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Original Article

BIOASSAY-GUIDED EVALUATION OF THE ANTIDIABETIC ACTIVITY OF *CLEOME RUTIDOSPERMA* DC

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ABSTRACT

Objective: An earlier anti-hyperglycemic study with crude extracts of *Cleome rutidosperma* indicated aqueous extract as the most effective. The present study was undertaken to in part identify the potent antihyperglycemic fraction from the aqueous extract of the plant, using bioassay guided fractionation.

Methods: Aqueous extract of *C. rutidosperma* were fractionated to obtain chloroform, ethyl acetate, n-butanol, methanol and aqueous fractions, which were tested for antidiabetic activity using acute Streptozotocin-Induced diabetic mice model. Further fractionation of the more active methanol fraction yielded 1st sub-fractions I- IX. The more active of these 1stsub-fractions were further re-fractionated to give 2ndsub-fractions (2SFC1 and 2SFC2). The more active of the 2ndsub-fractions (2SFC1) was purified further using preparative thin layer chromatography (TLC) and the resultant fractions (TLCFIC and TLCFIIC) were tested *in vivo*.

Results: The methanol fraction of *C. rutidosperma* significantly ($p < 0.05$) reduced blood glucose more than the other fractions, while the most active 1st sub-fraction from *in vivo* studies in mice was, chloroform: methanol (5: 5). Also, the more active of the 2ndsub-fractions was: 2SFC1. The preparative thin layer chromatography (TLC) results from *in vivo* studies indicated TLCFIC to be the most active.

Conclusion: The observed antidiabetic activity of the plant may be as a result the phytoconstituent of the plant. Therefore the fractionated component could be a new source of development of new plant based therapy for management of diabetes.

Keywords: Bioassay guided-fractionation, *Cleome rutidosperma*, Preparativee thin layer chromatography (TLC) and Streptozotocin-Induced diabetes.

INTRODUCTION

Bioassay (or biological assay) is the estimation of the activity or potency of a drug or other substance (e. g. plant extract) by comparing its effects on a test organism with that of a standard preparation. It is a type of scientific experiment conducted to measure the effects of a substance on a living organism and is essential in the development of new drugs and other scientific monitoring. The driving force behind much phytochemical research is the discovery of new biologically active compounds for medicinal or agricultural uses. Biological assays then must be carried out in order to identify promising plant extracts, to guide the separation and isolation, and to evaluate lead compounds. Identification of natural products from plants that may serve as valuable sources of bioactive agents for medicinal and agricultural uses largely depends on bioactivity-directed isolation [1].

Diabetes is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein [2] characterized by increased fasting and postprandial blood sugar levels. Diabetes mellitus is classified into two major subtypes: type I (insulin dependent diabetes mellitus, IDDM) and type II (non-insulin dependent diabetes mellitus, NIDDM). IDDM or juvenile-onset diabetes results from a cellular mediated autoimmune destruction of the β-cells of the pancreas [3].

However, NIDDM or adult-onset diabetes results from the development of insulin resistance and the affected individuals usually have insulin deficiency [4]. Type II diabetes is the most common form of diabetes constituting 90% of the diabetic population. In 1995 it was estimated that around 135 million people were affected from this condition and it was expected to affect 300 million by the year 2025 [5]. Management of diabetes without any side effect is still a challenge to the medical community. Several drugs such as biguanides, sulfonylurea and thiazolidenediones are presently available to treat diabetes mellitus [6]. The use of these drugs is restricted by their pharmacokinetics properties, secondary failure rates and an accompanying side effects [7].

Thus searching for a new class of compounds is essential to overcome diabetic problems ultimately leading to continuous search for alternative drugs [8]. The medicinal plants may provide the useful source of new oral hypoglycemic compounds for the development of pharmaceutical entities or as dietary adjunct to existing therapies [9]. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agents from medicinal plants has become more important. The ethnobotanical information reports state that about 800 plants may possess anti-diabetic potential [10]. Recently, the medicinal values of various plant extracts have been studied by many scientists in the field of diabetic research [11]. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare.

