

Research Article

Comparative Antihyperglycemic Effect of Petroleum Ether, Acetone, Ethanol and Aqueous Extracts of *Cleome ruidosperma* DC and *Senecio bialfrae* (Oliv. and Hiern) in Streptozotocin-induced Diabetic Mice

^{1,2}I.O. Okoro, ¹I.A. Umar, ¹S.E. Atawodi and ¹K.M. Anigo

¹Department of Biochemistry, Ahmadu Bello University, Samaru Zaria,

²Department of Biochemistry, Delta State University, Abraka, Delta State, Nigeria

Abstract: The present study aimed to investigate the acute antihyperglycemic effects of four different solvent extracts (Petroleum ether, acetone, absolute ethanol and water) of two plants: *Cleome ruidosperma* (leaves) and *Senecio bialfrae* (root) in STZ-induced diabetic mice and their *in vitro* antioxidant activities as well as their phytoconstituents. A single administration of the extracts at a dose of 500mg/kg body weight was done. All extracts as well as the standard drug, glibenclamide (at 600 µg/kg body weight) significantly lowered blood glucose level ($p < 0.05$) of the diabetic mice by 28.784±15.46- 40.866±15.61% for *Cleome ruidosperma* and 10.652±6.36- 19.858±7.65% for *Senecio bialfrae* within 6 hrs of extracts administration. The effect was more pronounced in the aqueous extracts of both plants. The median lethal dose (LD₅₀) of the extracts in rats was found to be > 5000 mg/kg body weight. The tested plant extracts displayed appreciable level of antioxidant activities by different assay methods: the ferric thiocyanate (FTC) method (26.42±1.75-56.46±2.83%), the thiobarbituric acid (TBA) method (18.43±1.67- 48.17±2.19%), reducing power method (22.76±1.87-68.45±1.07%) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method (26.23±0.94-66.12±1.41% inhibition). Quantitative phytochemical analysis of the extracts revealed the presence of Alkaloids (0.11±0.02-0.34±0.05%), Saponins (0.18±0.02-6.01±0.88%), Tannins (0.04±0.01-14.15±0.73%), Glycosides (0.002±0.001-0.113±0.040%), Phenols (0.02±0.01-0.21±0.02%), Flavonoids (0.012±0.003-0.433±0.170%) and Steroids (0.003±0.001-0.011±0.003%). Therefore, the results indicate that extracts of *Cleome ruidosperma* and *Senecio bialfrae* possess significant antidiabetic and *in vitro* antioxidant activities. They are also rich in phytochemicals.

Keywords: Antihyperglycemic effects, antioxidant, *Cleome ruidosperma*, phytoconstituents, *Senecio bialfrae* and STZ-induced diabetes

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in enzymes and high oxidative stress induced damage to pancreatic beta cells (Sharma *et al.*, 2010). It is also characterized by polyuria, albuminuria, renal enlargement and an increase in serum creatinine value (Sassy-Prigent *et al.*, 1995). Diabetes is becoming the third common killer of mankind, after cancer and cardiovascular disease, because of its high prevalence, morbidity and mortality (Li *et al.*, 2004). The presence of DM confers increased risk of many devastating complications such as cardiovascular disease, peripheral vascular disease complications such as coronary artery disease, stroke, neuropathy, renal failure, retinopathy amputations and blindness.

Several distinct types of diabetes mellitus exist and are caused by a complex interaction of genetics and environmental factors (Chitra *et al.*, 2010). According

to the World Health Organization estimate 3% of the world's populations (194 million) have diabetes and is expected to double (6.3%) by the year 2030 (Wild *et al.*, 2004) and much of this increase occurs in developing countries due to population growth, ageing, unhealthy diet, obesity and sedentary lifestyle (WHO, 2002) and in Nigeria, diabetes was found to be as high as 23.4% among the upper class socioeconomic group and 16% among the lower class socioeconomic group (Nwafor and Owhoji, 2001).

