidase. Ethyl acetate soluble and remaining aqueous fractions showed good activity against lipoxygenase. All the studied fractions showed very less toxicity *i.e.* <2.5%.

Keywords: *Rhynchosia pseudo-cajan* Cambess, antimicribial potential, disc diffusion method, *α*-glucosidase, lipoxygenase, hemolytic effects.

INTRODUCTION

The antiseptic qualities of the medicinal and aromatic plants as well as their extracts have been renowned since antiquity, while the attempts to illustrate these properties in the laboratory date back to early 1900s. Antimicrobial properties of the plant constituents, derived from wide variety of the plants have been assessed. From these studies it is clear that these plant secondary metabolites have great potential in the medical procedures and applications such as in the cosmetics, pharmaceutical and food industries (Dorman and Deans, 2000). So, the interest enlarge number of the traditional natural products has amplified. New natural pesticides can be developed excellently from various plant sources (Bobbarala *et al*., 2009). Fungal infections are the considerable source of mortality and morbidity in spite of advances in the medication and advent of the novel antifungal medicines (McNeil *et al*., 2001). Contrary to synthetic drugs the antimicrobials of the plant origin have no side effects and have vast therapeutic potential for healing of many infectious diseases (Janovska *et al*., 2003).

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Medicinal and aromatic plants are well known for producing certain bioactive molecules, which react with the other organisms present in environment thus inhibiting fungal or bacterial or growth. Such substances which inhibit growth of the pathogens and also have very less toxic to cells, are the very interesting sources for development of the novel antifungal and antimicrobial drugs (Chopra *et al*., 1992; Bruneton, 1995). As found in literature the terpenoids and flavonoids were found as very effective against the spectrum of tested bacteria (Kayser and Kolodziej, 1999; Friedman, *et al*., 2003). Terpenoids are amongst the chemicals which are responsible for culinary, medicinal and fragrant uses of the aromatic and medicinal plants (Dorman and Deans, 2000). Plant extracts mostly contain such types of compounds. Thus, it is significant to check different types of the medicinal plants for their antimicrobial and antioxidant potential.

Many flavonoids are the very effective inhibitors of the various enzymes. 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 enzymes (Walton and Brown, 1999).

Rhynchosia pseudo-cajan (Camb.) is an important medicinal plant, belongs to family Papillionaceae. It is known as "Lahrr" in Pakistan and widely distributed in Azad Jammu and Kashmir, Pakistan (Ajaib, 2008; Ali, 1977). Leaves are used as tonic in stomach disorders. Powder of leaves and bark is useful for digestive problems such as peptic ulcer (Ajaib, 2008). Fermented liquour is made by crude drugs of various *Rhynchosia* species that have anti-tumor, anti-oxidative, arthritis treating and preventive and skin whitening effects (Lee and Soon, 2006). *Rhynchosia pseudo-cajan*has been found to possess very strong antioxidant activity (Riaz *et al*., 2011). The phytochemical investigations on *Rhynchosia pseudo-cajan* Cambess. revealed presence of many terpenoids and flavonoids such as *β*-amyrin, mupinensisone, alpinetin, pinostrobin, 3-oxoleane-9(11),12-diene-30-oic acid, 4′,5,6-trihydroxy-7-methoxyflavone, 2′,4′,5,7-tetrahydroxyisoflavone and naringin (Riaz *et al*., 2012).

Keeping in view the medicinal importance of *Rhynchosia pseudo-cajan*, we have characterized various organic and aqueous fractions of this plant to assess their antibacterial activity, antifungal potential, enzyme inhibition activity and hemolytic activity, as such type of detailed work has not been performed yet on this plant. Antibacterial activity was checked against two types of gram-positive bacteria such as *Bacillus subtili s*and *Staphylococcus aureus* and two types of gram-negative bacteria such as *Escherichia coli* and *Pasturella multocida* 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, acetyl cholinesterase and lipoxygenase. The fractions were also checked for their toxicity by their hemolytic effects.

MATERIALS AND METHODS

Plant material

The plant, *Rhynchosia pseudo-cajan* Cambess, was collected from district Kotli, Azad Kashmir in July 2012. It was identified by a taxonomist, Dr. Muhammad Ajaib, Dept. of Botany, GC University, Lahore, Pakistan. The voucher specimen "GC.Herb.Bot.623" has been placed in herbarium of same university.