Cleome rutidosperma DC is a low-growing herb, up to 70 cm tall, found in waste grounds and grassy places with trifoliate leaves and small, violet-blue flowers, which turn pink as they age. The plant is native to West Africa, from Guinea to Nigeria, Zaire and Angola. It has become naturalized in various parts of tropical America as well as Southeast Asia [12]. *Cleome rutidosperma* has been well studied by different researchers. The analgesic, antipyretic, anti-inflammatory, antimicrobial, diuretic, laxative antioxidant, and antiplasmodial activities of the plant have already been reported [13-19]. *Cleome rutidosperma* is traditionally used in the treatment of paralysis, epilepsy, convulsions, spasm, earache, pain and skin disease [20].

Several works have been carried out in the past on *Cleome rutidosperma* to verify its folkloric uses. Earlier studies by the authors on the crude extracts of this plant have established some significant antidiabetic properties [21]. Therefore, the present work is an effort geared towards the bioassay-guided fractionation of the aqueous extracts of *C. rutidosperma* with a view to isolating and characterizing the constituents responsible for the antidiabetic properties observed in the plant.

MATERIALS AND METHODS

Reagents and chemicals

All reagents and chemicals used were of analytical grade purchased from Sigma Chemical Company, St. Louis, Missouri, USA.

Plant materials

The plant, *Cleome rutidosperma* was collected from Abraka in Delta State, Nigeria. It was identified at the Herbaruim Unit, Department of Plant Science, University of Benin, Benin City, Edo State-Nigeria, where the voucher specimen was deposited with number: UBHc0148.

Experimental animals

Male Wistar Albino rats weighing 120-200g and mice weighing 25-35g were obtained from National Veterinary and Research Institute (NVRI) Vom, Jos, Plateau State, Nigeria. The animals were allowed 3 weeks of acclimatization before commencement of an experiment. They were fed on the standard laboratory diet (Vital Feed Nig. Ltd, Jos, Nigeria) and water *ad libitum* throughout the experiment.

Treatment and extraction of plant samples

The plant sample was washed with distilled water and air-dried at room temperature, cut into small pieces and pulverized into fine powder using pestle and mortar. It was first extracted with petroleum ether and then distilled water by cold maceration method. Fifty grams(50g) powder of the plant was soaked in 200 ml of distilled water in airtight conical flask with daily shaking for three days at room temperature and was first filtered through double layered muslin cloth and then filtered through Whatman No 1filter paper. The filtrate was collected and concentrated at 50^0C using water bath and the dried extract was stored in the refrigerator at 4^0C till further use.

Bioassay guided fractionation of aqueous extracts

The crude aqueous extract of *Cleome rutidosperma* was fractionated by initially dissolving 110g of the extract in 500 mL of distilled water, mixed in a beaker and then filtered using No.1 What-man filter paper. The filtrate was thereafter transferred into a 1 L separating funnel and extracted with 5 × 250 mL n-butanol. The combined n-butanol fraction was concentrated using water bath at 50 ^{0}C. The aqueous layer was sequentially extracted with 3 × 250 mL chloroform, 3 × 250 mL ethyl acetate and 3 × 250 mL methanol. The combined chloroform, ethyl acetate and methanol fractions were separately concentrated using water bath at 50^0C. Finally, the remaining aqueous fraction was also concentrated using water bath at 50^0C.

Further fractionation of active methanol fraction (based on *in vivo* antihyperglycemic study of the above fractions) was done using silica gel-column chromatography; by loading it with 100g of silica gel (Qualikems, 60- 120 mesh). Four (4 g) grams of the methanol fraction was pre-adsorbed onto silica gel adsorbent (60–120 mesh) by firstly solubilizing it in little quantity of methanol, followed by addition of the silica gel (3 g) and then mixing.

The dried fraction-adsorbent mixture was then evenly loaded onto the top of the already packed column. The column was first eluted with 2 × 300 mL 100% chloroform followed successively by 2 × 300 mL chloroform- methanol in graded ratios (9:1), (8:2), (7:3), (6:4), (5:5), (4:6), (3:7), (2:8), (1:9) and finally (0:10). These 1st sub-fractions were collected and concentrated as described above.

