ANTIMICROBIAL AND ANTIHYPERGLYCEMIC ACTIVITIES OF *ACACIA MODESTA* LEAVES

Sunil Jawla[1*], Y. Kumar[1], M.S.Y. Khan [2]

[1]Department of Pharmaceutical Chemistry, I.T.S. Paramedical College (Pharmacy), Delhi-Meerut Road, Murad nagar, Ghaziabad, Uttar Pradesh, India - 201 206. [2]Department of Pharmaceutical Chemistry, Jamia Hamdard (Deemed University), Hamdard nagar, New Delhi, India - 110 062.

[*]**Correspondence author -**
Phone No. +911232-225380, 225381, 225382, +919456039139
Telefax: +911232-225380, 225381, 225382

Email address: suniljawla@its.edu.in

Running Head

Antimicrobial and antihyperglycemic activities of *A. modesta*

Summary

Acacia modesta twigs are used as miswak (tooth brush) for cleaning teeth and for various ailments traditionally in India and Pakistan. The present study was aimed to screen the antimicrobial and antihyperglycemic activity of *A. modesta* leaves.

Petroleum ether, EtOH, and EtOH: water (1:1) extracts of *Acacia modesta* leaves were investigated for antibacterial and antifungal activity against standard strains of *Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhimurium* and *Candida albicans, Cryptococcus albidus* against reference standards amikacin and clotrimazole respectively. The EtOH and EtOH: water (1:1) extracts were also administered to both normal and alloxan induced diabetic rats. The blood glucose levels were measured on 0, 1, 3, 5, 7th day after oral administration of extracts at doses of 100 and 300 mg/kg/day.

The EtOH and EtOH: water (1:1) extracts exhibited antimicrobial activity with minimum inhibitory concentrations ranging from 4.57-26.5 and 8.9-32.5 µg/ml respectively. Treatment of diabetic rats up to a week with the extracts reversed the permanent hyperglycemia. The EtOH extract (100 mg/kg) & EtOH: water extract (300 mg/kg) were found 12.34 and 6.08 % more potent hypoglycemic than standard oral hypoglycemic drug, glibenclamide 0.2 mg/kg b.w., respectively.

Key words

Leaves, zone of inhibition, minimum inhibitory concentration, antimicrobial activity, antihyperglycemic activity.

1. Introduction

Herbs are staging a comeback and herbal 'renaissance' is occurring all over the world. Due to alarming incidence of antibiotic resistance in bacteria and fungi; there is need for new and effective therapeutic agents for bacterial and fungal infections (1-2). Plants have been used for the treatment of various ailments since the ancient time and were explored for the discovery of potentially useful antimicrobial and antihyperglycemic leads (3-8).

Acacia modesta belongs to the family *Fabaceae* (subfamily-*Mimosoideae*). It is found in Pakistan (N.W.F Province Punjab, Baluchistan), India (Punjab, Utter Pradesh, Himachal Pradesh), and Afganistan. It is widely growing in sub-Himalayan tract and shivalik hills especially in Beas and Sutlej valleys. It is used as miswak (tooth brush) for teeth cleaning in various parts of India and Pakistan due to its antimicrobial properties. It is commonly known as Black sally, Phulai, Phulahi, Khaor, Paloz.

The chemical constituents of *Acacia modesta* reported are α amyrin, betulin, octacosanol and ε-sitosterol, γ sitosterol, pinitol, octacosanol, hentriacontanol and α-amino-β-oxalylaminopropionic acid (neurolathyrogen) (9-10). The leave, flower, fruit, gum, bark and wood of *A. modesta* wall is commonly used for medicinal and fuel purposes. The earliest chronicled mention is in the Ayurvedic treatise, the Charka Samhita (100 A.D.), which recommends miswak for cleaning teeth. The Bhavprakasha Nighantu, also known as the Indian Materia Medica (1500 A.D.), cites the plant for infections and diabetes. The other biological activity reported of *Acacia modesta* are antiacetylcholinsterase and butyrylcholinsterase (11), antidiabetic (12-13), antibacterial (14-17), gum washing and stopping bleeding (18). *A. modesta, A. nilotica* and *Pongamia glabra* and *Salvadora persica* twigs were found to be highly effective tools in removing oral deposits and exhibited antimicrobial properties (19). *A. modesta* possesses peripheral analgesic and anti-inflammatory properties, with analgesic effects partially associated with the opioid system (20).

In the present study, leaves of *A. modesta* were studied for antimicrobial and antihyperglycemic activities.

