Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 12, pp. 3719-3722, December, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

REVIEW ARTICLE

CHROMATOGRAPHIC AND ANTI-DIABETIC STUDIES ON ROOT EXTRACT OF *Acanthus montanus* (ACANTHACEAE)

***Odoh, U. E. and Ezugwu, C. O.**

Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka

ARTICLE INFO

Article History:
Received 01st September, 2013
Received in revised form
22nd October, 2013
Accepted 27th November, 2013
Published online 02nd December, 2013

Key words:
Acanthus montanus,
Chromatographic analysis,
Diabetes,
Hyperglycemic activity,
Toxicity.

ABSTRACT

The roots of *Acanthus montanus* were extracted with methanol and the extract successively extracted with n-hexane, petroleum ether, ethylacetate, diethylether and chloroform to investigate the anti-diabetic activity based on ethnomedicinal lead. Through a pilot study the ethylacetate fraction (EAF) was studied against alloxan-induced diabetic rats. Preliminary phytochemical analysis and acute toxicity studies were carried out in mice intraperitoneally. An attempt was made to resolve the EAF into its components using thin layer chromatography (TLC). Result of the anti-diabetic study shows significant dose-dependent reduction ($P < 0.05$) in blood sugar levels of both normoglycemic and hyperglycemic rats. When doses of 100, 200 and 300 mg/kg of the EAF were administered intraperitoneally to alloxan-induced diabetic rats, significant decrease in blood sugar level occurred (21.91, 38.12 and 49.20 % respectively) compared to the sugar lowering effect of glibenclamide (51.78 %). In normal rats, EAF (100, 200 and 300 mg/kg) exhibited a significant reduction of the blood sugar level of 19.20, 27.80 and 40.74 % respectively, while glibenclamide caused a 49.94 % reduction. Phytochemical tests showed the presence of alkaloids, flavonoids, glycosides, steroids, saponins, tannins and terpenoids. Acute toxicity test carried out in mice using Lorke's methods showed that the extract was safe, since no death was recorded at a dose of 5000 mg/kg. The study shows that the extract of the EAF of *Acanthus montanus* possessed a significant and dose-dependent hypoglycemic activity in normoglycemic and alloxan-induced diabetic rats and provides a pharmacological basis for the use of *Acanthus montanus* root in folk medicine in management of diabetes. Among the chromatographic solvent systems tested, chloroform: ethylacetate (6:4) gave the best resolution of the EAF giving the highest number of spots. The result of the chromatographic studies can be a guide for further studies to isolate and characterize the active principle(s) responsible for this activity.

INTRODUCTION

Diabetes mellitus, a heterogeneous disorder responsible for disruption of glucose homeostasis is becoming a serious health care challenge worldwide with an expected incidence of 239 million people by the year 2020 (Lakhdar, 2000). Diabetes mellitus has no cure irrespective of the widespread claim by traditional healers that they can cure it. It belongs to the groups of ailments that can presently only be managed. Amongst such incurable illnesses are AIDS, cancer and hypertension. Diabetes mellitus is characterized by an elevation of blood glucose caused by a relative or absolute deficiency of insulin (Mycek *et al.*, 2000). These effects results in imbalance between the biological action and normal secretion (Judd and Ramani, 1999). The disease affects nearly 10 % of the population (Joy and Kittan, 1999). Different groups of oral hypoglycemic agents are currently available with characteristic profiles of side effects (Prout, 1974; Holtman and Turner, 1991; Willams and Pickup, 1991; Kameswara, *et al.*, 1977).

Corresponding author:* ***Odoh, U. E.*
Department of Pharmacognosy, Faculty of Pharmaceutical Sciences,University of Nigeria, Nsukka.