The most active 1st sub-fractions from *in vivo* studies was: chloroform: methanol (5: 5). This 1st sub-fraction was further re-fractionated in a column (25 × 3 cm) loaded with silica gel (Qualikems, 60- 120 mesh) as described above. The column was first eluted with 2 × 300 mL 100% chloroform followed by 2 × 300 mL chloroform- methanol (5:5) and 2 × 300 mL chloroform- methanol (0:10). Second sub-fractions were collected and on the basis of their Rf values, similar sub fractions were pooled together to give 2nd sub-fraction C1 (2SFC1) and 2nd sub-fraction C2 (2SFC2). These sub fractions were tested for their blood glucose lowering effect in STZ-induced diabetic mice.

The more active of the 2nd sub-fractions was 2SFC1. It was purified further using preparative thin layer chromatography (TLC) with Ethyl acetate: Methanol: Acetic acid (7:2:1) solvent system. The resultant spots: spots I and II (SIC and SIIC) were tested for antidiabetic activity *in vivo*.

Intra-peritoneal acute toxicity studies (LD_{50})

In our previous work on the oral acute toxicity of extracts of *Cleome rutidosperma* [21], we found the LD_{50} of the plants to be > 5000mg/ kg body weight. Consequently in this study, we decided to carry out the intra-peritoneal acute toxicity of the plant in order to ascertain the safe doses of the plant sub fractions to be administered to animals through this route. The intra-peritoneal LD_{50} determination was done in two phases by the method of Lorke [22] using 13 rats. In phase one, rats were divided into 3 groups of 3 rats each and were treated with 10, 100 and 1000 mg 1st sub-fractions/kg body weight. The rats were observed for clinical signs and symptoms of toxicity within 24 h. Based on the results of phase one, the second phase was done which consisted of 4 rats divided into 4 groups of 1 rat each and were treated with 1200, 1600, 2900 and 5000 mg 1st sub-fractions/kg body weight. Clinical signs and symptoms of toxic effects and mortality were then observed for 72 hours. The median Lethal Dose (LD) was then calculated.

Comparison of the effects of oral and intra-peritoneal routes of administration of 1st Sub-fractions of *C. rutidosperma* on fasting blood glucose level in STZ-induced diabetic mice

In order to compare the effect of oral and intra-peritoneal routes of administration of the most active 1st Sub-fraction of *C. rutidosperma* on fasting blood glucose level, 125mg/kg body weight of the sub fraction was administered to STZ-induced diabetic mice. Here, diabetes was induced as described previously [27]. Fifteen mice were randomly allocated to negative control and treatment groups of five mice each as follows:

Group I: Diabetic control mice.

Group II: Diabetic mice given 125mg/kg body weight of the sub fraction of *Cleome rutidosperma* (intraperitoneal).

Group III: Diabetic mice given 125mg/kg body weight of the sub fraction of *Cleome rutidosperma* (oral).

Fasting blood glucose concentration was first determined in overnight fasted mice by the enzymatic glucose oxidase method using a commercial glucometer (Accu-chek® Active, Roche diagnostic, Mannheim, Germany), following which the plant sub fraction was administered through oral/ intra-peritoneal routes. Blood glucose values were then estimated hourly for 6 hour.

Statistical analysis

The results are presented as mean ± SD and were analysed using ANOVA followed by Tukey kramer multiple comparison test in Graphpad Prism, version 6.0 0 (Graph Pad Software, San Diego, CA, USA) and values of $P< 0.05$ were considered significant.

RESULTS

The effects of the different fractions of *C. rutidosperma* on fasting blood glucose (FBG) levels of STZ-induced diabetic mice are shown in fig. 1. Significant decrease ($p<0.05$) in FBG was seen in the treated diabetic mice, particularly the group treated with methanol fraction of the plant. Thus, the methanol fraction is the most active fraction of the plant. The phytochemical screening of the methanol fraction indicated that the fraction was rich in phytochemicals as shown in table 1.

Shown in fig. 2 are the effects of different 1st Sub-fractions of *C. rutidosperma* and glibenclamide on fasting blood glucose level of STZ-induced diabetic mice. A significant decrease ($p>0.05$) in FBG was seen in the diabetic mice treated with the 1stsub-fraction eluted with chloroform: methanol (5: 5). Phytochemical screening of this sub-fraction revealed the presence of all phytoconstituents present in the methanol fractions of the plant except Saponnins and Steroids (Table 2).