From ancient period, people have been using medicinal plants for the treatment of diabetes and WHO estimates that 80% of the populations presently use herbal medicine for primary health care (Atmakuri and Dathi, 2010). Anti-diabetic plants have the ability to restore the function of damaged pancreatic tissue by increasing the insulin or inhibiting the intestinal absorption of glucose (Malviya *et al.*, 2010). Administration of appropriate antioxidants from plant source could prevent or retard the diabetic complications to some extent (Muthulingam, 2010).

Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important. Plant drugs and herbal formulation are frequently considered to be less toxic and free from side effects than synthetic ones (WHO, 1985). Thus, 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 (Kavishankar *et al.*, 2011).

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich source of free radical scavengers (Zheng and Wang, 2001; Cai *et al.*, 2003; Gracelin *et al.*, 2012). They are also antioxidant compounds which possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial and antiviral activities (Sala *et al.*, 2002; Gracelin *et al.*, 2012). Ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes and other diseases associated with ageing (Ashokkumar *et al.*, 2008). Phytochemicals isolated from plant sources have been used for the prevention and treatment of cancer, heart disease, diabetes mellitus and high blood pressure (Waltner-Law *et al.*, 2002).

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 (Waterhouse and Mitchell, 1998). It is commonly known as Spider plant or fringed spider flower with local names of “garseya” (in Hausa), “àkidimmōó” (Igbo) and “ètare” (Yoruba), in Nigeria. *Cleome rutidosperma* has been well studied by different researchers. The analgesic, antipyretic, anti-inflammatory, locomotory, antimicrobial, diuretic, laxative, antioxidant and antiplasmodial activities of the plant have already been reported (Bidla *et al.*, 2004; Bose *et al.*, 2004, 2005, 2006, 2007, 2008, 2010). *Cleome rutidosperma* is traditionally used in the treatment of paralysis, epilepsy, convulsions, spasm, ear-ache, pain and skin disease (Burkill, 1985). However, there is paucity of literature on the anti-diabetic potential of this plant.

Senecio biafrae (Oliv. and Hiern), also known as English spinach is a perennial climbing herb which naturally occurs in African forest zones, from Guinea to Uganda. It's local names in Nigeria are: “ota eke” (Igbo) and “worowo” or “ròròwò” (Yoruba). It is one of the green leafy vegetables consumed in Sierra Leone, Ghana, Benin Republic, Nigeria, Cameroon and Gabon (Adebooye, 2004). Also, leaves of *Senecio biafrae* contain various secondary metabolites such as dihydroisocoumarins, terpenoids, sesquiterpenes or

amino acids (Dairo and Adanlawo, 2007; Tabopda *et al.*, 2009). It is equally known for its therapeutic virtues, notably in Nigeria where it is used in the treatment of diabetes or pulmonary defects (Burkill, 1985; Iwu, 1993; Adebayo, 2009). The leaves and stems of the plant are used either macerated in water or in palm wine by traditional healers of our locality to treat cases of women infertility (Lienou *et al.*, 2010). Although, the medicinal properties of this plant have been extensively studied, its antidiabetic property is largely unknown.

Therefore, this study was undertaken to evaluate the antihyperglycemic effectiveness of four different solvent extracts of *Cleome rutidosperma* leaves and *Senecio biafrae* roots in STZ-induced diabetic mice as well as the *in vitro* antioxidant activities and phytochemical constituents of the extracts.

MATERIALS AND METHODS

Chemicals: Streptozotocin and Glibenclamide were purchased from Sigma chemicals (St Louis U.S.A). All other chemicals and reagents used were of analytical grade.

Plant materials: The plants (*Cleome rutidosperma* and *Senecio biafrae*) were collected from Abraka in Delta State, Nigeria and Akoko in Ondo State, Nigeria, respectively. They were identified by Dr. H.A. Akinnobosun of the Herbarium Unit, Department of Plant Science, University of Benin, Benin City, Edo State-Nigeria, where voucher specimens were deposited with numbers: UBHc0148 and UBHs0149.

