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Research article

THE STEROIDAL COMPONENT AND HYPOGLYCAEMIC EFFECT OF STEM BARK EXTRACT OF *MITRAGYNA INERMIS* (WILD) O. KUNDZE (RUBIACEAE) IN ALLOXAN INDUCED DIABETIC WISTAR RATS.

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ABSTRACT: Among other ethno medicinal uses, *Mitragyna inermis* have been claimed by traditional medical practitioners in northern Nigeria to be an effective anti-diabetic agent. The hypoglycaemic effect of the ethanol extract of *M. inermis* on blood glucose levels of alloxan induced diabetic albino rats have been investigated. The results revealed that the plant possessed hypoglycaemic activity. Doses of 250, 350 and 450mgkg^{-1} body weight intraperitoneally (i.p.) were administered to the rats but the 350mg/kg^{-1} dose exhibited the highest hypoglycaemic potentials. The LD_{50} value (1600mgkg^{-1}) recorded on the ethanol extract of stem-bark of *M. inermis* indicated that it is slightly toxic but could be used safe on human at lower doses. Silica gel column fractionation of the ethanol extract of *M. inermis* led to the isolation of a pure compound **1,** 5-cholesten-3-phenyl-22, 24-β-diketone. ^{1}H and ^{13}C NMR and IR spectroscopy have been used jointly to determine the conformation of the compound.
Keywords: Steroids, Stem bark, *Mitragyna inermis*

INTRODUCTION

Mitragyna inermis is grown in the sub-saharan Africa (Konkon *et al*., 2008; Igoli *et al*., 2005; Inngierdingen *et al.,* 2004; Adjanohoun *et al.,* 1986; Burkill, 1985). It is known as "Giyayya/Etiyayya" in Hausa (northern Nigeria). The plant is a low branching tree and 16m, height. Its bole is 60cm diameter, scaly grayish bark and flower head is white. *M. inermis* is grown on damp perennially flooded, sites, swampy savannah, or inland site of coastal mangrove. The plant is common across the region from Mauritania to West Cameroon and into the Congo basin and Sudan (Burkill, 1985).

M. inermis is well known for its ornamental and medicinal purposes. Its medicinal applications, however, depends as practiced by inhabitants of different geographical areas. It is used for the treatment of diabetes, ulcer, pile, dysentery and bone pain among the Hausa/Fulani tribes of northern Nigeria. The plant is also used in association with *Cocos nuciefera* to treat jaundice in Ivory Coast (Konkon *et al*., 2008; Adjanohoun *et al.,* 1986). Decoctions prepared from the leaves, stems, and roots of the plant are reported to be used against malaria and boils in Mali (Inngjerdingen *et al.,* 2004; Azas *et al*., 2002). Other noticeable uses of the plant include, pain killer, arthritis, epilepsy, rheumatism, nasopharyngeal affliction, stomach trouble and venereal diseases (Burkil, 1985). Biological investigations on *M. inermis* revealed its antibacterial and anticonvulsant effects (*Zongo et al.,* 2009; Mu'azu and Kaita, 2008; Asase *et al.,* 2008). The hypotensive, cardiothoracic and vasodilatory properties of the aqueous extract of the plant was also reported (Ouedrago *et al.,* 2004).

The phytochemical analysis carried out on the leaf extracts of *M. inermis* revealed the presence of steroids, tritepenoids, polyphenols, flavonoids, catecnic tannins, saponins and alkaloids. The alkaloids, speciophylline and uncarine were isolated from the leaves. While glycosides (Inermiside I and II) were also isolated from the stem-bark of the plant (Cheng *et al*., 2002).

However, there are no scientific reports on the antidiabetic effect of *M.inermis*. The present study therefore was aimed to investigate the hypoglycaemic activity of the ethanol extract obtained from the stem bark of *M. inermis* in alloxan – induced diabetic rats. The study was also aimed to isolate an anti diabetic components from the extract of *M. inermis*.