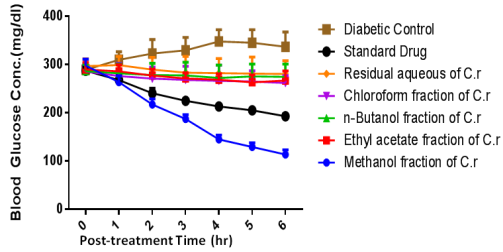

Fig. 1: Effects of different fractions of *C. rutidosperma* (C. r.) and standard drug (Glibenclamide) on fasting blood glucose level of STZ-induced diabetic mice.

Table 3 present the results of acute toxicity of 1st subfractions of *C. rutidosperma* leaves in rats. The different groups were treated with *C. rutidosperma* showed no specific abnormalities signs and mortalities in the first phase, but the behavior of rats changed in the second phase with signs such as fatigue and loss of appetite, and there was 100% mortality for all groups. Comparison of the effects of oral and intra-peritoneal routes of administration of different 1st sub-fractions of *C. rutidosperma* (c. r.) on fasting blood glucose level in STZ-induced diabetic mice is shown in fig. 3. The intraperitoneal administration of 125 mg/ kg of *C. rutidosperma* sub-fraction caused a significant ($p<0.05$) reduction in FBG than the same dose of same sub-fraction of the plant administered orally. Comparatively, the intra-peritoneal route of administration demonstrated the best effect.

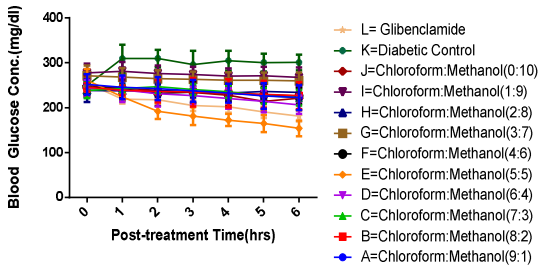

Fig. 2: Effects of different 1st sub-fractions of *C. rutidosperma* (C. r.) and Glibenclamide on fasting blood glucose level of STZ-induced diabetic mice.

Table 1: Phytochemical Constituents of the Methanol Fraction of *C. rutidosperma*

Constituents	Tests Results	
Carbohydrates	Molisch	+
Cardiac glycosides	Kelle Killiam	+
Saponnins	Frothing	+
Steriod	Liberman Buchard	+
Triterpene	Liberman Buchard	+
Tannins	Ferric Chloride	+
Flavonoid	Sodium hydroxide	+
Alkaloid	Mayers	+
	Dragendorff	+
	Wagners	+
Phenols	Pyridins-ferric chloride	+

(+) – Presence of phytochemical compound, (–) – Absence of phytochemical compound

Table 2: Phytochemical Constituents of the Most Active 1stSub-fractions of *C. rutidosperma*

Constituents	Tests Results	
Carbohydrates	Molisch	+
Cardiac glycosides	Kelle Killiam	+
Saponnins	Frothing	-
Steroid	Liberman Buchard	-
Triterpene	Liberman Buchard	+
Tannins	Ferric Chloride	+
Flavonoid	Sodium hydroxide	+
Alkaloid	Mayers	+
	Dragendorff	+
	Wagners	+
Phenols	Pyridins-ferric chloride	+

(+) – Presence of phytochemical compound, (–) – Absence of phytochemical compound

Table 3: Acute Toxicity of the Sub fractions of *C. rutidosperma* Leaves in Rats by Intraperitoneal Route after 72 hours of Observation

Group	Number of rats	Doses (mg/kg)	*C. rutidosperma*	
			Number of rats died	Percentage of rats died
1	3	10	0	0
2	3	100	0	0
3	3	1000	0	0
1	1	1200	1	100
2	1	1600	1	100
3	1	2900	1	100
4	1	5000	1	100
LD_{50}			1095.45 mg/kg body weight	