2. Materials and Methods

2.1. Collection of Plant material

The leaves of *Acacia modesta* were collected from Village Konha, District Solan (Himachal Pradesh) in the month of January 2010. The plant was identified by plant taxonomist Dr. A.K. Sharma, Department of Botany, Multanimal Modi (P.G.) College, Modinagar, Ghaziabad (U.P.), India. Voucher specimens (MMCM/02/057) were deposited in the Herbarium of the Department of Botany, Multanimal Modi (P.G.) College, Modinagar, Ghaziabad (U.P.), India, for future reference.

2.2 Preparation and Extraction of Plant Material

The leaves were shade dried and powdered in electrical grinder. Powdered leaves (500 g) were successively extracted for 48 hours with petroleum ether, EtOH using continuous hot soxhlation. The residue was further soaked in the conical flask containing EtOH: water (1:1), and shaked for 48 hours at 120-130 rpm. The weights of crude extracts obtained were 2.5g (0.5%), 45.6g (9.1%) & 55.2g (11%, w/w, yield) for petroleum ether, EtOH and EtOH: water (1:1) extracts respectively.

2.3. Phytochemical Analysis

The leaves extracts were further subjected to preliminary quantitative tests for the presence of carbohydrate, protein, steroid, glycoside, saponin, alkaloid, tannin, phenolic compound and flavonoid according to standard quantitative and qualitative methods (21-22).

2.4. Microorganisms

Bacterial and fungal strains were obtained from Department of Pharmaceutical sciences, I.T.S Paramedical College (Pharmacy) and I.T.S Dental College, Murad Nagar India. Mueller Hinton agar and

Saboraud's dextrose agar (SDA) were procured from Himedia Laboratories.

2.5. Disc Diffusion Method

Antibacterial activity against standard strains of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (MTCC-121), *Bacillus cereus* (MTCC-430), *Klebsiella pneumoniae* (109), *Proteus mirabilis* (MTCC-1429), *Salmonella typhimurium* (MTCC-98), *Streptococcus pneumoniae* (MTCC-2672) using amikacin as reference, and antifungal activity against standard strains of *Candida albicans* (MTCC-183), *Cryptococcus albidus* (MTCC-2661) using clotrimazole as reference were evaluated using the disc diffusion method. Sterile filter paper discs (Whatman No. 1, diameter 5 mm) were loaded with 100 µl of leaves extracts (5 mg/disc), reference drugs; amikacin (10 µg/disc; for bacteria) and clotrimazole (20 µg/disc; for fungi). The discs were completely saturated with the extracts and dried. The bacteria and fungi were cultured in Muller Hinton Broth (MHB) and Potato Dextrose Broth (PDB) respectively and incubated at 37˚C for 24 hours. Then, the active cultures were inoculated into 10 ml of cultures (MHB/PDB) and incubated at 37˚C for 15 hours. Microorganisms were diluted with MHB/PDB to obtain bacterial/ fungi count of 5-10X10^5 CFU/ml. The loaded discs were placed on tryptone agar plates (for bacteria) and Saboraud's dextrose agar plates (for fungi) inoculated on the surface with each microorganism culture (0.01 ml) and incubated at 37ºC, for 24-48 hrs. The discs were tested in triplicate, including blank (disc containing solvent only) and the standard drugs. Zones of inhibition and the mean of zones of inhibition were calculated (23-25).

2.6. Minimum Inhibitory Concentration

Minimum inhibitory concentrations (MIC's) of all the extracts were determined by micro dilution method (26). It is carried out by the disc diffusion test of different concentration of the plant extracts. The minimum concentration of extracts that inhibits the growth of bacteria and fungi were noted as MIC values (Table 3).

2.7. Experimental animals

Male albino Wistar rats (**150-200g**) of age 8-12 weeks old were procured from Institutional Animal House (Reg. No. 1044/c/07/CPCEA), I.T.S Paramedical College, Murad Nagar, Ghaziabad, Uttar Prasesh, India, for the present study. Rats were housed under standard conditions (25 °C, 12 h light and 12 h dark cycle, 60% humidity, water *ad libitum*), and acclimatized to the laboratory conditions for 7 days.

2.8. Blood glucose level determination

Blood glucose levels (mg/100ml) were determined using an Accu-Check active (Roche Diagnostic GmbH, Germany), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time intervals.