Extraction and fractionation

The shade-dried and ground whole plant (15 kg) was extracted exhaustively with the methanol (20L × 5) at the room temperature. Methanolic extract was evaporated on rota-vapour to yield the residue (950 g), that was dissolved in the distilled water (2 L) and after this partitioned with *n*-hexane (1.3 L × 4), $CHCl_3$ (1.3 L × 4), EtOAc (1.3 L × 4) and *n*-BuOH (1.3 L × 4) respectively. All these organic fractions as well as remaining water fraction were concentrated separately on rota-vapour. The yields of *n*-hexane soluble fraction, $CHCl_3$ soluble fraction, EtOAc soluble fraction, *n*-BuOH soluble fraction and remaining aqueous fraction were 203g, 226g 198g, 185g and 138g, respectively. The residues obtained were further used to assess their *in vitro* antibacterial and antifungal potential, enzyme inhibition and hemolytic activities.

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 (100 µL), contained 10^7 CFU/ml (colony-forming units) of bacteria cells on the Nutrient agar medium. The 9 mm 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 4 ºC 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.

Enzyme inhibition assays

α-Glucosidase assay

The *α*-glucosidase inhibition activity was done by the slightly modified standard method (Pierre *et al.*, 1978).

The reaction mixture contained 70µL of 50mM phosphate buffer saline having pH 6.8 and 10µL (0.5mM) of test compound, followed by adding 10µL (0.057 units) enzyme, thus making total volume upto 100µL. Contents were mixed thoroughly and incubated at 37ºC for 10 minutes. Then reading was taken at 400 nm. Then 10µL of 0.5mM substrate (*p*-nitrophenylglu-copyranoside) was added to initiate the reaction. Acarbose was taken as the positive control. It was incubated for 30 minutes at 37ºC and then absorbance was measured, using Synergy HT micro plate reader, at 400 nm. All readings were taken in triplicates. Calculation of % inhibition was done by following equation:

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

Butyryl cholinesterase and Acetyl cholinesterase assay

Butyryl cholinesterase (BChE) and Acetyl cholinesterase (AChE) inhibition activity was performed by the slightly modified standard method (Ellman *et al.,* 1961). The reaction mixture contained 60µL (50mM) $Na_2H\ PO_4$ buffer having pH 7.7 and ten µL (0.5mM $well^{-1}$) test compound, followed by adding 10µL BChE(0.5 unit $well^{-1}$), thus making total volume up to 100mL. Contents were mixed thoroughly and absorbance was noted at 405 nm. Then the reaction mixture was incubated at 37ºC for 10mins. Then 10µL (0.5mM $well^{-1}$) of substrate (butyrylthiocholine bromide) was added to initiate the reaction followed by adding 10 µL DTNB (0.5mM $well^{-1}$). It was incubated for 30 minutes and then at 37ºC and then absorbance was noted at 405 nm using Synergy HT micro plate reader. Eserine (0.5mM $well^{-1}$) was taken as the positive control. After 30min of incubation at 37ºC, absorbance was measured at 405 nm. Synergy HT (BioTek, USA) 96-well plate reader was used in all experiments. All readings were taken in triplicates. Calculation of % inhibition was done by following equation:

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

Lipoxygenase assay

Lipoxygenase (LOX) inhibition activity was checked by slightly modified standard method (Baylac and Racine, 2003*;* Evans, 1987; Tappel, 1953). Reaction mixture contained sodium phosphate buffer, 140µL (100mM) having pH 8.0, 15µL(600U) purified lipoxygenase enzyme (Sigma, USA) and 20 µL test compound thus making total volume up to 100mL. Contents were mixed thoroughly and absorbance was noted at 234 nm. It was incubated at 25°C for 10 minutes. Then 25µL substrate solution was added to initiate the reaction. Absorbance change was noted at 234 nm after 6 minutes, using Synergy HT (BioTek, USA) 96-well plate reader. Baicalein (0.5 mM $well^{-1}$) was taken as positive control. Calculation of percentage inhibition was done by following formula.

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

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).

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 (3 mL), freshly obtained, was taken and centrifuged at 1000x*g*for 5 minutes. After discarding plasma, cells were washed with 5 mL of chilled (4ºC) 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 37ºC 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,100 µL of supernatant was taken, and then diluted with chilled (4ºC) 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 lysisfor each sample was done by following formula:

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

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

RESULTS

Antibacterial activity

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.

The results of antibacterial activity have been summarized in fig. 1. It was observed that *n*-hexane soluble fraction showed very less activity. Chloroform fraction and ethyl

acetate soluble fraction showed good activity against *Staphylococcus aureus* (26 and 22 mm respectively), *Bacillus subtilis* (26 and 28 mm respectively) and *Escherichia coli* (22 and 20 mm, respectively). The *n*-butanol soluble fraction showed good activity against *Staphylococcus aureus* (20 mm) and *Bacillus subtilis* (28 mm) while aqueous fraction was found only active against *Bacillus subtilis* (22 mm). The results were compared with Streptomycin Sulfate, a reference antibacterial drug. These observations have been made on the basis of measurements of zones of growth inhibition in mm.