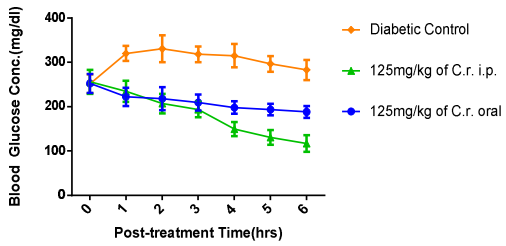

Fig. 3: Comparison of the effects of oral and intraperitoneal routes of administration of different 1st sub-fractions of *C. rutidosperma* (C. r.) on fasting blood glucose level in STZ-induced diabetic mice

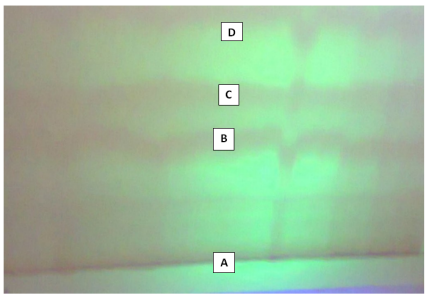

Plate 1: Preparative TLC fractionation of the Most Active 2nd Sub-fraction of *C. rutidosperma*, A= Origin, B= TLC fraction I(TLCFIC), C= TLC fraction II(TLCFIIC), D= Solvent front

Shown in fig. 4 are the effects of the 2nd sub-fractions of *C. rutidosperma* (C. r.) and glibenclamide on fasting blood glucose level of STZ-induced diabetic mice. Comparatively, the 2nd sub-fraction 1 gave the highest glucose lowering effect for this plant

The effects of the constituent(s) of preparative TLC spots of *C. rutidosperma* (Plate 1) and glibenclamide on fasting blood glucose level of STZ-induced diabetic mice are shown in fig. 5. Significant ($p < 0.05$) reduction in FBG was demonstrated by TLCFIC.

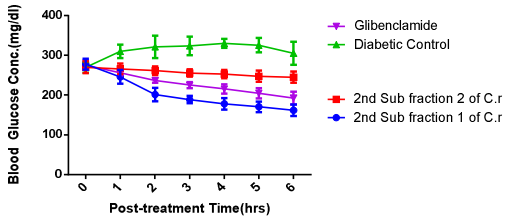

Fig. 4: Effects of different 2nd sub-fractions of *C. rutidosperma* (C. r.) and Glibenclamide on fasting blood glucose level of STZ-induced diabetic mice

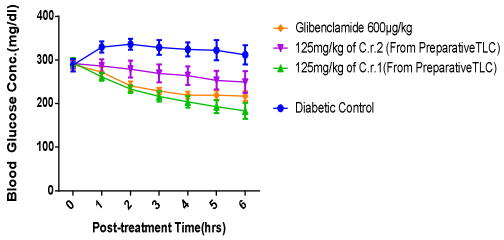

Fig. 5: Effects of constituent of different preparative TLC spots of *C. rutidosperma* (C. r.) and Glibenclamide on fasting blood glucose level of STZ-induced diabetic mice

DISCUSSION

Based on promising antihyperglycemic results from previous work on the plant investigated in this study [21], we attempted to isolate and identify the antidiabetic principle in the plant by carrying out partial purification of the most active extract of the plant(*Cleome rutidosperma*). Firstly, the aqueous extract of the plant was partitioned between methanol, chloroform, ethyl acetate and n-butanol

Table 4: Maximum Percentage decrease in FBG of Most Active fraction at different fractionation stages of *C. Rutidosperma* in STZ-induced Diabetic Mice

Fractionation Stage/Fraction	Maximum % Decrease in FBG
1 (Methanol fractions)	43.49 ±6.33[a]
2 (1st Sub fractions)	40.97 ±3.33[a]
3 (2nd Sub fractions)	41.73 ±4.78[a]
4 (Spots from preparative TLC)	37.76 ±4.08[a]

Values are mean of five determinations ± SD; Values with different superscripts down the column differ significantly ($p<0.05$).