The search for anti-diabetic agents with little or no side effects is a continuous process. The plant kingdom presents a wide field for prospecting effective oral hypoglycemic agent. More than 300 species of plant have been reported to possess hypoglycemic activity (Rahman and Zaman, 1989). However, only few have been investigated, despite the World Health Organization (WHO) recommendation that traditional plant remedy for diabetics warrant further evaluation (WHO, 1980). Extracts of plants have been used as traditional remedies for the treatment of diabetes mellitus in many parts of the world. In Africa too, traditional healers use plant extracts for treatment of diabetes mellitus. Some of these plants, which have been ascertained to posses varying degrees of hypoglycemic activity are; *Bridelia* ferruginea (Iwu, 1980), *Vernonia amygdalina* (Akah and Okafor, 1992), *Picralima nitida* (Inyagha, 1999), *Arachis hypognea* (Biblis *et al*, 2002), *Prosopis Africana* (Okide *et al.*, 2003), *Loranthus micranthus* (Osadebe *et al.*, 2004) *Mucuna slonaei* (Ezugwu *et al.*, 2005), *Detarium microcarpum* (Odoh *et al.*, 2005), *Sphenostylis sternocarpa* (Odoh *et al.*, 2007), etc.

Acanthus montanus commonly known as bears breach, mountain thistle or alligator plant is a striking small shrub with

sparse branches and soft stem. It is a native of Tropical West Africa (County and Jore, 1998) and commonly found in South Eastern Nigeria. *Acanthus montanus* has been found useful in the traditional treatment of several diseases. The Igede people of Nigeria use the ground leaves of *Acanthus montanus* to treat boils, skin infections and hypertension and as an antitussive (Igoli *et al*, 2005). In Democratic of Congo the plant is used to treat urogenital infections, urethral pain, endo-metritis, urinary diseases, diabetes, cystitis and leucorrhoea (Undie, 2005). It has also been reported to have intestinal smooth muscle relaxant activity (Adeyemi *et al*, 2004) as well as antiepileptic activity (Noumi and Fouzi, 2003).

MATERIALS AND METHODS

Collection of plant materials

The *Acanthus montanus* roots used in this experiment were collected from Nsukka, Enugu State in June, 2008 and identified by Mr. A.O.Ozioko of Bioresources Development and Conservation Programme Centre, Nsukka, Enugu State. The voucher specimen (UN/PCOG/08/398) was kept at the Herbarium of Department of Pharmacognosy, University of Nigeria, Nsukka. The fresh roots were carefully washed in water, dried at room temperature and milled to a coarse powder.

Chemicals/Reagents/Equipment

The reagents were sourced commercially and include methanol, hydrochloric acid, alloxan monohydrate, chloroform and Tween 20 (Sigma, USA), glibenclamide (Merck, Germany), silicia gel GF254, ethlyacetate, petroleum ether, n-hexane and diethylether (Hopkin and Williams, England), Accuchek glucometer and Accuchek strips (Manesty, England).

Extraction

A 500 g quantity of the powdered root material was exhaustively extracted in a soxhlet extractor using 95 % methanol for 42 h. The methanol extract was extracted successively with petroleum ether, n-hexane, diethylether, ethylacetate and chloroform. These fractions were concentrated for further studies at reduced temperature and pressure in a rotary evaporator.

Animals

Albino rats weighing 130-220 g and mice weighing 14-21 g were used in the experiment. They were obtained from the Animal House of Zoology Department of University of Nigeria, Nsukka. The animals were housed in white metallic cages and kept in a room where a 12-hour light/dark cycle was maintained with free access to water and food for seven days to acclimatize the animals to the laboratory conditions.

Phytochemical studies

The chemical constituents of the methanol extract of the leaf were investigated using standard methods (Harbourne, 1998; Trease and Evans, 1994). The classes tested for include alkaloids, glycosides, carbohydrates, reducing sugars, saponins, tannins, flavonoids and steroids.

Acute toxicity test (LD_{50})

The LD_{50} of the EAF were determined using the method of Lorke (1983). Nine mice were used for preliminary test. They were divided into three groups of three mice each. The three groups were given 10, 100 and 1000 mg/kg of the EAF prepared in 20 % (v/v) Tween 20 in water, intraperitoneally. The mice were observed for lethal effects for 24 h and number of deaths was recorded. Other doses corresponding to 2000, 3000, 4000 and 5000 mg/kg were given to four mice respectively. The mice were observed for 24 h and number of deaths was recorded.