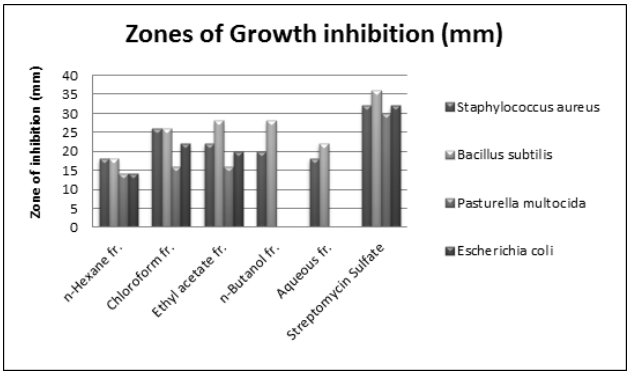


Fig. 1: Zones of inhibition (mm) of various fractions of *Rhynchosia pseudo-cajan* against Gram-positive and Gram-negative bacteria

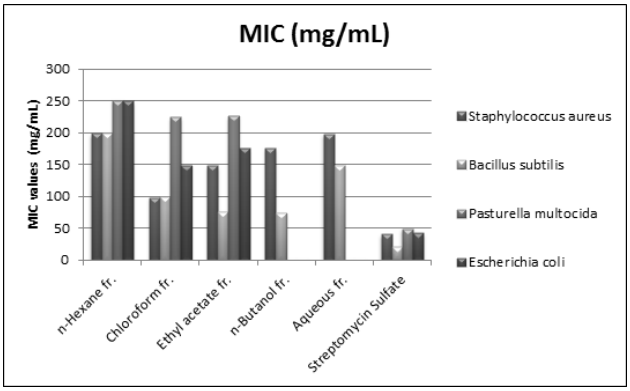


Fig. 2: MIC of various fractions of *Rhynchosia pseudo-cajan* against Gram-positive and Gram-negative bacteria.

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. Lowest MIC was shown by *n*-butanol fraction and ethyl acetate soluble fraction against *Bacillus subtilis i.e.* 74 and 76 mg/ml respectively. Chloroform soluble fraction also showed good value of MIC against *Staphylococcus aureus* and *Bacillus subtilis i.e*. 98 and 99, respectively.

Antifungal Activity

Antifungal activity of all the studied fractions of *Rhynchosia pseudo-cajan* 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. It was observed from the results (fig. 3) that *n*-hexane soluble fraction showed very less activity. Chloroform, EtOAc and *n*-BuOH soluble fractions showed good activity only against *G. lucidum* i.e. 20, 22 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.

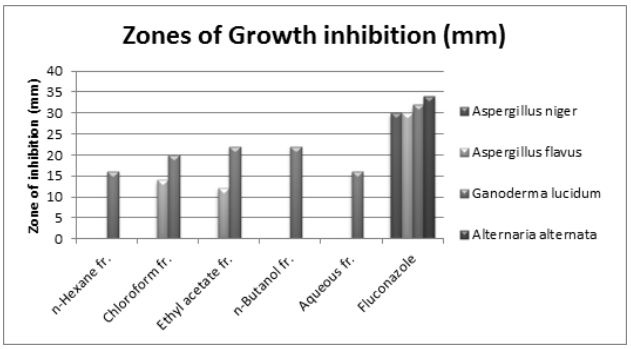


Fig. 3: Zones of inhibition (mm) of various fractions of *Rhynchosia pseudo-cajan* against fungi.

MIC was also calculated and results have been summarized in fig. 4. Chloroform, ethyl acetate and *n*-BuOH fraction showed good MIC values against *G. lucidum i.e.* 178, 150 and 149mg/mL, respectively. Statistical analysis of variance (ANOVA) and the Duncan *t*-test supported the experimental results obtained. The results mentioned as good were found significant ($p<0$).