The highest reduction of fasting blood glucose from *in vivo* study of the fractions was obtained from the methanol fraction. This fraction was further purified in stages to give various sub fractions. A GC-MS analysis of the final most active of the subfractions was carried out. Phytochemical screening of the methanol fraction revealed the presence of carbohydrates, cardiac glycosides, saponnins, steriod, triterpene, tannins, flavonoid, alkaloid and phenols.

Contrary to the expected results from activity-guided assays, the antidiabetic activity of the more active fraction from preparative TLC (TLCFIC)-the purer fraction, was found to be lower than the initial methanol fraction at stage one of fractionation. This proposes that the bioactive principles in the initial fractions may act synergistically to produce the antidiabetic effect, and fractionation might have removed some of the compounds. Indeed, synergistic antidiabetic effects of several antidiabetic plants have been previously reported [23]. It has also been reported that the reasons behind apparent loss or total absence of any biological activity include the amount at which a particular potential metabolite is produced, synergistic or antagonistic relationship among the molecules when the crude extracts are tested for the biological activities. These are some variables that can significantly alter the efficacy of a particular metabolite in a plant [24, 25].

LD50 determination has remained a useful tool in the safety assessment of substances. In spite of its several criticisms [26, 27]. The LD50 of the most active subfraction of *Cleome rutidosperma* was calculated to be 1,095.45mg/kg. According to the classification of Clarke and Clarke [29], substances that have an intraperitoneal LD50 between 50 and 500mg/kg are considered toxic and Onyeyilli et al [29] categorized an intraperitoneal LD50 of 1400mg/kg under low toxicity. Thus, the estimation of median lethal dose (LD50) of intrapentoneal subfraction of the plant (1,095.45mg/kg body weight), is suggestive of toxicity. Therefore, the subfraction of *Cleome rutidosperma* may not be safe when taken through the intraperitoneal route. To evaluate the most effective route of administration of fractions of *C. rutidosperma,* a comparative

study was done between oral and intra-peritoneal routes. The results showed the intra-peritoneal route of administration as the most effective. This finding is in agreement with the work of Garg [30] who pointed out that the pharmacokinetics disposition of a drug using intraperitoneal route of administration is faster and more effective in comparison with that of the oral route.