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

Treatment and extraction of plant samples: The plant samples were washed with distilled water and air-dried at room temperature, cut into small pieces and pulverized into fine powder using pestle and mortar. They were sequentially extracted with solvents of increasing polarity (petroleum ether, acetone, ethanol and water), using the method of Arokiyaraj *et al.* (2009), as published by Jeyaseelan *et al.* (2011). Fifty grams powder of the plant was initially soaked in 200 mL of petroleum ether in airtight conical flask with daily shaking for three days at room temperature and were first filtered through doubled layered muslin cloth and then filtered through Whatman No 1 filter paper and the filtrates were collected into airtight bottles. The

petroleum ether was removed from the filtrate under reduced pressure using rotary evaporator at low temperature. The residue was dried and used for the acetone extraction followed by ethanol and water using procedure similar to the one carried out for the petroleum ether extraction. The crude extracts were stored in refrigerator at 4°C until used.

Induction of experimental diabetes: Streptozotocin was dissolved in 10 mM citrate buffer pH 4.5 (Archana *et al.*, 2001). The mice (diabetic control and test mice) were injected intra-peritoneally with portions (1 µL/g) of this solution at a dose of 60 mg per kg body weight after an overnight fast. 3 days after induction of diabetes, the mice with plasma glucose more than 200 mg/dL were considered as diabetic mice and were allowed for 2 weeks diabetic stabilization before being used for the experiment.

Quantitative phytochemical analysis: The different solvent extracts were subjected to quantitative phytochemical tests to determine the secondary metabolites present therein using standard procedures. Alkaloid and Flavonoids determinations were done using Harborne (1973) method; Tannin determination by Van-Burden and Robinson (1981) method; determination of Saponin by Obdoni and Ochuko (2001) method; evaluation of Glycosides was by the method described by El-Olemy *et al.* (1994). Phenolic Compounds by Hagerman *et al.* (2000) method and estimation of Steroids was done by the method of Evans (1996).

In vitro antioxidant properties of extracts: The *In vitro* antioxidant activities of plant extracts were done by different standard assay methods. The Ferric Thiocyanate (FTC) assay was carried out as described in the method of Osawa and Namiki (1981); Thiobarbituric Acid (TBA) assay by the method of Ottolenghi (1959); Reducing power was done according to the method described Oyaizu (1986), while the DPPH radical scavenging assay was by the method of Liyana-Pathiranan and Shahidi (2005).

Acute toxicity studies (LD₅₀): Acute toxicity studies of the plants extracts were conducted to ascertain the safe doses of the plant extracts to be administered to animals

in this study. Oral LD₅₀ determination was done by the method of Lorke (1983) using 13 rats. The median Lethal Dose (LD₅₀) was then calculated.

Evaluation of antihyperglycemic activity of the extracts: The assay of the antihyperglycemic activity of the four extracts of each plant involved administration of single dose of 500 mg/kg body weight of the extracts using mice weighing 25 to 35 g. Forty mice were randomly allocated to negative/positive control and treatment groups of five mice each as follows:

- Group I** : Normal untreated mice
- Group II** : Diabetic control mice
- Group III** : Diabetic+standard drug
- Group IV-VII** : Diabetic mice given 500 mg/kg body weight of the different extracts of *Cleome ruidosperma*

The groupings above were repeated for the extracts of the second plant (*Senecio biafrae*).

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 extracts were administered orally using gastric cannula. Blood glucose values were then estimated hourly for 6 h. The control group (II), received distilled water in place of the extract. Similarly, a standard antidiabetic drug (Glibenclamide 600 µg/kg) was administered orally to the group III animals and glucose determined at the same time intervals for the same duration according to the procedure of Aderibigbe *et al.* (2001).

Statistical analysis: Data are reported as mean±SD and were analyzed statistically using One way ANOVA followed by Tukey kramer multiple comparison test and values of p<0.05 were considered significant.

RESULTS

Phytoconstituents of *Cleome ruidosperma* and *Senecio biafrae*: The quantitative phytochemicals in different extracts of the plants are shown in Table 1.