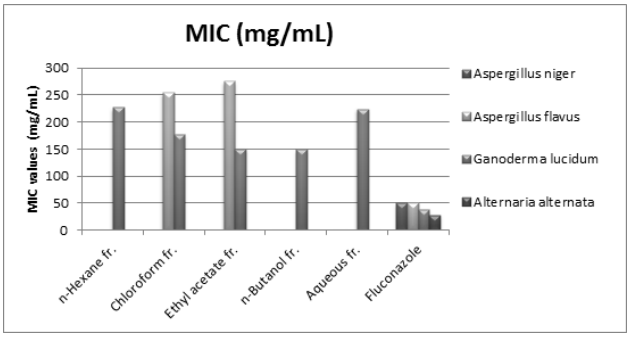
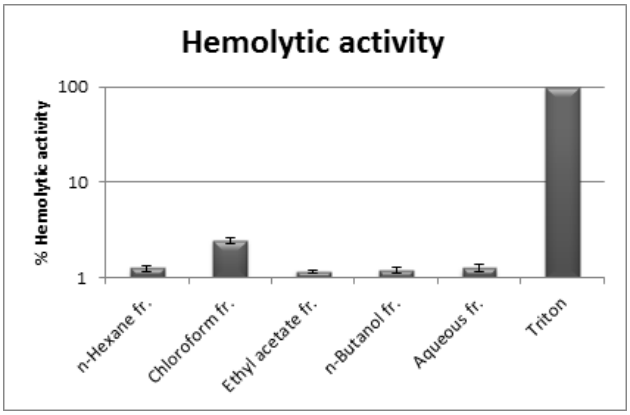


Fig. 4: MIC of various fractions of *Rhynchosia pseudo-cajan*against fungi.

Enzyme inhibitory potential

Enzyme inhibition activities of the studied fractions were checked against four enzymes i.e. α-glucosidase, butyryl cholinesterase, acetyl cholinesterase and lipoxygenase and the results have been summarized in the table 1. It was observed from the results (table 1) that remaining aqueous fraction possessed very good activity against α-glucosidase, even greater then acarbose, a reference standard drug. It showed 85.43±0.16% inhibition of enzyme at concentration of 0.1 mg/mL. Its IC_{50} value was calculated as 29.81±0.12 µg/mL as compared to acarbose

which showed IC_{50} value 38.62±0.04 µg/mL. Chloroform and ethyl acetate soluble fractions also showed good activity having IC_{50} values 99.13±0.55 and 87.46±0.69 µg/mL, respectively. Chloroform and aqueous fraction showed moderate activity against butyryl cholinesterase having IC_{50} values 71.61±0.58 and 54.11±0.21µg/mL, respectively. None of the fractions showed activity against acetyl cholinesterase. Ethyl acetate soluble and remaining aqueous fraction showed good activity against lipoxygenase, having IC_{50} values 39.57±0.91 and 41.42±0.25µg/mL, respectively as compared to baicalein, a reference standard, which showed IC_{50} value 22.4±1.3 µg/mL.



Note: Y-axis has been displayed by using log 10 based scale.

Fig. 5: Hemolytic activities of various fractions of *Rhynchosia pseudo-cajan.*

Hemolytic activity

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 *Rhynchosia pseudo-cajan* showed very less toxicity. The *n*-hexane, $CHCl_3$, EtOAc, *n*-BuOH soluble and remaining aqueous fraction showed toxicity values 1.27±0.09%, 2.45±0.17%, 1.17±0.06%, 1.21±0.07% and 1.27±0.10 %, respectively.

DISCUSSION

Antibacterial activity

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 appressoria, lignifications and defensive proteins. These metabolites inhibit the spore germination of microbes. Parthenolide present in *Tanacetum parthenium* is very effective against the bacterial infections (Walton and Brown, 1999).

The results (fig. 1) mentioned as good were found significant ($p<0.05$). As found in literature, the OH group present in phenolics, steroids, coumarins and flavonoids especially at C-6 and C-8 position of flavonoids 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). The good antibacterial activity of chloroform fraction was attributed to the presence of icosyl-*p*-hydroxybenzoate, mupinensinone, alpine tin and pinostrobin in this fraction (Riaz *et al*., 2012) because these contain active hydroxyl groups. The good antibacterial activity of EtOAc soluble fraction was attributed to the presence of 12-oleanen-3-ol and the flavonoids such as naringin, 4′,5,6-trihydroxy-7-methoxy flavone and 2′,4′,5,7-tetrahydroxy isoflavone isolated from this fraction (Riaz *et al*., 2012).

Antifungal activity

The bioactive molecules, present in medicinal plants, inhibit the fungal growth.Some of the fungitoxic compounds from the plants are: Luteone (in *Lupinu salbus*), Sakuranetin (in *Ribus nigrum*), and Nobiletin (in *Citrus* spp.). Thus good antifungal activity shown by polar fractions might be attributed to presence of many terpenoids and flavonoids in polar fractions (Riaz*et al*., 2012) as discussed earliar.