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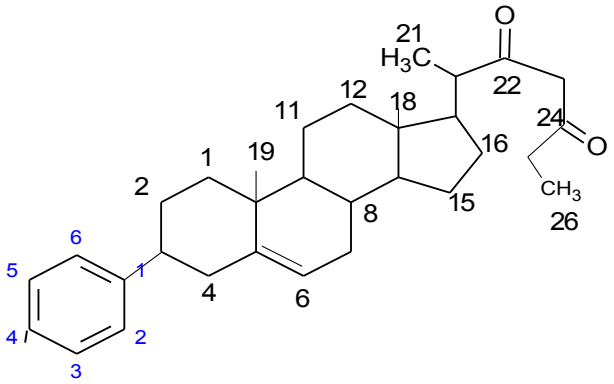

Compound 1

MATERIALS AND METHODS

Collection of Plant Materials

Fresh stem bark of *M. inermis* were collected from bush at Magenzaka Village in Safana Local Government Area of Katsina State (Nigeria) on 29th July, 2010. The plant was locally identified by Baba Sani of Government Pilot Secondary School, Batsari and authenticated by Dr Bala Sidi (Botanist) of Biological Sciences Department, Bayero University, Kano and Voucher specimen with accession No. 137 and plant No. 61 was deposited at the herbarium unit.

Extraction of Plant Materials

The plant materials were chopped, air dried for several days, pounded and sieved into powder using mortar, pestle and sieve. The powder (600g) was percolated with 5.0L of ethanol (96%v/v) for two weeks with occasional shaking for effective extraction. The ethanol extract was decanted and concentrated at 40°C using rotary evaporator. The crude extract (96.84g) obtained was kept in a deep freezer until used.

Isolation of Compound MI – 1 – 15

The ethanol extract (28g) of the stem bark of *M. inermis* was chromatographed on silica gel (170g) column and eluted in this order with n-hexane, n-hexane/chloroform (1:1), chloroform, chloroform/ethyl acetate (1:1), ethyl acetate, ethyl acetate/methanol (1:1), and methanol. The eluants were collected in portions of 100ml. Different fractions were collected into weighed beakers. Similar fractions were combined on the basis of their thin layer chromatography patterns. Fraction MI – 1 – 15 eluted with chloroform/ethyl acetate (1:1) gave a brownish semi solid compound (1.006g), 5-cholesten – 3 – phenyl 22, 24 – β- diketone 1. FT-IR (film) (cm^{-1}) 2926, 2348, 1601, 1268, 738 and 460.

^{1}HN MR (Acetone, 200 MHz) gave signals at δ1.007 (3H, d, J=6.8 Hz, 4H – 12), 0.21 (3H, δ, 3H – 26); ^{13}CN MR (Acetone, 50 MH_z) data see Tables 2, 3.

Animals

Wistar strain albino rats of both sex (120-220g) were purchased from the Pharmacology and Clinical Pharmacy Department, Ahmadu Bello University, Zaria and used for this study. They were kept in standard cages at room temperature and 12h day/night cycle in the animal house at the Pharmacology Department, Bayero University, Kano. The animals were feed freely on commercial feed (Vital feed) and water.

Acute Toxicity

The lethal dose LD_{50} of the extract was determined by the method described by Lorke (1983). In this method, 9 rats were divided into 3 groups 3 rats each in the first phase. The rats were injected intraperitoneally (ip) with the extract at single doses 100, 200 and 300$mgkg^{-1}$ and observed for signs of toxicity or death. In the final phase, another three groups (n=3) of 3 rats each were given 500, 1000 and 2,500$mgkg^{-1}$ (i.p) of extract and were observed for 24h for signs of toxicity or death. The median lethal dose LD_{50} was calculated from the final phase.

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Induction of *Diabetes mellitus* in the Animals: The animals were fasted. For 12h before commencement of the experiment but were allowed free access to water. Their fasting Blood FBG (55-120mg/dl) were also taken prior inducement. Diabetes was induced by a single intraperitoneally (i.p) injection of 150mg/kg of alloxan monohydrates dissolved in normal saline . the rats were kept for the next 24h on % glucose solution bottle in their cages to prevent hypoglycemia (Stanley and Venugal, 2001). After a period of 38 h, surviving rats with blood glucose level (BGL) greater than 200 mg/dl (BGL>200mg/dl) were considered diabetes and used for this research work.

Treatment of Diabetes Rats: The alloxan-induced diabetic rats were randomly assigned into five groups (A-E) of four rats each. Group A, B, and C, were treated with 250, 350 and 450mg/kg of ethanol extract respectively. Group D was the control group and was treated with distilled water only. Group E was treated with a standard anti-diabetic drug, glibenclamide. All the treatments were done intraperitoneally with a single dose.

Determination of Blood Glucose Levels (BGL): Blood samples for blood glucose determinations were collected from the tail tip at intervals of 0,2,4,6,8 24h. Determination of the blood glucose levels were done by the glucose-oxidase principle (Beach and Turner, 1958) using the One Touch Basic and results were reported as mg/dl (Rheney and Kirk, 2000).

Statistical Analysis

The results of BGL were presented as mean±SEM.

