EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 7 (July-2015) pp. 147-152

www.ijcrar.com

Castalagin from *Anogeissus acuminata* (Roxb.ex.DC) Guill. Ex. Perr, a potent Hypoglycaemic Agent

M. Z. Zaruwa[1]*, J. Manosroi[2], T. Akihisa[3] and A. Manosroi[2]

[1]Adamawa State University, Nigeria
[2]Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand
[3]School of Chemical Engineering, Nihon University, Surugadai, Tokyo, Japan
Corresponding author

KEYWORDS

Castalagin, *Anogeissus acuminate*, Hypoglycaemic Agent

A B S T R A C T

The antihyperglycemic activity of a pure compound from *Anogeissus acuminata* (Roxb. Ex. DC) Guill and Perr. was investigated after isolation and characterization. The compound Castalagin was tested for its hypoglycaemic activity by monitoring the fasting blood glucose on alloxan induced diabetic mice. Castalagin was characterized using 1H and 13C-NMR and two-dimensional correlation spectroscopy COSY, NOSEY, HMQC and HMBC. Castalagin significantly reduced ($p<0.05$) fasting blood glucose by 65.06% in comparison with insulin. This observation opens a new role for castalagin as a potent hypoglycemic agent in the treatment and management of diabetes mellitus.

Introduction

The Increase in the Diabetes mellitus (DM) globally prevalence has been documented, with an estimated 171 million people worldwide suffering from it (Tunrayo, 2013, Wild *et al.*, 2004). This figure has been predicted to likely double by 2030 (Shaw *et al.*, 2010). Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycaemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level (Mani Kandaselvi *et al.*, 2012). It increases the risk of several complications such asretinopathy, microangiopathy, and nephropathy (Sanye *et al.*, 2013).

As a result of the high cost, side effect and the paucity of drugs in many communities especially in developing countries, many DM patients resort to the use of medicinal plants and other remedies.

The use of medicinal plants around the world, especially in developing countries such as Thailand and Nigeria is inevitable considering the socio-economic status of the majority of people in these countries. Plant materials which are being used as traditional medicine for the treatment of DM are considered good sources for a new drug or a lead to make a new drug (Mani Kandaselvi

et al., 2012). Amongst such plants are *Anogeissus acuminata* (Manosroi *et al*.,2011), *Abelmoschus moschatus Medik, Acacia Arabica* (Lam) Wild., *Allium sativum* L. (garlic), *Aloe barbadensis* Mill and *Momordica charantia* L. (Kavishankar *et al*., 2011).

Anogeisus acuminata, commonly known as button tree belongs to the family C*ombretacea* (Rangoon creeper family). It is a genus of trees native to South Asia, the Arabian Peninsula and Africa. The hypoglycemic efficiency of its aqueous extract has earlier been reported and observed to be comparable to insulin with five folds free radicalscavenging activity of ascorbic acid (Manosroi *et al*.,2011). Hemamalini *et al*., (2010) reported its use in the treatment of painful inflammatory conditions in India.Its anti-HIV and anti-snake venom activities have been well documented (Rimando *et al*., 1994; Dahareand Jain, 2010).

This research is aimed at investigating the antihyperglycemic activity of *A. acuminata* as well as isolation technique as described by Rimando *et al*., (1994). Thus, identifying new antidiabetic compounds to the already existing ones.

Materials and Methods

Instrumentation

^{1}H- and ^{13}C-NMR spectra (400 and 100 MHz), respectively and two-dimensional correlation spectroscopy COSY, NOSEY, HMQC, andHMBC, were recorded ona Bruckner AV-400 spectrometer in MeOD. Chemical shifts are reported in δ (ppm)values. Thin-layerchromatography were carried out on precoated silica gel 60 F254 plates (E.Merck,0.25 mm), and detected under UV light (254 nm) and spraying with ceric sulfatereagent. Silica gel 230—400mesh (E.Merck,Germany) was used column chromatography (CC) gradient elution withthe solvent mixtures indicated in each case respectively. The EI-MS was recordedon a JEOL JMS-HX-110 mass spectrometer. HPLC separation was performed onreverse phase recyclingHPLC (LC908) using column L80 at a flow rate of 4 mL/minand 66 atm.

Plant Materials

Anogeissus acuminata was collected from Mae FaLuang (Akha Lahu) village, Chiang Rai, Thailand. It was identified at the Chiang Mai University Herbariumand given a voucher number (Voucher No. 09 to 41).The bark was air dried and was milled into powdered form.