Enzyme inhibitory potential

Many flavonoids as well as hydoxycinnamic 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 enzymes for example quercetin is used for the inhibition of 5-lipoxygenase and the xanthine oxidase is inhibited by C-7 hydroxyl group of the flavonoids (Walton and Brown, 1999).

The major function of the *α*-glucosidase is to hydrolyze1, 4glycosidic linkage from non-reducing end of *α*-linked oligosaccharide, *α*-glucosides and *α*-glucans substrates, to produce the *α*-D-glucose (Chiba, 1997). The *α*-Glucosidase inhibitors are the molecules or the compounds that are used as the oral anti-diabetic drugs, for patients with the type-2 diabetic mellitus. Postprandial hyperglycemia has very important role in the development of the type-2 diabetes, and the complications associated with this disease such as neuropathy, nephropathy, macroangipathy and microangiopathy (Baron, 1998). Enzyme inhibitors can retard liberation of the *D*-glucose of disaccharides and oligosaccharides from the dietary complex carbohydrates and hence delay absorption of glucose, resulting in the reduced

Table 1: Enzyme inhibition activities of various fractions of *Rhynchosia pseudo-cajan* 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 fraction	1.41±0.09	NIL	1.58±0.16	NIL	1.12±0.08	NIL	2.62±0.20	NIL
$CHCl_3$ fraction	56.39±0.69	99.13±0.55	71.01±0.87	71.61±0.58	29.55±0.95	NIL	14.54±0.97	NIL
EtOAc fraction	61.36±0.25	87.46±0.69	22.12±0.66	NIL	3.05±0.49	NIL	66.23±0.31	39.57±0.91
n-BuOH fraction	9.48±0.58	NIL	6.52±0.76	NIL	36.18±0.37	NIL	22.98±0.82	NIL
Aqueous fraction	85.43±0.16	29.81±0.12	57.12±0.36	54.11±0.21	45.61±0.47	NIL	62.97±0.55	41.42±0.25
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

postprandial hyperglycemia (Harold and Lebovitz, 1997). Thus, inhibition of *α*-glucosidase enzyme is considered as essential in managing the type-2 diabetes.

Acetyl and butyrylcholinesterases terminate acetylcholine at the cholinergic synapses (Cygler *et al*., 1993). AChE catalyze the hydrolysis of acetylcholine, a neurotransmitter, and thus termination of nerve impulse takes place (Quinn, 1987). It has been found that BChE is present in the Alzheimer's plaques in significantly higher quantities. So, search for the new cholinesterase inhibitors has been considered as significant and the ongoing strategy to establish new drugs for treatment of the Alzheimer's disease as well as other related diseases (Bertaccini and Substance, 1982). Cholinesterase inhibitors enhance the amount of acetylcholine, available for neuromuscular and neuronal transmission, by their ability to reversibly or irreversibly (Gauthier, 2001). Varieties of neuromuscular and neurological disorders involve the diminution of the cholinergic activity. The ligands which can cause inhibition of breakdown of acetylcholine are often considered as most effective treatments. Lipoxygenase enzymes catalyze the addition of molecular oxygen to unsaturated fatty acids, which containcis-1, 4-pentadiene system, and produce hydro peroxides (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).

As obvious from the results that chloroform, ethyl acetate and aqueous fractions showed good activities against enzymes, so these might be taken into consideration for further pharmacological studies.

Hemolytic activity

Plant derived natural compounds have been gained much attention due to their potential to act as chemo preventive and cytotoxic activity. To perform hemolytic assay is very important to determine whether the specific drug that possesses antioxidant, antimicrobial and other bioactivities, can be used in the pharmacological applications. The *in vitro* hemolytic activities are now-a-days becoming new area of research in the drug lead discoveries (Mukherjee and Rajasekaran, 2010). 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 plants (Oliveira *et al*., 2009). 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, *n*-BuOH and remaining aqueous fraction of plant have potent antimicrobial effects against wide spectrum of bacteria.$CHCl_3$, EtOAc, *n*-BuOH soluble fractions showed good activity against fungus *G. lucidum*. Remaining aqueous fraction possessed very good activity against α-glucosidase, even greater then acarbose, a reference standard drug. Its IC_{50} value was found as 29.81±0.12µg/mL as compared to acarbose (IC_{50} 38.62±0.04µg/mL). $CHCl_3$ and EtOAc soluble fractions also showed good activity against *α*-glucosidase. EtOAc and remaining aqueous fraction also showed good activity against lipoxygenase. All the studied fractions showed very less toxicity i.e. <2.5%. 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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