RESULTS AND DISCUSSION

There were no signs of toxicity observed in the rats throughout the 24 h of extract administration in the first phase (results not shown). In the second phase, particularly in the third group, there was a decrease in feed intake from 8 h of extract administration, consequently, there was 3/3 death recorded on this group. The LD_{50} was calculated as 1600 mg/kg. This value indicated that the extract was very slightly toxic to the test rats.

The change in glucose concentrations in diabetic and normal rats at different time intervals after intraperitoneal administration of the three doses, 250, 350 and 450 mg/kg of the stem – bark of *M. inermis,* glibenclamide and control groups is shown in Table 1.

The 250 mg/kg dose showed only a short term hypoglycaemic effect as it reduced the glucose concentration in diabetic rats to about 25% after 2 h of treatment compared with normal rats. Similar effect was recorded on 450 mg kg^{-1} dose in which a slight reduction in glucose level was observed at 6h interval. A significant reduction ($P<0.05$) was observed in the glucose levels of the diabetic rats at 350mg kg^{-1} compared with control normal rats. The reduction was noticed throughout the experiment (Table 1). This result is comparable with the hypoglycaemic effect of glibenclamide, an anti-diabetic drug. From the results (Table 1), the ethanol extract of the stem-bark of *M. inermis* can reverse the effect of alloxan-induced diabetes. Possible mechanisms by which the plant extract brings about its hypoglycaemic action may be by increasing either the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from the bound form or the extract may enhance peripheral utilization of glucose or reduce glucose absorption from the gastrointestinal tract (Stanley Mainzen Prince and Menon, 2002).

5-cholestene – 3 – phenyl – 22, 24 – β – diketone.

FT-IR spectra confirmed the aromatic υ (ring) 1601 cm^{-1}. The absorption at 1268 cm^{-1} is ketonic (C=0 absorption). The finger band at 737 cm^{-1} is also an indicative of carbonyl group. The band at 2926 cm^{-1} suggests the C-H str, for methyl and/or methylene groups.

The ^{1}HNMR spectra displayed diagnostic signals due to the influence of the ring system on the side chain methyl and methylene groups in the proposed compound. The presence of an aromatic ring is indicated by multiple at δ 6.8 and the aromatic pattern reveals mon-substitution. The unsaturation (H-C=C) was shown at δ5.3 (5H). The ^{13}C NMR spectral data (Table 3), further supported the identification of compound 1 as 5 – cholesten – 3 – phenyl – 22, 24 – β – diketone.

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Table 1: Effects of Ethanol Stem Bark Ethanol Extract of *Mitragyna inermis* on Alloxan-induced Diabetic Albino Rats.

Treatment	Blood Glucose Levels (mg/dl)					
	O h	2 h	4 h	6 h	8 h	24 h
Control (dist water)	202±0.00	395±22.76	470±0.00	448±3.54	412±43.13	299±0.00
Glibenclamide	376±21.80^{ns}	342±18.4^{a}	317±59.25^{a}	262±23.70^{a}	342±60.81^{a}	278±39.80^{a}
250mg/kg	422±26.68^{ns}	315±72.57^{a}	406±48.51^{a}	411±46.69^{a}	464±49.83^{ns}	516±50.68^{ns}
350mg/kg	453±89.58^{ns}	393±77.61^{a}	348±75.06^{a}	347±73.1^{a}	341±75.30^{a}	327±52.15^{a}
450mg/kg	398±28.10^{ns}	414±3106^{a}	427±29.28^{ns}	356±24.95^{a}	447±32.88^{a}	420±41.45^{ns}

Values are given as mean±SEM. Experimental groups are compared with control and standard drug groups.
a=P<0.05 = significant
ns = not significant
n=4

Table 2: ^{13}CNMR (200MHz. Acetone) data for compound MI-1-15

Carbon	PPM
1^{1}	115.570
2^{1}	131.351
3^{1}	129.098
4^{1}	132.482
5^{1}	118.484
6^{1}	115.001

Table 3: ^{13}CNMR (200MHz. Acetone) data for compound M1 – 1 – 15

Carbon	PPM
1.	75.969
2.	70.793
3.	114.265
4.	76.991
5.	105.586
6.	99.555
7.	74.753
8.	73.767
9.	87.628
10.	74.950
11.	55.991
12.	85.003
13.	77.295
14.	94.357
15.	64.662
16.	73.046
17.	88.698
18.	54.413
19.	48.062
20.	62.128
21.	55.831
22.	205.748
23.	102.233
24.	120.115
25.	100.161
26.	54.253

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CONCLUSION

The experiment evidence obtained in the present laboratory animal study indicated that ethanol extract of stem-bark of *Mitragyna inermis* possessed anti-diabetic properties. Silica gel column fractionation of the ethanol extract led to the isolation of compound d MI-1-15 which proposed to be 5-cholesten – 3 – phenyl – 22, 24 – β – diketone. Research work is in progress to study the hypoglycaemic effect of the isolated compound on rats.