Hypoglycemic studies

Determination of hypoglycemic effect on normoglycemic rats

Twenty-five healthy rats weighing 130-180 g were used for this experiment. The animals were divided into five groups each containing five animals. They were fasted overnight for 12 h. At the end of the fasting period, different doses of the extract were given to the animals intraperitoneally. Group I received 100 mg/kg of EAF, Group 2 received 200 mg/kg of EAF, Group 3 received 300 mg/kg of EAF, Group 4 received 10 mg/kg of glibenclamide as a positive control and Group 5 received 2 ml/kg of 20 % (v/v) Tween 20 (negative control). At 0, 1, 3 and 6 h, blood samples were withdrawn from the tail vein of each rat and the blood sugar level determined using the glucometer.

Determination of hypoglycemic effect on hyperglycemic rats

Twenty-five healthy albino rats weighing 180 – 220 g were used for the experiment. The albino rats were weighed and fasted for 12 h. Hyperglycemia was induced by intravenous administration of 80 mg/kg of alloxan monohydrates prepared in normal saline. The animals were allowed free access to water and food for 7 days. On the eighth day, the animals whose blood glucose level were above 150 mg/kg were selected and divided into five groups (n=5). Group 1 received 100 mg/kg of EAF, Group 2 received 200 mg/kg of EAF, Group 3 received 300 mg/kg of EAF, Group 4 received 10 mg/kg of glibenclamide and Group 5 received 2 ml/kg of 20 % (v/v) Tween 20. Blood samples were collected from the tail veins of the rats at 0, 1, 3 and 6 h respectively after treatment and the blood sugar levels were determined.

Chromatographic analysis

Thin-layer chromatography was used to resolve EAF following a standard procedure (Touchstone, 1992). Clean glass plates (20 cm by 20 cm) were silica gel GF254 to a thickness of 0.25 cm using Kenso CJK 520 spreader coated with. The coated plates were air-dried and activated in an oven for 1 h at 110 ^{0}C. The plates were then cooled to room temperature. The EAF was dissolved in methanol and the dissolved extract was spotted on the plates, 2 cm from the edge of the plates. The

plates were allowed to dry at room temperature. The spotted plates were developed in different solvent systems: chloroform; methanol (1:1), chloroform: petroleum ether (7:3), chloroform: n-hexane (8:2), chloroform: diethyl ether (9:1) and chloroform: ethyl acetate (6:4).

Statistical analysis

The results expressed as Mean ± S.E.M were analyzed by student's t-test. Values of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The yield of the methanol extract and EAF were 15.32 and 6.48 % (w/w) respectively. The acute toxicity studies showed that at the dose of 5000 mg/kg of the EAF there was no death, which means that they were relatively safe, and can be used at studied doses in management of diabetes. Administration of EAF at 100, 200 and 300 mg/kg body weight, significantly ($P < 0.05$) reduced the blood glucose level of normal and alloxan-induced diabetic rats in a dose-dependent manner as shown in Tables 1 and 2. In both the normoglycemic and alloxan-induced diabetic rats, EAF produced marked reduction in the blood sugar level within 3 h post-administration and reached its peak in 6 h. When the anti-diabetic effect was compared with the glibenclamide, EAF was almost as potent as the standard.

Table 1. Hypoglycemic activity of the ethylacetate fraction (EAF) of *Acanthus montanus* root on the fasting blood sugar levels of normal rats

Treatment	Dose mg/kg	Mean Blood sugar (MBS) Levels (mg/dl)					Percentage Maximum Reduction (%)
		0 h	1 h	3 h	6 h	9 h	
EAF	100	72.39 ± 1.06	69.85 ± 0.77	65.25 ± 3.12*	58.49 ± 4.39*	63.70 ± 2.89	19.20
	200	87.51 ± 1.32	84. 05 ± 2.03	74.32 ± 1.23*	63.15 ±1.47*	70.64 ± 0.63	27.84
	300	75.09 ± 1.72	71.80 ± 1.53	62.85 ± 0.64*	44.50 ± 3.13*	52.50 ± 2.14	40.74
Glibenclamide	10	90.50 ± 2.35	83.24 ± 1.54	69.80 ± 1.31*	45.30 ± 4.35*	57.08 ± 6.00	49.94
2 ml/kg Tween 20	-	72.50 ± 1.18	72.50 ± 3.46	71.08 ± 4.89	71.25 ± 2.26	71 20 ± 2.38	1.96

