

ANTIDIABETIC ACTIVITY AND ISOLATION OF BIOACTIVE COMPOUNDS FROM *HYDROLEA ZEYLANICA*

Borkar Vijay S.*¹, Senthil Kumaran K.², Senthil Kumar KL.¹, Gangurde Hemant H.³, Chordiya Mayur A.³

¹Padmavathi College of Pharm. and Res. Institute, Dharmapuri (TN). NH 7, Periyanaahalli, Tamil Nadu 635205, India

²K.K College of Pharmacy, Gerugambakkam, Chennai, Tamil Nadu 600122, India

³SSDJ College of Pharmacy, Chandwad (MS) India. 423101 Maharashtra - India

Submitted: 12-05-2015

Revised: 13-06-2015

Accepted: 02-07-2015

Corresponding author
Borkar Vijay S.

Email:
vijayborkar79@gmail.com

ABSTRACT

The whole plant of *Hydrolea zeylanica* (HZ) (Hydrophyllaceae family) was coarsely powdered and extracted with ethanol using soxhlet apparatus. The ethanol extract was then fractionated successively using various polarity ranges of solvents and screened for *in-vivo* antidiabetic activity using streptozotocin induced diabetic male wistar rats. The phytochemical investigation of all the fractions and powdered drug analysis was performed. Among the fractions evaluated, chloroform fraction showed highest decrease in blood glucose, total cholesterol and serum triglyceride level as 75.11%, 59.77% and 35.98% respectively when treated at 50mg/mL concentration. As the chloroform fraction has shown better potency towards antidiabetic activity, was subjected to chromatographic separation and three compounds stigmasterol, kaempferol and p- coumaric acid were isolated and characterized by various spectroscopic techniques. The overall results tend to suggest the antidiabetic activity of HZ and principal source of presumed bioactive compounds which may be responsible for many of the pharmacological properties.

Key word: *Hydrolea zeylanica*, antidiabetic, total cholesterol, triglycerides, *in vivo*.

INTRODUCTION

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycemia and glucose intolerance, as a lack of insulin, defective insulin action, or both. Such complications arise due to insufficiency of the regulatory systems for catabolism and anabolism of carbohydrates, lipids and proteins originate from defective insulin secretion, insulin action, or both (Shillito, 1988). The effective prevention and control programmes for diabetes are very much needed to control the prevalence of diabetes mellitus globally (WHO, 1944). The World Health Organization has listed 21,000 plants which are used for medicinal purposes around the world. Among these, 2500 species are in India. India is the largest producer of medicinal herbs endowed with a wide diversity of agro-climatic conditions and is called as botanical garden of the world (Seth and Sharma, 2004). Many of the traditional Indian medicinal plants are found to have anti diabetic potential (Arumugam, *et al.*, 2013). Number of active

molecules have been evaluated for treating hyperglycemia and validated for anti diabetic properties (Nwamarah, *et al.*, 2015) (Noor, *et al.*, 2013). In the present research work the plant selected is HZ. In India it is widely distributed; and is reported to have antiseptic properties. The leaves are considered to possess cleansing and antiseptic properties. Paste of whole plant with coconut oil is applied in minor cuts, wounds and boils as antiseptic for quick healing (Bhagyaleena and Gopalan, 2012). HZ has not been investigated before for antidiabetic activity hence in the present study the various fractions obtained from ethanolic extract was evaluated for anti diabetic activity and attempt has also been made to isolate some bioactive compounds those may be responsible for the antidiabetic activity.

MATERIAL AND METHODS

Hydrolea zeylanica is widely distributed; annual, seasonally submerged, emergent; an erect or diffuse, succulent herb, flowers blue, glandular hairy, seeds numerous and minute (Bhagyaleena and Gopalan, 2012). The plant

was collected from Dhamapuri, Tamilnadu, India and was authenticated from Botanical survey of India. The whole plant material was cleaned thoroughly and dried under shade.