REFERENCES

Asase, A., Kokubun, T., Grayer, J., Kite, G., Simmonds, M.S.J., Yeboah, A.A.O., and Odatten, G.T. (2008). Chemical Constituents and anti-malarial activity of medicinal plants from Ghana: *Cassia sieberiana, Haematostapis bateri, M. inermis* and *Pseudocedrela kotschyi. Phytother. Res.* 22: 1013 – 1016.

Azas, N., Lawrencin, N., Delmas, F., Digiorgio, C., Gasqnet, and Timo – David, P. (2002). Synergistic *in vitro* anti malaria activity of plant extracts used as traditional herbal remedies in Mali. *Biomedical and life sciences; parasitology research* vo. 88 (2). Pp 165 – 171.

Adjanohoun, E.J., Ahyi, M.R.A., Ake, A.I., Akpagana, K. and P. Chibon *et al,* (1986). Contributions aux etudes ethnobotaniques et floristiques au Togo. *Medicine Traditionelle et pharmaopee. Agence de Cooperation Culturelle et Technique,* Paris, France. Pp. 671.

Beach, E.F. and Turner, J.J. (1958). An enzymatic methods for glucose determination in the fluis. *Clin. Chem.* 4:462-465.

Burkill, H.M. (1985). The useful plants of West Africa Vol. 4, *Royal Botanical Garden* Kew (K).

Cheng, Z.H., Yua, B.Y. and Yang, X.W. (2002). 27-Nor-triterpenoids glycosides from *M. inermis. Phytochemistry,* 61: 379 – 382.

Igoli, J.O., Ogaji, O.G., Tor-Anyiin, T.A., Igoli, N.P. (2005). Traditional medicine practice amongst the Igede people o Nigeria. Part II. Africa J. Trad. CAM. 2 (2): 134 – 152.

Innjerdingen, K., Nergard, C.S., Diallo, D., Mounkoro, P.P. and Paulsen, B.S. (2004). An ethnopharmacological survey of plants used for wound healing in Dongoland, Mali. *West Africa J. Ethnopharmacol.,* 92: 233 – 244.

Konkon, N.G. Adejoungona, A.L., Manda, P., Simagn, D., Nguiesan, K.E and Kone, B.D. (2008). Toxicological and Phytochemical Screening Study of *M. inermis* (Wils) O. Kuntze (*Rub.*). Anti diabetic Plant. *J. Medicinal Plant Research* 2 (10), Pp. 279 – 284.

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Arch. Toxicol.* Pp. 275 – 287.

Mu'azu, J and Kaita, A.H. (2008). A review of tropical plants used in the treatment of epilepsy amongst the Hausa/Fulani tribes of northern Nigeria. *Africa Journal of Traditional, Complementary and Alternative Medicines.* Vol. 5 (4), Pp. 387 – 390.

Ouedraogo, S., Ranlayo, H.R., Ndiaye, M., Kabore, Z.I., Guissous, I.P., Bucher, B. and Andrianotsitohaina, R. (2004). Cardiovascular properties of aqueous extract from *M. inermis* (Wild). *J. Ethnopharmacol.,* 93: 35 – 350.

Rheney, C.C. and Kirk, K.K. (2000). Performance of three blood glucose meter. *Ann. Parmacother.* 34 (3): 317 – 321.

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Stanley Mainzen Prince and Menon (2003). Hypoglycaemic and Hypolipidaemic Action of Alcohol Extract of *Tinospora cordifolia* Roots in chemical induced diabetes in rats. *Phytother. Res. 17* (410 – 413).

Stanley, M.P. and Venugopal, M.P. (2001). Anti-oxidant action of *Tinospora cordifolia* roots extract in alloxan diabetic rats. *Phytother Res.* 15: 213 – 218.

Zongo, C., Etieme Francis, O.A., Savadogo, A., Luois, C.O., Jean, K. and Alfred, S.T. (2000). *In-vitro* Antibacterial properties of Total Alkaloids Extract from *M. inermis* (Wild) O. Kuntze: a west African Traditional Medicinal Plant. *Asian Journal of Plant Science,* 8: 172 – 177.