Values are expressed as mean ± SEM, * P<0.05, n=5

Table 2. Hypoglycemic activity of the ethylacetate fraction (EAF) of *Acanthus montanus* root on the fasting blood sugar levels of alloxan-induced diabetic rats

Treatment	Dose mg/kg	Mean Blood sugar (MBS) Levels (mg/dl)					Percentage Maximum Reduction (%)
		0 h	1 h	3 h	6 h	9 h	
EAF	100	183.50 ± 1.56	178.60 ± 3.90	167.84 ± 2.76*	143.30 ± 1.81*	152.62 ±7.43	21.91
	200	186.50 ± 0.23	179.85 ± 4.20	154.30 ± 5.30*	115.40 ± 6.11*	127.35 ± 2.55	38.12
	300	163.70 ± 1.33	157.38 ±7.23	139.51 ± 0.98*	82.50 ± 3.50*	91.65 ± 2.15	49.60
Glibenclamide	10	165.5 ± 1.81	158.4 ± 1.92	137.54 ± 3.14*	79.30 ± 4.51*	85.65 ± 0.54	51.78
2 ml/kg Tween 20	-	176.7 ± 0.26	176.30 ± 0.11	175.82 ± 0.56	174.02 ± 1.47	176.34 ± 0.77	1.52

Values are expressed as mean ± SEM, * P<0.05, n=5.

In alloxan-induced diabetic rats, the EAF exhibited significant ($P < 0.05$) dose-dependent reduction of 21.91, 38.12 and 49.20 % of the blood sugar levels at the doses of 100, 200 and 300 mg/kg doses respectively while 51.78 % reduction of the blood sugar level was observed for 10 mg/kg of glibenclamide after 6 h of treatment. In the normoglycemic rats, EAF at the doses of 100, 200 and 300 mg/kg exhibited significant ($P < 0.05$) dose-dependent reduction of the blood sugar levels of 19.20, 27.80 and 40.74 % was respectively, while glibenclamide caused a 49.94 % reduction of the blood sugar levels. Phytochemical studies showed that the EAF contains alkaloids, flavonoids, glycosides, saponins, tannins, steroids and terpenoids. These constituents are likely to be responsible for the observed significant activity of the fraction, either singly or in synergy with one another. Some plants that contain alkaloids have been reported to have hypoglycemic activity (Bever and Zahad, 1979). So the hypoglycemic activity associated with EAF may be attributed to the presence of alkaloids and flavonoids. These are mainly phenolic compounds, which have been reported to have anti-diabetic effects (Farjou *et al.*, 1987). The precise mechanism by which this fraction lowers blood glucose is however not clear. The hypoglycemic action of EAF could also be due to the possible enhancement of the peripheral utilization of glucose or by increasing the pancreatic secretion of insulin from the cells of islets of langerhans or its release from bound insulin (Pari and Amarnath, 2004). This insulin inhibits conversion of triglycerides, free fatty acids and the conversion of glycogen to glucose. This suggestion is supported by the fact that despite pre-treatment of the rats with alloxan, which is known to cause permanent destruction of pancreatic β- cells (Zarrow *et al.*, 1964), hypoglycemic effect was still observed in alloxan-induced diabetic rats. It could also, be suggested to a mechanism similar to that of the Glibenclamide (Daonil®), which is one of sulphonylureas. Sulphonylureas as oral hypoglycemic agent cause hypoglycemia by activating the pancreatic β-cells to stimulate insulin release. It also believed that some plants that induce hypoglycemia perform this function by removing inactivating compounds through the SH groups in these activating compounds. Similarly, some hypoglycemic plants containing anthocyanosides appear to act by improving vascularization of the pancreas. Others act by blocking oxidative enzyme of the Kreb's cycle (succinic dehydrogenase and cytochrome oxidase), thus increasing anaerobic glycosides and decreasing gluconeogenesis and entailing an increased rate of transfer of