2.9. Acute toxicity studies

Acute oral toxicity was conducted according to OECD (OECD/OCDE 2001, 425) guidelines based on up and down procedure. A limit dose (5000mg/kg;oral) of *A. modesta* leaves extracts were used to estimate LD_{50}. The doses (100 and 300 mg/kg/day) of EtOH extracts of *A. modesta* leaves were used in the present study (27).

2.10. Induction of Diabetes

Diabetes was induced in albino rats (Wistar strain) by a single intraperitoneal injection of alloxan monohydrate (CDH, Bombay) in normal saline (120 mg/kg) after overnight fasting for 12 h. The fasting blood glucose level was measured after 48 h of alloxan injection. The rats with effective and permanent elevated plasma glucose levels (above 300mg/100ml) were used.

2.11. Effect of extracts on glucose-loaded normal rats

The oral glucose tolerance test was carried out after overnight fasting (16 h) of the normal rats. Vehicle (distilled water), EtOH extracts of the leaves of *A. modesta* at two doses (100 and 300 mg/kg) and standard oral hypoglycemic agent- glibenclamide

(Daonil® Sanofi Aventis Pharma. Ltd. Mumbai, India) (0.2 mg/kg) were administered to six different groups of rats (n=6). Glucose (4 g/kg) was fed 60 min after treatment with leaves extracts. Blood samples were withdrawn from the tip of tail after 0, 30, 60, 90, 120, 240 and 360 minutes from normal control and experimental animals for estimation of blood glucose levels.

2.12. Measurement of blood glucose level in diabetic rats up to 7 day

Rats were divided randomly in four groups of 6 rats each. After overnight fasting; diabetic rats were treated orally with vehicle, EtOH extracts of *A. modesta* leaves (100 and 300 mg/kg) and glibenclamide (0.2 mg/kg) daily up to 7 days. Blood samples were collected from the tip of tail on 0, 1, 3, 5, 7th day from control and experimental animals.

2.13. Statistical analysis of data

Results were analyzed statistically on the GraphPad instat version 5 software using Student's t test for paired data and one way ANOVA using Dunnett's Multiple Comparison Test. A difference in the mean values of $P<0.05$ were considered significant.

3. Result and discussion

Phytochemical screening of *A. modesta* leaves showed presence various phytochemicals (Table 1). The antibacterial and antifungal properties of leaves extracts against medically important pathogens were measured by the presence or absence of zones of inhibition and the MIC values. The antibacterial activity of the EtOH and EtOH: water (1:1) extracts of the *A. modesta* leaves were very effective against most of the bacteria tested and especially against *Bacillus* species and *Pseudomonas aeruginosa*. The EtOH extract was shown to have significant antifungal activity against *Candida albicans*, *Cryptococcus albidus* (Table 2).

Table 1. The Qualitative Phytochemical Analysis of the extracts of *A. modesta* leaves.

Ext.	Carbohydrate	Protein	A. A	Steroid	Alkaloid	Glycoside	Tannin	Flavonoid	Saponin
A	-	-	-	+	-	-	-	+	-
B	+	-	-	+	+	+	+	+	+
C	+	-	-	-	+	+	+	+	+

A- Petroleum ether extract; B - EtOH extract; C - EtOH: water (1:1) extract; A.A- Amino acid; "+"- Presence; "-" –Absence

Table 2. Antimicrobial activity of extracts of *A. modesta* leaves by disc diffusion assay.

Microorganism	Zone of Inhibition (mm)				
	A	**B**	**C**	**Amik**	**Clotr**
Staphylococcus aureus (ATCC-25923)	Na	6.5±1.9	4.2±0.9	13.9±0.12	Nd
Escherichia coli (MTCC- 443)	Na	9.0±1.5	5.00±1.2	2.1±0.9	Nd
Pseudomonas aeruginosa (ATCC-9027)	Na	12.1±0.7	11.2±1.1	13.1±0.7	Nd
Bacillus subtilis (MTCC-121)	Na	12.7±0.3	11.0±0.9	13.9±1.0	Nd
Bacillus cereus (MTCC-430)	Na	14.6±0.7	9.0±1.5	17.0±0.9	Nd
Klebsiella pneumonia (MTCC-109)	Na	4.3±0.3	6.3±1.6	5.3±1.3	Nd
Proteus mirabilis (MTCC-1429)	Na	2.3±1.5	5.3±0.4	3.0±1.5	Nd
Salmonella typhimurium (MTCC-98)	Na	1.0±1.1	1.5±0.2	12.7±0.4	Nd
Streptococcus pneumonia (MTCC-2672)	Na	4.3±0.6	1.8±0.4	5.0±0.8	Nd
Candida albicans (MTCC-183)	Na	7.0±0.7	1.0±0.9	Nd	13.6±0.8
Cryptococcus albidus (MTCC-2661)	Na	6.5±0.6	2.1±1.5	Nd	13.9±0.7