CONCLUSION

The fractions of *Cleome rutidosperma* showed significant antidiabetic activities with minimal toxicity when gave orally. Therefore the fractionated components could be new sources of development of new plant based therapy for management of diabetes.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Ukwueze SE, Osadebe POa, Ezenobi NO. Bioassay-guided evaluation of the antibacterial activity of *Loranthus* species of the African mistletoe. Int J Pharm Biomed Res 2013;4(2):79-82.
2. Kahn CR, Shechter Y. Insulin oral hypoglycemic agents and the pharmacology of the endocrine pancreas in the pharmacological basis of therapeutics. In: Gillman AG, Rail TW, Nies AS, Taylor P (eds) Insulin oral hypoglycemic agents and the pharmacology of the endocrine pancreas in the pharmacological basis of therapeutics. Pergamon press, New York; 1991. p. 1463–95.
3. Aikinson MA, Maclaren NK. The pathogenesis of insulin dependent diabetes. N Engl J Med 1994;331:1428–36.
4. Takeshi K, Shoichi N, Yasunori K. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. Diabetes Res Clin Pract 2002;55:65–85.
5. King H, Aubert RE, Herman WH. Global burden of diabetes 1995–2025:prevalence, numerical estimates, and projection. Diabetes Care 1998;21:1414–31.
6. De-Fronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. In: International Text book of Diabetes mellitus, 2nd edn. Chichester, John Wiley, England; 1997. p. 635–712.
7. Donath MY, Ehses JA. Type I and type II diabetes: NOD the diabetes we thought it was. Proc Natl Acad Sci 2006;103:12217–8.
8. Hansotia T, Drucker DJ. GIP and GLP-1 as incretin hormones: lessons from single and double incretin receptor knockout mice. Regul Pept 2005;128:125-34.
9. Pepato MT, Mori DM, Baviera AM. Fruit of the jambolan tree (Eugenia jambolana) and experimental diabetes. J Ethnopharmacol 2005;96:43-8.
10. Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia*: a review. J Ethnopharmacol 2004;93:123–32.
11. Jain AK, Mohan G, Vairale RS. Folklore claims on some medicinal plants used by Bheel tribe. Ind J Tradit Knowl 2010;9(1):105–7.
12. Waterhouse B, Mitchell A. Northern australia quarantine strategy weeds target list. AQIS Miscellaneous Publication, Canberra; 1998. p. 29.
13. Bidla G, Titanji VPK, Joko B, El-Ghazali G, Bolad A, Berzins K. Antiplasmodial activity of seven plants used in African folk medicine. Indian J Pharmacol 2004;36(4):245-6.
14. Bose A, Saravanan VS, Karunanidhi N. Analgesic and locomotor activity of extracts of *cleome rutidosperma* DC. Indian J Pharm Sci 2004;66:795-7.
15. Bose A, Gupta JK, Ghosh T. Antimicrobial activity of certain extracts of *Cleome rutidosperma*. Indian J Nat Prod 2005;21:39-41.
16. Bose A, Mondal S, Gupta JK, Dash GK, Ghosh T, Si S. Studies on diuretic and laxative activity of ethanol extract and its fractions of *Cleome rutidosperma* aerial parts. Pharmacogn Mag 2006;2(7):178-82.
17. Bose A, Gupta JK, Dash GK. Ghosh T, Si S. Diuretic and antibacterial activity of aqueous extract of *Cleome rutidosperma*. DC. Ind J Pharm Sci 2007;69(2):292.
18. Bose A, Mondal S, Gupta JK. Antioxidant and free radical scavenging activities of *Cleome rutidosperma*. Oriental Pharm Exp Med 2008;8:135-45.
19. Bose A, Smith PJ, Lategan CA. Studies on *In Vitro* antiplasmodial activity of *Cleome rutidosperma*. Acta Polanica Pharm-Drug Res 2010;67:315-8.
20. Burkill HM. The useful plants of west tropical Africa, vol. 1 (Families A-D), 2nd ed. Royal Botanic Gardens, United Kingdom; 1985. p. 960.
21. Okoro IO, Umar IA, Atawodi SE, Anigo KM. Antidiabetic effect of *Cleome rutidosperma* DC and *Senecio biafrae* (Oliv. & Hiern) extracts in streptozotocin-induced diabetic rats. Int J Pharm Sci Res 2014;5(6):2480-97.
22. Lorke D. A new approach to acute toxicity testing. Arch Toxicol 1983;54:275-87.
23. Atangwho IJ, Ebong PE, Eyong EU, Asmawi MZ, Ahmad M.Synergistic antidiabetic activity of Vernonia amygdalina and Azadirachta indica: biochemical effects and possible mechanism. J Ethnopharmacol. 2012; 141(3):878-87.
24. Osadebe PO, Ukwueze SE. Comparative study of the antimicrobial and Phytochemical properties of mistletoe leaves sourced from six host trees. J Biol Res Biotechnol 2004; 2:18–23.
25. Wagner ML, Teresa F, Elida A, Rafeal, AG.Comparison of micro- and macromolecular criollo mistletoe (Ligaria cuneifolia (R. et. P) Tiegh and European mistletoe (Viscum album L). Pharmaceutical Act Buenos Aires 1996; 15(2):99-105.
26. Ozolua RI, Eshiotsemie S, Tafamel IGE. Acute and sub–acute toxicological assessment of aqueous leaf extract of Bryophyllum pinnatum (Lam.) in Sprague –Dawley rats. Am J Pharmacol Toxicol 2010;5(3):145–57.
27. Shivananda Nayak BS. Evaluation of wound healing activity of ethanolic extract of Morinda citrifolia L. Leaf Evid – Based comp. Alternat Med 2009;6:351-6.
28. Clarke CG, Clarke ML. Veterinary Toxicology, 1st Ed. Bailliere Tindall. London; 1977. p. 10–20.
29. Onyeyili PA, Sandabe UK, Chibuzo GA, Balewa A. Studies on the effect of the stem bark extract of Ficus thonningi in the nervous system. Biosci Res Commun 2000;12:34-7.
30. Garg SK. Veterinary Toxicology, CBS Publishers and Distributors, Darya Ganj, New Delhi, India; 2004. p. 321.