Table 3. TLC on ethylacetate fraction (EAF)

Solvent system	No of bands	Visible Colour of bands
Chloroform: methanol	0	No colour separation
Chloroform: petroleum ether	3	Light green, yellow, light brown
Chloroform: diethylether	4	Light brown, green, yellow, yellowish green
Chloroform: ethylacetate	5	Light brown, green, yellow, dark brown, yellowish brown
Chloroform: n- hexane	2	Light green, yellow

Table 4. RF values of TLC bands of ethylacetate fraction (EAF)

Band	RF Value	Colour
1	0.143	Light brown
2	0.286	Green
3	0.354	Yellow
4	0.464	Dark brown
5	0.644	Yellowish brown

glucose from the blood to the tissue (Bever and Zahad, 1979). From the thin layer chromatographic studies, on trail of many solvent systems, the chloroform-ethylacetate (6:4) gave the best separation of the EAF with five visible bands (Table 3) and the Rf values of 0.143, 0.286, 0.357, 0.464 and 0.643 as shown in Table 4.

Conclusion

The root of *Acanthus montanus* do possesses hypoglycemic properties in normal and alloxan-induced diabetic rats. This study provides a pharmacological basis for the use of this plant by traditional healers in the management of diabetes mellitus. The result of the chromatographic studies can be a guide for further studies to isolate and characterize the active principle(s) responsible for this activity.

REFERENCES

Adeyemi, O. O, Okpo S. O. and Young-Nwafor, C. C. 2004. The relaxant activity of the methanolic extract of *Acanthus montanus* on intestinal smooth muscles. *Journal of Ethnopharmacology*, 68, (1-3), 169 – 173.

Akah, P A and Okafor, C. l 1992. Blood sugar lowering Effects of *Venonia Amydalina Del* in an experimental Rabbit model, *phytothe*r. Res. 6: 171-173.

Bever. B. O. and Zahad, G. R. 1979. Plants with oral hypoglycemic action. J. Crude Drug Res., 17: 139 – 196.

Biblis, L. S., Shehu, R. A. and Abubakar, M. U. 2002. Hypoglycemic effect of *Arachis hypognea*. *Phytomed*., 9: 553-555.

County, L. and Jore, J. H. 1998. Acanthus montanus. *The Home Owner's Journal*. Vol. B97: 78.

Ezugwu, C. O. and Odoh, U. E. and Ajali, U. 2005. Blood sugar lowering effect gum extract of *Mucuna sloanei* Fawc. and Randle (Fam. Papillionaceae) seed. *Bio*. *Research*, 3 (2): 49-51.

Farjou, I. B., AL- Ani, M. and Guirges, S. Y. 1987. Lowering of blood glucose in diabetic rabbits by Artemisia extract. *J. Faculty Med., Baghdad*, 92: 137 – 141.

Harborune , J. B. 1998. Textbook of Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Chapman and hall Ltd: London p12.

Holtman, R. R. and Turner, R. C. 1991. Oral agents and insulin in the treatment of NIDDM. In: Pickup J. C., Williams G. (eds.) Textbook of Diabetes, Blackwell, and Oxford. pp 467-469.

Hood, A. M. and Lowburry, E. J. L. 1954. Anthocyanins in bananas. Nature, London, 173: 402-3.

Igoli, J. O., Ogaji, O. G., Tor-Anyiin, T. A. and Igoli, N. P. 2005. Traditional Medicine Practice Amongst the Igede people of Nigeria Part 11. *Afri. J. Compli. Alter. Med.,* Vol. 2, No. 2: 134-152.

Inyagha, S. I. 1999. Antidiabetic effect of *Picralima nitida* aqueous extract: A mechanism independent of insulin release from pancreatic cell. *Nig J. Nat. Prod and Med*.; 3, 66-67.

Iwu, M. M. 19801. Anti-diabetic properties of *Bridelia ferruginea* leaves. *Planta Medica*. 39: 247.