Extraction of plant

The shade dried whole plant was coarsely powdered and extracted with ethanol using soxhlet apparatus for 72h. The ethanol extract was then concentrated and fractioned successively using petroleum ether, hexane, chloroform, ethyl acetate and ethanol to obtain various fractions. The phytochemical investigation was carried out (Kokate, *et al.*, 2007) (Table I) and powdered drug analysis for the determination of ash values was performed by usual methods (WHO, 1988) (Table II).

Ethical clearance

All the experimental protocols were permitted and approved by the Institutional Animal Ethical Committee (IAEC); number CPCSEA /PCP/IAEC/PHD/119/12. The experimental animals were treated as per the guideline of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA)

Acute and sub acute toxicity study (Aboudoulatif, *et al.*, 2010) (Rastogi, *et al.*, 2015).

Animal Model

Healthy female wistar rats (150-200g) were selected and housed in well ventilated rooms (12h light/dark cycle) at $27\pm 5^\circ$, relative humidity $50\%\pm 5$ and fed with standard diet, water and libitum.

Experimental dsign

The three female rats each sequentially were treated with a single dose of 5g/kg of ethanolic extract at intervals of 48h and observed individually for acute toxicity signs and behavioral changes 1h post dosing and at least once daily for 14 days.

Anti diabetic activity (Ariful and Rafiqul, 2011) (Koushik, *et al.*, 2010).

Preparation of doses

Streptozotocin (45mg/mL) was prepared in 0.1mol/L citrate buffer, pH 4.5. Chloroform, ethyl acetate and ethanol fraction was

suspended in distilled water to produce 50mg/mL suspension.

Animal model

Male wistar rats (150-200g) were used for the study and kept in well ventilated animal house (12h light/dark cycles) at $27\pm 5^\circ$, relative humidity $50\%\pm 5$, fed with standard diet, water and libitum. Animals were fasted for 16h prior to drug administration allowing access only to water and deprived of both food and water during the experiment.

Experimental design

All the rats were randomly divided into 7 groups each containing 5 rats. Group I- normal control, group II- normal metformin control, group III- diabetic control, group IV- diabetic metformin control and group V, VI and VII- diabetic treated with chloroform, ethyl acetate and ethanol fraction respectively at 50 mg /mL each. Group II and IV were treated with metformin HCl (150mg/kg, i.p.). The standard drug and test samples were fed orally with intra gastric tube for 24h experiment. Diabetes was induced (Group III-VII) by intra peritoneal injection 1mL/kg, of freshly prepared Streptozotocin (45mg/kg), after base line glucose was estimated. After 48h blood samples were withdrawn from the tail vein of the rats and the blood glucose level was estimated using Glucometer (BioLand, Germany). Animals with blood glucose levels above 11.1mmol/L were selected for the study considering the condition of diabetes was established.

Collection of blood and serum samples

After treatment, blood samples were collected from tail vein of each rat of all groups before and at 0, 1, 2, 3, 6, 10, 16, and 24th h and analyzed for blood glucose content (Table III). Then all the rats were sacrificed, about 1-2mL blood were collected from the heart directly with the syringes, centrifuged (4000 rpm, 10min), serum was collected and total cholesterol (TC) and serum triglycerides (TG) was determined (Table IV).

Biochemical analysis

Serum total cholesterol and triglycerides was estimated at 505 and 546nm respectively

using cholesterol oxidase/p-amino antipyrine (CHO/PAP) method and glycerol 3-phosphate oxidase (GPO) method respectively according to manufacturer's protocol.

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean \pm SEM. Differences between the control and the treatments were tested for significance using ANOVA followed by Dunnett's *t*-test. *p* value < 0.05 were considered as significant using Graph Pad Instat version 3.01.

Isolation and characterization of the compound

Chloroform fraction was selected for column chromatography since it has shown better antidiabetic effect comparing to other and eluted with solvent mixtures of increasing polarity. All fractions showing single spot on TLC plate were pulled together, purified and observed for R_f value. The isolated compounds were characterized with the aid of UV, IR, ¹HNMR and MS. The three compounds were isolated and found to be stigmasterol, kaempferol and *p*-coumaric acid (Figure 1).