Extraction and Fractionation of the Extract

650g of the powdered sample was extracted with 3000ml of 80% v/v MeOH (CH_3OH) using soxhlet extractor. The crude methanolic extract was filtered and evaporated under reduced pressure. The resultant residue was dissolved in*n* – chloroform ($CHCl_3$). The solution was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in distilled water and fractionated by liquid phase partition with EtOAc ($C_2H_5OCH_2COO-$): BuOH: MeOH (8:2:2). All sub-fractions were evaporated under reduced pressure. The *n* – chloroform extract was loaded onto silica gel for column chromatography and fractionated using chloroform-MeOH: MeOH: formic acid (9:10:1). Solvents used were arrived at after testing by Thin Layer Chromatography. Elutes were collected according to each band and were evaporated by a rotary evaporator and yields calculated.

Antihyperglycemic Activity

Male, Sprague - dawley rats weighing between 160-180g were grouped randomly consisting of 6 rats. They were maintained on standard pellet and water ad-libitum. All methods used were ethically approved by the ChiangMai University's Animal Ethics Committee. ProtocolNumber: 40/2552. All animals were fasted overnight prior to investigation.

Diabetes was induced by a single intraperitoneal injection of 180 mg/kg of alloxan monohydrate in normal saline water in a volume of about 3 mL. 100, 200, 400mg/kg body weight of the obtained extract and fractions of *A.acuminata* were intraperitoneally administered to the 0.5iu/kg of Insulin and 1.0 mg/kg Glibenclamide were administered as the positive control groups respectively.The negative control group was administered distilled water.

Fasting blood glucose (FBG) was assessed hourly for a period of 4 hours using the method Zaruwa *et al.* (2012).

Purification and Isolation of Bioactive Compound

The sub-fraction with the highest potency was subjected to purification by column gel chromatography technique. 8.0g of the fraction was loaded into ODS gel in a 90 × 4.5cm column and semi-pure or pure fractions were eluted with 300ml diluted methanol and collected atintervals. The eluted fractions were run on analytical TLC to ascertain its purity. Eluents with similar spots were combined and further separated using reverse phase recycling HPLC with a solvent system of MeOH:H_2O (55:45).A pure compound was obtained after several recycling.

Structural Elucidation

The structure of the isolated compound was elucidated using the nuclear magnetic resonance (NMR) and EI – MS.

Statistical Analysis

Statistical significance was established using one-way analysis of variance, and datawere reported as mean ± standard error mean. Significant difference was established at$p$ <0.05. Statistical analyses were carried out using SPSS for Windows, version 17.0(SPSS Inc., Chicago, IL).

Ethical clearance

All methods were ethically approved by the Chiang Mai University`s Animal Ethics Committee. Protocol Number 40/2552.

Results and Discussion

The shift from synthetic medicine to natural remedies has led to the discovery and development of novel drugs for the treatment and management of various ailments. In this study, the antihyperglycaemic property of *A. acuminata* was investigated and its bioactive component isolated.

The methanol extract obtained from the soxhlet extraction of *A. acuminata*powder gave a yield of 18.05% w/w. After partitioning, the yields of methanol and chloroform fractions were 1.39% and 16.5% respectively. Silica gel column chromatography gave three sub fractions of $CHCl_3$(0.91%), $CHCl_3$: MeOH (0.15%) and MeOH (0.30%), respectively. After liquid partitioning, the methanolic fraction gave the following sub-fractions EtOAc (0.42%), $CHCl_3$ (0%), C_4H_5OH (13.63%) and MeOH (2.49%) respectively.

Table.1 ^{1}H – NMR (400 MHz) Spectroscopic Data for Castalagin (D_2O)

Position	$\delta(H^O)$
CH (1)	**5.65** (brs)
CH (2)	**5.06** (d, J = 7.3)
CH (3)	**5.00** (d, J = 6.9)
CH (4)	**5.09** (d, J = 6.9)
CH (5)	**5.49** (d, J = 6.9)
CH2 (6)	**4.12** (d, J = 12.8)
	4.90 (d, J = 11.9)
CH (2')[iii]	**6.90** (s)
CH (2')[iv]	**6.75** (s)
CH (2')[v]	**6.70** (s)