Joy, K. L. and Kittan, R. 1999. Anti-diabetic activity of *Picorrlira kurroa* extract. *J. Ethnopharm.,* 167: 143-148.

Judd, L. and Ramani, R. 1999. Pharmacological management of type 2 diabetes mellitus: Current and Future Therapies in Pharmacy, Times, October, 85-93.

Kameswara, R. B., Giri R, Kesavulu, M. M. and Apparao, C. H. 1997. Herbal medicines in the treatment of diabetes mellitus. *Manphar Vaidya Patrika* I (4,5): 33-35.

Lakhdar, D. 2000. An epidemic of diabetes in the Arab world, Diabetes Inter, 10: 66.

Lorke, D. 1983. A new approach to practical acute toxicity testing: *Archieves of Toxicology*. 54: 275-287.

Mycek, J. M., Richard, A. H. and Pamella, C. C. 2000. Insulin and Oral Hypoglycemic Drugs In: Lippincott's Illustrated *Reviews Pharmacology*, 2nd ed., pp. 255-257.

Noumi, E. and Fouzi, F.L. 2003. Antiepileptic activity of *Acanthus montanus* on experimental animals. Pharmaceutical Biology (*Formally Journal of Pharmcognosy*), Vol 41, No. 5, 330 – 339.

Odoh, U E, Ezugwu C. O, Ezea, S. C. and Ezejiofor, M. A. 2007. Investigation of anti-diabetic properties of aqueous extract of *Sphenostylis sternocarpa* seeds (Fam. Papillionaceae). *J. Med. Pharm. Sci.,* Vol. 3, No.2: 54-57.

Odoh, U. E., Ezugwu, C. O., Nwodo, N. J. and Ajali, U. 2005. Anti-diabetic property of *Detarium microcarpum* Guill and Perr (Fam. Ceasalpinaceae). *Afr. J.Pharm. Res. Dev. (AJPRD).* Vol. 1 (1): 39-41.

Okide, G. B., Odoh, U. E. and Ezugwu, C. O. 2003. Hypoglycemic activity of the gum extract of *Prosopis Africana* seeds. *J. Trop. Med. Plant*, vol. 4(1): 47-49.

Osadebe, P. O., Okide, G. B. and Akabogu, I. C. (2004). Study on anti- diabetic activities of crude methanolic extracts of *Loranthus micranthus* (Linn) sourced from five different host trees. *J. Ethnopharmacol.,* 3342: 1-6.

Pari, L. and Amarnath, S. 2004. Antidiabetic activity of *Boerhavia diffusa* L.: Effect on hepatic key enzymes in experimental diabetes. *J. Ethnopharmacol*., 91: 109-113.

Prout, T. E. 1974. In: Mallaisse W J, Pirart J (eds) Proceedings (V111) of Congress of International Diabetes Federation. *Excerpta Medica*. Amsterdam, 162.

Rahman, A. U. and Zaman, K. 1989. Medicinal Plant with hypoglycemic activity. J. Ethnopharmacol*.,* 26: 1-33.

Touchstone, J. C. 1992. Practice of Thin Layer Chromatography. John Wiley and Sons, New York, p. 264.

Trease, G. E. and Evans, W. C. 1994. Textbook of Pharmacognosy, 13th ed., Balliere Tindall, London, pp. 167-197.

Didie V. S. 2005. Plant remedies from Democratic Republic of Congo. *Medicinal Plant Database African Research Museum*. HT 20, H (104).

WHO, 1980. World Health Organisation Experts Committee on Diabetic Mellitus Second Reports. Technical Report Series, 646, Ed. Invena Della Betta; Milano, pp 66.

Williams, G. and Pickup, J. C. 1991. New Drugs in the Management of Diabetes Mellitus. In: Pickup J.C., Williams, G. (eds) Textbook of Diabetes, Blackwell, oxford, pp. 977-993.

Zarrrow, Z. X., Yochim, J. M. and Mc Carthy, J. L. 1964. Experimental Endocriminology, A Source Book of Basic Techniques. Academic Press, New York, pp. 406-509.

****