RESULT AND DISCUSSION

Acute and sub acute toxicity was observed with a single dose of 5g/kg of ethanolic extract at intervals of 48h. There were no signs of mortality or acute toxicity in 3 female rats. The antidiabetic effect of various fractions of the ethanolic extracts of *HZ* on the blood glucose, serum TC and serum TG levels were investigated in the control and Streptozotocin induced diabetic rats using metformin HCl as standard antidiabetic agent.

Effect of various fractions on blood glucose, total cholesterol and triglyceride

Decrease in blood glucose level was observed in animals treated with various fractions (single dose-50mg/mL) at 0, 1, 2, 3, 6, 10, 16, and 24th hours (Figure 2). The significant reduction at 24th hour of the experiment ($p < 0.05$) for chloroform, ethyl acetate and ethanol fraction showed of 75.11%, 24.58% and 38.22% respectively. Metformin caused maximum reduction of blood glucose

level of 82.31% in comparison to controlled diabetic rats. After 24h treatment decrease in total cholesterol and triglyceride on diabetic rats was observed (Figure 3). Chloroform, ethyl acetate and ethanol fraction showed decrease in total cholesterol level of 59.77%, 38.18% and 28.85% respectively. The triglyceride was decreased by 35.98%, 26.18%, and 12.57% for chloroform, ethyl acetate and ethanol fraction respectively. Metformin was able to reduce total cholesterol and triglyceride by 62.85% and 43.76% respectively comparing to diabetic control groups.

Isolation and characterization of the compounds

Compound 1, Stigmasterol, white needle shaped crystal, M. P. 138-140 °C, R_f value 0.64 with steroid in nature was isolated at 30:20 (CHCl₃:CH₃OH). UV, λ_{max} , 276 nm, CH₃OH, FTIR (KBr, cm⁻¹) 3525.02 (-OH), 3242.35 (-CH=CH-), 3079.95 (=C-H-), 2936.00 (-CH-), 1615.18 (C=C), 1468.32 (CH₂)_n, 1370.05 [CH₂(CH₃)₂], 1020.50 (C-O), 830.25 cm⁻¹(C-H vibration of unsaturated part). ¹HNMR (400 MHz, CDCl₃) δ : 0.77560, 0.84281, 0.92660, 1.05401, 1.25401, 1.54300 (CH₃), 3.4013 (-CH-), 5.4300 (H-cyclic), 5.1809, 5.0092 (-CH=CH-). Mass at m/z 427[M]⁺ corresponding to C₂₉H₄₈O with other characteristic fragmentations at m/z : 412[CH₃]⁺, 394[H₂O]⁺, 308[C₆H₁₄]⁺, and 264[C₃H₈]⁺. The above spectral data was compared with reported data (Ahmed *et al.*, 2013 and Sajjadi *et al.*, 2013”).

Compound 2, Kaempferol white crystalline powder, M. P. 278 °C, R_f value 0.48 with phenolic nature was isolated at 30:70 (CHCl₃:C₂H₅OH). UV, λ_{max} , 365 nm, CH₃OH, FTIR (KBr, cm⁻¹) 3445, 3330 (Ar-OH), 2955, 2930- (Ar-CH), 2860, 2620, 1668 (C=C), 1615 (C=C), 1579, 1510 (Ar-C=C), 1450, 1409 (C=C), 948, 820, 1120 (substituted ring). ¹HNMR (CDCl₃, 400 MHz) δ : 5.1752 (Ar-OH), 5.9688(Ar-OH), 7.3252(Ar-OH), 6.8766(Ar-H), 12.7041, 9.4066, 14.7629(cyclic-OH). Mass at m/z 286.72[M]⁺ corresponding to C₁₅H₁₀O₆ and other fragments at m/z 258 [H₂O], 241[C₂H₂], 213[C₃H₈O], 185[H₂O], 167 [C₂H₄O], 123[CH₄O], 91[CH₄O], and 58. The above spectral data was compared with reported data (Nazni, *et al.*, 2014; Gudej, 2003).

Table I. Phytochemical investigation on fractions of *Hydrolea zeylanica*.