Each value represents mean ± SEM (n = 3); A - Petroleum ether extract; B - EtOH extract; C - Ethanol: water (1:1) extract; Amik – Amikacin; Clotr – Clotrimazole; Na-No activity; Nd – Not done

However petroleum ether extract did not inhibit the growth of any of the tested bacterial and fungal species. Since EtOH and EtOH: water (1:1) extracts were shown to be effective, hence MICs of these extracts were performed. The EtOH and EtOH: water (1:1) extracts showed MICs of 4.57-26.5 and 8.9-32.5 µg/ml respectively (Table 3). EtOH extract was highly effective against *Pseudomonas aeruginosa, Bacillus subtilis* and *Bacillus cereus* with an MIC of 4.57, 6.59, 8.06 µg/ml. Both extracts showed effective inhibition towards *Candida albicans* and *Cryptococcus albidus*. MIC studies further supported the data obtained by agar diffusion assay indicating zones of inhibition. This study proves the traditional use of *A. modesta* as antibacterials.

Table 3. Minimum Inhibitory Concentrations of extracts of *A. modesta* leaves.

Microorganism	MIC (µg/ml)			
	B	**C**	**Amik**	**Clotr**
Staphylococcus aureus (ATCC-25923)	13.21	21.70	0.93	Nd
Escherichia coli (MTCC- 443)	21.52	32.50	3.90	Nd
Pseudomonas aeruginosa (ATCC-9027)	4.57	13.25	0.98	Nd
Bacillus subtilis (MTCC-121)	6.59	15.50	0.48	Nd
Bacillus cereus (MTCC-430)	8.06	21.25	3.20	Nd
Klebsiella pneumonia (MTCC-109)	21.50	22.00	0.48	Nd
Proteus mirabilis (MTCC-1429)	22.00	21.91	6.69	Nd
Salmonella typhimurium (MTCC-98)	26.50	13.07	0.89	Nd
Streptococcus pneumonia (MTCC-2672)	22.00	23.60	0.53	Nd
Candida albicans (MTCC-183)	5.50	9.50	Nd	0.48
Cryptococcus albidus (MTCC-2661)	7.80	8.90	Nd	0.57

MIC-Minimum Inhibitory Concentration; B - EtOH extract; C - EtOH: Water (1:1) extract, nd – Not done; Amik – Amikacin; Clotr – Clotrimazole

The EtOH & EtOH : water (1:1) extracts of *A. modesta* leaves at the dose of 100 and 300 mg/kg reduced the blood glucose level significantly after 30 minutes in glucose loaded rats; which was comparable to the glibenclamide (0.2 mg/kg) ($P<0.05$)(Table 4).

Further, treatment of diabetic rats up to 7 days with leaves extracts at 100 and 300 mg/kg/day, p.o. significantly reversed the permanent hyperglycemia induced by alloxan monohydrate. The highest anti-hyperglycemic effect was observed by the EtOH extract of leaves at 100 mg/kg. The EtOH extract (100 mg/kg) & EtOH: water extract (300 mg/kg) were found 12.34 and 6.08 % more potent than standard oral hypoglycemic drug, glibenclamide 0.2 mg/kg b.w., respectively (Table 5). The LD_{50} for *A. modesta* extracts were found to be >5000mg/kg (p.o.) in albino wister rats. Morbidity and sign of toxicity was not observed in any of the five normal rats tested with extracts.

Table 4. Antihyperglycemic effect of *A. modesta* leaves extracts in glucose loaded normal hyperglycemic rats.

Treatment	Mean blood glucose concentration ±SEM (mg/dl)						
	0 min	**30 min**	**60 min#**	**90 min**	**120 min**	**240 min**	**360 min**
Control	94.67±1.76	95.00±1.65	95.33±1.33	156.50±2.16	137.83±4.03	113.50±2.91	99.67±2.39
Glib (0.2 mg/kg)	96.33±1.76	78.17±3.46***	65.67±2.75***	93.00±3.28***	81.67±3.01***	66.33±3.03***	76.00±3.39***
B (100 mg/kg)	92.50±1.45	96.00±1.53	92.33±1.02	142.33±4.26**	136.17±2.47	104.33±2.20	95.50±1.34
B (300 mg/kg)	92.67±1.17	91.67±1.78	86.33±1.67**	127.67±3.09***	116.83±2.06***	95.50±2.99**	91.33±2.46
C (100 mg/kg)	93.33±1.41	91.83±1.17	90.67±1.87	134.33±2.31***	128.17±2.52	97.00±2.52**	92.17±1.97
C (300 mg/kg)	92.83±1.30	93.00±1.53	93.50±1.78	136.17±1.30***	131.17±2.32	98.17±3.82**	95.50±1.77