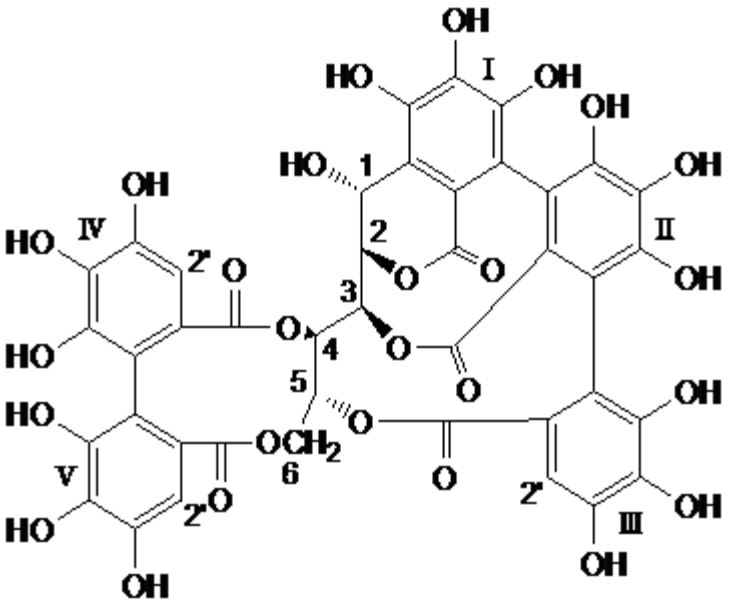

Figure.1 Structure of castalgin

Table.2 Hypoglycemic effect of the isolated compound Castalgin from *A. acuminate*

	0 h	1 h	2 h	3 h	4 h	FBG(%) reduction
DW (0.5ml)	233.00 ± 0.37	247.60 ± 0.74	268.20 ± 0.33	234.60 ± 0.45	246.0 ± 16.55	0.00
Ins (0.5iu/kg)	313.00 ± 0.54	330.0 ± 0.78[a,b,c]	90.60 ± 0.06[a,b,c]	87.80 ± 0.79[a,b,c]	88.40 ± 0.06[a,b,c]	71.95
Glb(1.0mg/kg)	237.6 ± 0.61	193.6 ± 0.61[a,b,c]	146.4 ± 0.37[a,b,c]	130.8 ± 0.37[a,b,c]	130.8 ± 0.73[a,b,c]	45.62
N (group 1)	212.4 ± 0.43	265.0 ± 0.82	329.4 ± 0.02	275.6 ± 0.42	207.2 ± 0.76	2.45
2N(group 2)	290.00 ± 0.77	367.00 ± 0.41	406.80 ± 0.31	402.80 ± 0.31	370.40 ± 0.28	0.00
5N(group 3)	314.80 ± 0.86	412.20 ± 0.34	354.8 ± 0.06	143.6 ± 0.27[a,b,c]	110.0 ± 0.85[a,b,c]	65.06

Note: values = mean ± SD; n = 6: (a) statistical significant ($p < 0.05$) as compared with group 1; (b) statistical significant ($p < 0.05$) as compared with group 2; (c) statistical significant ($p < 0.05$) as compared with group 3.

The hypoglycaemic effects of methanol sub fractions of *A.acuminata* are depicted in figure 1. The MeOH, BuOH and EtOAc sub fractions showed a dose dependent effect, significantly ($p < 0.05$) reducing fasting blood glucose by 42.45%, 31.46% and 14.59 %, respectively at the 4^{th} h with 400mg/kg b.wt extract. MeOH sub fraction reduction (42.45%) was 0.47 and 0.91 fold of insulin and glibenclamide, respectively. The high activity observed in the MeOH extract may be attributed to synergetic properties of all the fractions. This is further buttressed by the reduced activity on fractionation. Several studies have reported reduction in biological activities on fractionation of plant extracts compared to the crude (Chanda *et al*., 2013).

The chloroform sub fraction also showed significant ($p<0.05$) reduction in fasting blood glucoseat 2, 3 and 4 h as shown in figure 2. All the chloroform sub fractions was observed to significantly ($p<0.05$) reduce FBG by 10.49%, 10.34% and 29.96% respectively at 400mg/kg b.wt. at 4 h. The observed reduction of FBG by the MeOH extract and subfractions indicates a hypoglycaemic potential of *A. acuminata* which corresponds to several reported antidiabetic properties of the plant (Zaruwa *et al*., 2012).