Phyto Constituents	Petroleum Ether	Hexane	Chloroform	Ethyl Acetate	Ethanol Residue
Alkaloids	-	+	+	-	-
Carbohydrates	-	-	-	+	+
Tannins & Phenols	-	-	+	-	-
Glycosides	-	-	-	+	+
Flavones & flavonoids	+	-	+	-	-
Steroids	-	+	+	-	-
Proteins	-	-	-	-	-
Amino acids	-	-	-	-	-
Saponins	-	-	-	-	-

+, Positive test; -, Negative test

Table II. Ash values of powdered drug of *Hydrolea zeylanica*.

Physical Constants	Ash value (%) w/w			Average (%)w/w
	I	II	III	
Total Ash	15.53	15.57	15.59	15.49
Acid insoluble ash	3.11	3.15	3.13	3.13
Water soluble ash	6.36	6.38	6.33	6.35
Loss on drying	9.55	9.47	9.48	9.50s

Table III. Effect of various fractions of *Hydrolea zeylanica* on blood glucose level in diabetic rats on one day treatment.

Groups (Treatment)	Blood glucose level (mmol/L) at Hours							
	0	1	2	3	6	10	16	24
I	7.27±	7.28 ±	7.29 ±	7.30±	7.31±	7.30 ±	7.31±	7.33 ±
10mL saline	0.0044	0.0048	0.0044	0.0054	0.0031	0.0044	0.0031	0.0044
II	7.28 ±	7.29 ±	7.28 ±	6.21±	5.20 ±	5.16 ±	4.15 ±	4.12 ±
150mg/kg	0.0044	0.0044	0.0048	0.44	0.0044	0.0067	0.0037	0.0024
III	11.02 ±	11.52±	11.98±	12.55±	13.85±	14.15±	14.65±	15.85±
10mL saline	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037
IV	11.03 ±	10.51±	10.05±	9.75±	8.65 ±	7.08 ±	6.16 ±	6.05 ±
150mg/kg	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0083
V	11.12 ±	11.06±	10.05±	9.25±	8.05±	7.80±	7.50 ±	6.35 ±
50mg/mL	0.0037	0.0037	0.0037	0.0037	0.0037	0.001	0.0037	0.0083
VI	11.15±	11.12±	11.05±	10.95±	9.75±	9.18±	9.03 ±	8.95 ±
50mg/mL	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037
VII	11.21±	11.18±	11.12±	11.08±	10.88±	9.07±	9.06 ±	8.11 ±
50mg/mL	0.0060	0.0037	0.0037	0.0037	0.0037	0.0037	0.0037	0.0044