SEM - Standard error of the mean; N=6, # Glucose load (4g/kg), **p<0.01, significantly different compared to control, ***p<0.001, significantly different compared to control, Glib. - Glibenclamide, B - EtOH extract; C – EtOH: water (1:1) extract.

Table 5. Antihyperglycemic effect of *A. modesta* leaves extracts in diabetic rats up to 7 days.

Treatment[#]	**Mean blood glucose concentration ±SEM (mg/dl)**				
	0 day	**1st day**	**3rd day**	**5th day**	**7th day**
Control	95.50±2.43	93.83±1.62	96.00±1.34	96.83±1.91	94.83±2.30
Diabetic control	309.00±2.63	309.17±2.68	311.33±3.45	316.17±5.17	316.33±3.28
Glib. (0.2 mg/kg)	313.83±4.37	284.67±6.64**	236.17±6.63***	178.00±5.40***	93.17±3.41***
B (100 mg/kg)	315.17±4.09	274.33±4.19***	232.67±3.59***	155.83±4.85***	84.50±2.50***
B (300 mg/kg)	314.17±3.30	270.83±1.94***	231.33±5.60***	149.17±5.87***	81.67±3.16***
C (100 mg/kg)	316.67±3.76	286.83±3.85**	244.17±7.36***	163.33±4.75***	87.50±2.65***
C (300 mg/kg)	313.33±3.99	286.83±4.14**	245.83±5.58***	165.33±7.71***	89.67±2.98***

#mg/kg/day for 7 days, S.E.M - Standard error of the mean; N=6, **p<0.01, significantly different compared to diabetic control, ***p<0.001, significantly different compared to diabetic control. Glib. – Glibenclamide, B - EtOH extract; C – EtOH: water (1:1) extract.

The present antimicrobial and antihyperglycemic study is the first systematic positive report on the efficacy of EtOH and EtOH: water (1:1) extracts of *A. modesta* leaves. The polar extracts might be effective due to the fact that the majority of the traditional medicines were prepared using water as the medium.

4. Conclusion

The EtOH and EtOH: water (1:1) extracts showed antimicrobial activity towards both Gram-positive, Gram-negative organisms and the fungi which support the traditional use of this plant in the Ayurvedic treatise and the Charka Samhita (100 A.D.).

The present study also suggests that EtOH and EtOH: water (1:1) extracts of *A. modesta* leaves have potent anti-hyperglycemic properties. Treatment up to week with these extracts reversed the permanent hyperglycemia induced by alloxan. Phytochemical screening showed the presence of glycosides, flavonoids, saponins and tannins in the leaves extracts of *A. modesta*. Many reports suggested the hypoglycemic properties of flavonoids (28-30). This may be the reason for antidiabetic properties of *A. modesta* leaves. Further biochemical, toxicological and pharmacological investigations are required to better characterize the active principle(s) responsible for antimicrobial and antidiabetic properties.

5. Acknowledgements

We would like to thank Mr. Harikesh Kalonia, University Institute of Pharmaceutical sciences, Punjab University, India, for his excellent technical support and Department of Microbiology, I.T.S. Dental College, Murad nagar, Ghaziabad, India for providing necessary facilities to carry out above stated work.