The MeOH sub fraction from the liquid partitioning of methanolic extract of *A.acuminata* showed the most effective hypoglycaemic activity and was thus further purified, from which castalagin was isolated. Its ^{13}C- and ^{1}H NMR Chemical shift values and structure are depicted in table 1 and figure 3 respectively.
5000mg/kg (0.0001mg/0.5ml) b.wt castalagin (crude extract equivalent) exhibited significant reduction ($p<0.05$) (65.06%) in fasting blood glucose as shown in table 1. This effect was comparable to the insulin effect but significantly ($p<0.05$) higher than the glibenclamide effect. Though castalagin has never been mentioned for the treatment of DM, its parent compound tannins have been reported to be an efficient insulin enhancer (Anderson and Polansky, 2002). Other studies have reported the role of tannins in efficiently controlling DM, whether used as drugs or in foods (Nyunaiet *et al.*, 2007; Broadhurst *et al.*, 2000; Iwu, 1983). Ellagitannins from other plant species have previously been reported to have hypoglycemic activity (Klein *et al.*, 2007; Maria *et al*, 2010). Therefore the new role of castalagin as a hypoglycaemic agent agrees with these findings and may be regarded as the bioactive component of *A. acuminata* responsible for its antidiabetic properties.

Conclusion

The significant hypoglycaemic activity exhibited by castalagin isolated from *A.acuminata* in this study, opens a new area in the pharmacological application of the compound as a suitable agent for the treatment and management of DM for the first time. The hypoglycaemic effect observed from castalagin elicits further investigation on the full potentials of this compound in the management of DM and related diseases.

References

Anderson, R. A., Polansky, M. M. (2002). Tea enhances insulin activity. *J. Agric Chem* 50: 7182 – 7186.

Broadhurst, C. L., Polansky, M. M., Anderson, R. A. (2000). Insulin like biological activity of culinary and medicinal plant aqueous extracts in vitro. *J Agric Food Chem.,* 48(3): 849 – 852.

Hemamalini, K., Prasad Naik, K. O., Ashok, P. (2010). Anti-inflammatory and

analgesic effect of methanolic extract of *Anogeissus acuminata* leaf. *Int J Pharm Biomed Res* 1(3): 98 – 101. Iwu, M. M. (1983). The hypoglycaemic properties of *Bridellia ferruginea*. *Fitoterapia*54(6): 243 – 248.

Kavishankar, G. B., Lakshimidevi, N., Mahadeva Murthy, S., Prakash, H. S. and Niranjana, S. R. (2011). Diabetes and medicinal plants. A review. *Int J Pharm Biomed Sci* 2(3): 65-80.

Klein, G., Kim, J., Himmeldirk, K., Cao, Y., Chen, X. (2007). Anti-diabetes and anti-obesity activity of *Lagerstroemia speciosa*. *eCAM* 4(4): 401 – 407.

Mani Kandaselvi, S., Subalaksuni, R., Thinagarabu, R. and Ravikumar, R. (2012). Evaluation of antidiabetic potential of ethanolic extract of leaves of *Ficus bengalensis* Linn. *Int J Pharm and Bio Sci*. 3(2): 108-114.

Manosroi, J., Zaruwa, M. Z**.,** Manosroi, A. (2011). Potent hypoglycemic effect of Nigerian anti-diabetic medicinal plants. *Journal of Complementary and Integrative medicine* 8(1): 1- 16.

Maria, D. P. T., Gunawan, P., Kawabata, J. (2010). Novel α-glucosidase inhibitors from *Macaranga tanarius* leaves. *Food Chem* 123 (2): 384 – 389.

Nyunai, N., Njikam, N., Abdennebi, E., Mbafor, J. T., Lamnaouer, D. (2009). Hypoglycemic and antihyperglycemic activity of *Ageratum conyzoides* L. in rats. *Afr. J. Trad. CAM*6(2): 123 – 130.

Rimando, A. M., Pezzuto, J. M., Farnsworth, N. R., Santisuk, T., Reutraku, V., Kawanishi, K. (1994). New Lignans from *Anogeissus acuminata* with HIV-1 reverse transcriptase inhibitory activity. *J Nat Prod*. 57(7): 896 – 904.

Sanye, S. K., Ghiware, N. B., Kawade, R. M., Gunjkar, V. N. and Vadvalkar, S. M. (2013). A short review on chronic complications in diabetes mellitus. *Int J of Res and Chem* 3(4): 842-845.

Shaw, J. E., Sicree, R. A. and Zimmet, P. Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice* 87: 4-14.

Tunrayo, A. T. (2013). Assessment of nutritional status of diabetic patients in Ogun State, Nigeria. *American Journal of Human Ecology* 2(4): 120 - 126.

Zaruwa, M. Z., Manosroi, J., Akihisa, T., Manosroi, W. Manosroi, A. (2012). Anti-diabetic activity of *Anogeissus acuminata* a medicinal plant selected from the Thai medicinal plant database Manosroi II. *Wudpecker Journal of Medicinal Plants* 1(1): 008-015.