All values are expressed as mean ± S.E.M., n=5 in each group, * $p < 0.05$

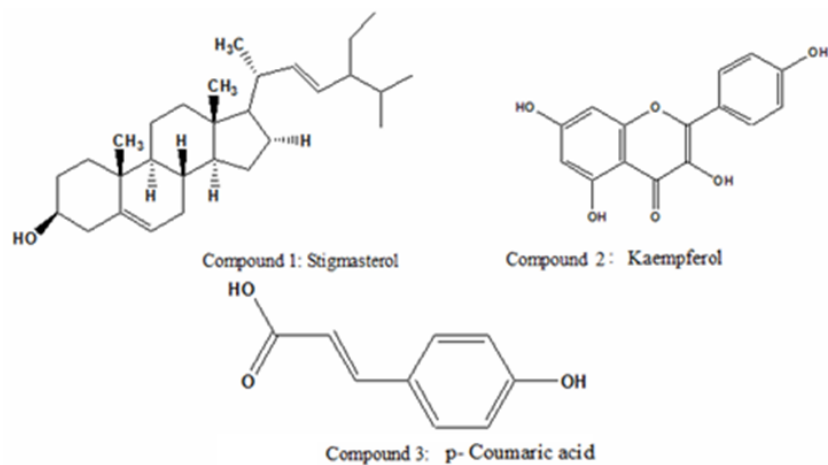


Figure 1, Isolated compound of *Hydrolea zeylanica*

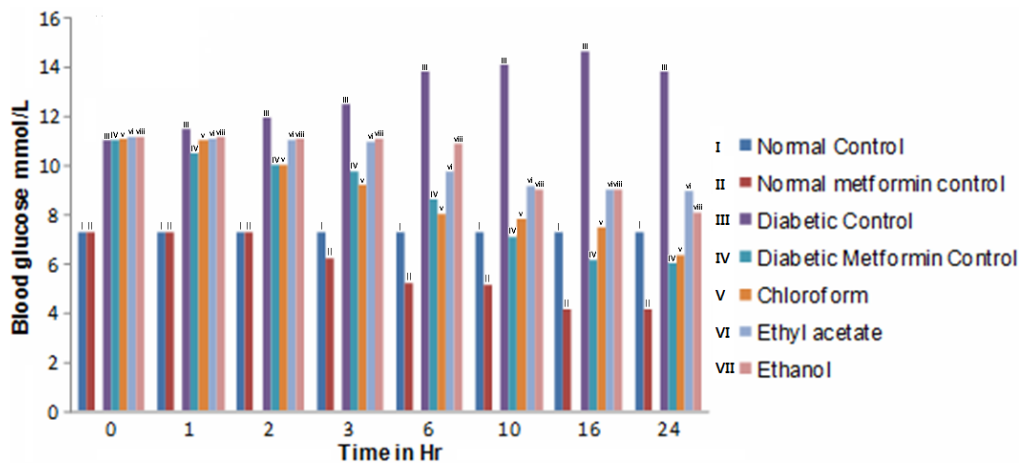


Figure 2. Effect of various fractions of *Hydrolea zeylanica* on blood glucose level in diabetic rats on one treatment

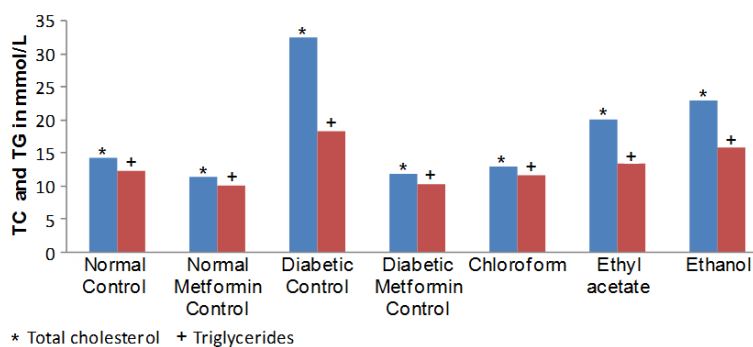


Figure 3. Effect of various of *Hydrolea zeylanica* on total cholesterol and serum glycerides in diabetic rats after one day treatment

Compound 3, p-Coumaric acid, white needles, M. P. 187-189°C, R_f value 0.89 with acidic in nature was isolated at 20:90 (CHCl₃:CH₃CH₂COOCH₃). UV, λ_{max}, 310nm, CH₃OH, FTIR (KBr, cm⁻¹) 3391.5 (Ar-OH), 2830.6 (=CH), 2560.3 (-CH=CH), 1655.2 (C=C), 1599 (-CH₂), 1509.1, 1465, 1425, 1320. ¹HNMR (CDCl₃, 400 MHz) δ: 6.58 (2H, -C=C-), 6.9 (2H, -C=C-), 5.20 (Ar-OH), 9.17 (CH=CH), 12.76 (OH- carboxylic). Mass at m/z 163.04 corresponding to C₉H₈O₃ with other fragments at 119 [CO₂]⁺, 91[C₂H₄], 63 [C₂H₄]. The above spectral data was compared with reported data (Yi Ming Chiang *et al.*) (Ali A *et al.*).

CONCLUSION

The chloroform fraction of ethanolic extract of HZ was found to be most effective in lowering of blood glucose, total cholesterol and triglycerides level in Streptozotocin induced diabetic rats. This reduction could be a result of the antioxidant effect of the flavonoids which was found to be present in chloroform fraction. The chloroform fraction was chromatographed to separate the bioactive constituents. Three compounds stigmasterol, kaempferol and p-coumaric acid were isolated and characterized by various spectroscopic techniques. The spectral and physical data of isolated compounds were matched with earlier reports. In conclusion HZ may be composed of principal source of presumed bioactive compounds which may be responsible for many of the pharmacological properties.