6. References

1. Bhavnani SM, Ballow CH. New agents for Gram-positive bacteria. Current Opin Microbiol 2000; 3:528-534.
2. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. J Ethnopharmacol 1998; 62:183-193.
3. Cowan MM. Plant products as antimicrobial agents. Clin.Microbiol Rev 1999; 12:564-582.
4. Chariandy CM, Seaforth CE, Phelps RH, Pollard GV, Khambay BPS. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. J Ethnopharmacol 1999; 64:265-270.
5. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov 2005; 4(3):206-220.
6. Bnouham M, Ziyyat A, Mekhfi H, Tahri A, Legssyer A. Medicinal plants with potential antidiabetic activity. Int. J. Diabetes & Metabolism 2006; 14:1-25.
7. Frode TS, Medeiros YS. Animal models to test drugs with potential antidiabetic activity. J Ethnopharmacol 2008; 115:173–183.
8. Akansha, Srivastava AK, Maurya R. Antihyperglycemic activity of compounds isolated from Indian medicinal plants. Indian J Exp Biol2010; 46:294-298.
9. Joshi KC, Tholia MK, Sharma T, Chemical examination of *Acacia modesta*. Planta Med 1975; 27(3):281-283.
10. Quereshi MY, Pilbeam DJ, Evans CS, Bell EA. The Neurolathryogen, α-Amino-β-oxalylaminopropionic Acid in Legume Seeds. Phytochemistry 1977;16:477-479.
11. Khan RA, Bukhari IA, Nawaz SA, Choudhary MI. Acetylcholinesterase and butyrylcholinesterase inhibitory potential of some Pakistani medicinal plants. J Basic Appl Sci 2006; 2:7-10.
12. Rahman AU, Zaman K. Medicinal plants with hypoglycemic activity. J Ethnopharmacol 1989; 26:1-55.
13. Singh KN, Chandra V, Barthwal KC. Hypoglycemic activity of *Acacia arabica*, *Acacia benthami* and *Acacia modesta* leguminous seed diets in normal young albino rats. Ind J Physiol Pharmacol 1975; 19:167-168.
14. Iqram F, Khan MA. In-vitro evaluation of plant extracts and antagonistic fungi againstXanthomonas campestris pv. Citri (Hasse) dye. Pak J Bot 2003; 35(5):967-970.

15. Asghar R, Ahmad M, Zafar M, Akram A, Mahmood J, Hassan M. Antibacterial Efficacy of *Acacia modesta* Wall Miswak against dental pathigens. J Biol Sci 2003; 6(24): 2024-2025.
16. Rashid A, Hashmi H. *In vitro* Susceptibility of Some Gram Positive and Gram Negative Strains of Bacteria and Fungi to root extracts of *Acacia modesta*. Pak J Biol Sci 1999; 2(3): 746-749.
17. Akhtar MA, Bhatti R, Aslam M. Antimicrobial activity of plants decoctions against Xanthomonas campertris. J Trop Agri 1997; 74:226-228.
18. Salim M, Ahmad M. Chemistry of the medicinal plants of genus Acacia. J Hamdard Med 1998; 41:63-67.
19. Saeed A. *Salvadora persica* its position and heritage in Islamic density. J Hamdard Medicus 1988; 1:75-76.
20. Bukhari IA, Khan RA, Gilani AH, Ahmed S, Saeed SA. Analgesic, anti-inflammatory and anti-platelet activities of the methanolic extract of *Acacia modesta* leaves. Inflammopharmacol 2010; 18: 187-196.
21. Evans WC, ed. Trease and Evans Pharmacognosy, 15th ed., Sounders-an imprint of Elsevier, New Delhi, India.
22. Harborne JB. Phytochemical methods: A Guide to Modern Techniques of Plant Analysis, 3rd ed., Springer (India), New Delhi, India.
23. Hewitt W, Vincent S. Theory and Application of Microbiological Assay. Academic Press, San Diego, USA.
24. Singh B, Sahu PM, Sharma MK. Anti-inflammatory and antimicrobial activities of triterpenoids from *Strobilanthes callosus* Nees. Phytomedicine. 2002; 9: 355-359.
25. Ahameethunia AR, Hopper W. Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria. BMC Complementary and Alternative Medicine 2010, 10:6 (accessed on line: http://www.biomedcentral.com/1472-6882/10/6)
26. Jayaguru P, Raghunathan M. Group I intron renders differential susceptibility of Candida albicans to Bleomycin Mol Biol Rep 2007; 34: 11-17.
27. Organization for Economic Co-operation and Development (OECD).OECD guidelines for the testing of chemicals. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure

2008. (Available from http://lysander.sourceoecd.org. Accessed on March 21, 2009).
28. Adaramoye OA, Adeyemi EO. Hypoglycaemic and hypolipidaemic effects of fractions from kolaviron, a biflavonoid complex from *Garcinia kola* in streptozotocin-induced diabetes mellitus rats. J Pharm Pharmacol 2006; 58: 121-128.
29. Al-Awwadi NAJ, Poucheret P, Cassanas G, et al. Antidiabetic activity of red wine polyphenolic extract, ethanol, or both in streptozotocin-treated rats. J Agric Food Chem 2004; 52:1008–1016
30. Jayaprakasam B, Vareed SK, Olson LK, Nair M. Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits. J Agric Food Chem 2005; 53(1): 28–31.