ACKNOWLEDGMENT

Authors are thankful to the Principal, Padmavathi College of Pharmacy and Research Institute, Dharmapuri (TN) for providing necessary laboratory facility to complete the work.

REFERENCES

- Ahmed Y., Rahman S., Akhtar P., Islam F., Rahman M., Yaakob Z., 2013. Isolation of steroids from n-hexane extract of the leaves of *Saurauia raxburghii*. *Int. Food. Res. J.* 20: 2939-2943.
- Ali A., Meredith AW., Thomas HR., 2013. Techniques for analysis for plant phenolic compounds. *Molecules*.18: 2338-2375.
- Ariful Islam MD., Rafiqul Islam MD., 2011. Antidiabetic and hypolipidemic effects of different extracts of *Coccinia cordifolia* L. on normal and streptozotocin induced diabetic rats. *Pak. J. Pharm. Sci.* 24: 331-338.
- Arumugam G., manjula P., Parri N., 2013. A review: Anti diabetic medicinal plants used for diabetes mellitus. *J. Acute. Disease*.196-200.
- Bhagyaleena P., Gopalan R., 2012. Aquatic medicinal plants in ponds of palakkad, Kerala, India. *J. Pharm. Biol. Sci.* 2: 29-35.
- Gudej J., 2003. Kaempferol and quercetin glycosides from *Ribes ideus* L. leaves. *Acta Poloniae. Pharm. Drug. Res.* 60: 313-316.
- Kokate CK., Purohit AP., Gokhale SB., 2007, *Practical Pharmacognosy*. 30th ed. Nirali Prakashan, India. pp. 593-597.
- Koushik P., Koushik D., Khokra SL., Sharma A., 2010. Antidiabetic activity of the plant *Abutilon indicum* in streptozotocin-induced experimental diabetes in rats. *Int. J. Pharmacog. Phytochem. Res.*2:45-49
- Nazni P., Dharmaligam R., 2014. Isolation and separation of phenolic compound from coriander flowers. *Int. J. Agric. Food Sci.* 4:13-21.
- Noor A., Bansal VS., Vijayalakshmi MA., 2013. Current update on anti-diabetic biomolecules from key traditional Indian medicinal plants. *Current Sci.* 104: 721-727.
- Nwamarah JU., Otitoju O., Otitoju GTO., 2015. Chemical composition and anti-diabetic properties of *Jatropha curcas* leaves extract on alloxan induced diabetic wistar rats. *Afr. J. Biotech.*14: 1056-1066.
- Piero MN., Nzaro GM., Njagi JM., 2014. Diabetes mellitus – a devastating metabolic disorder. *Asian. J. Biomed. Pharm. Sci.* 4: 1-7.

- Rastogi A., Srihari S., Gayathri M., 2015. Antidiabetic activity of methanolic extract of *Hygrobila auriculata* in adult male wistar rats. *J. Pharm. Sci. Res.* 7: 98-102.
- Sajjadi SE., Shokoohinia Y., Mehrmiri P., 2013. Isolation and characterization of steroids, phthalide and essential oil of the fruits of *Kelussia odoratissima* Mozaff., an endemic mountain celery. *Res. Pharm. Sci.* 8: 35-41.
- Seth SD., Sharma B., 2004. Medicinal plants of India. *Indian J. Med. Res.* 120: 9-115.
- Shillitoe RW. 1988. Psychology and diabetes: Psychosocial factors in management and control.
- World Health Organization. 1994. *Prevention of diabetes mellitus*, Technical Report Series no. 844: Geneva. World Health Organization.
- World Health Organization. 1998. *Quality Control Methods for Medicinal Plants Materials*: Geneva. World Health Organization.
- Yi Ming Chiang *et al.* 2003. Cytotoxic constituents of the Leaves of *Calocedrus formosana*. *J. Chin. Chem. Soc.* 50: 161-166.