Table 1: Concentration of phytochemicals in different extracts of *Cleome ruidosperma* and *Senecio biafrae*

| Plant/Extract | Alkaloids (%) | Saponins (%) | Tannins (%) | Glycosides (%) | Phenol (%) | Favonoids (%) | Steroids (%) |
|---------------|-------------------------|-------------------------|-------------------------|---------------------------|-------------------------|---------------------------|--------------------------|
| AQCR | 0.29±0.01 ^a | 3.11±0.89 ^a | 12.03±0.81 ^a | 0.033±0.009 ^a | 0.18±0.01 ^a | 0.433±0.170 ^a | 0.004±0.002 ^a |
| ECR | 0.18±0.02 ^a | 4.12±0.81 ^a | 10.44±0.95 ^a | 0.004±0.004 ^a | 0.21±0.02 ^a | 0.310±0.078 ^a | 0.011±0.003 ^a |
| ACR | 0.27±0.01 ^a | 6.01±0.88 ^b | 14.15±0.73 ^b | 0.011±0.004 ^a | 0.10±0.02 ^b | 0.263±0.070 ^{ab} | 0.003±0.002 ^a |
| PCR | 0.11±0.02 ^b | 2.31±0.50 ^a | 5.48±0.89 ^c | 0.002±0.001 ^a | 0.14±0.02 ^{ab} | 0.183±0.058 ^{ab} | 0.004±0.002 ^a |
| AQSB | 0.26±0.02 ^a | 0.81±0.13 ^a | 0.05±0.02 ^d | 0.113±0.040 ^b | 0.04±0.01 ^c | 0.035±0.006 ^b | 0.011±0.002 ^a |
| ESB | 0.31±0.04 ^{ac} | 0.22±0.03 ^{ac} | 0.11±0.02 ^d | 0.053±0.017 ^{ab} | 0.04±0.02 ^c | 0.011±0.003 ^b | 0.021±0.005 ^b |
| ASB | 0.34±0.05 ^{ac} | 0.33±0.09 ^{ac} | 0.04±0.01 ^d | 0.073±0.026 ^{ab} | 0.02±0.01 ^c | 0.021±0.008 ^b | 0.003±0.001 ^a |
| PSB | 0.28±0.04 ^a | 0.18±0.02 ^{ac} | 0.04±0.02 ^d | 0.030±0.008 ^{ab} | 0.03±0.01 ^c | 0.012±0.003 ^b | 0.004±0.001 ^a |

Values are mean of three determinations±SD; Values with different superscript down the column differ significantly (p<0.05); Where: AQCR= Aqueous Extract of *Cleome ruidosperma*; ECR= Ethanol extract of *Cleome ruidosperma*; ACR= acetone extract of *Cleome ruidosperma*; PCR= Petroleum ether extract of *Cleome ruidosperma*; AQSB = Aqueous extract of *Senecio biafrae*; ESB = Ethanol extract of *Senecio biafrae*; ASB = Acetone extract of *Senecio biafrae*; PSB = Petroleum ether extract of *Senecio biafrae*

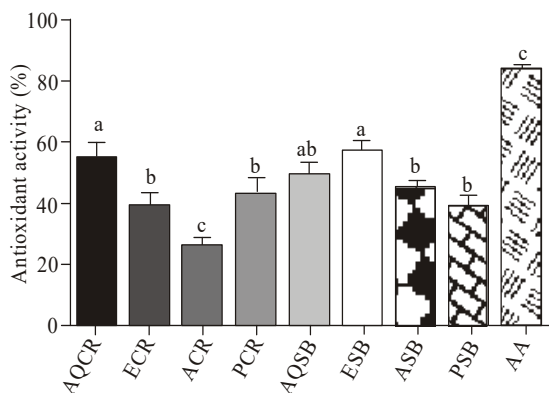


Fig. 1: *In vitro* antioxidant activity of plant extracts using the FTC method; Bars with different letters differ significantly ($p < 0.05$) Where: AQCR = Aqueous Extract of *Cleome rutidosperma*; ECR = Ethanol extract of *Cleome rutidosperma*; ACR = Acetone extract of *Cleome rutidosperma*; PCR = Petroleum ether extract of *Cleome rutidosperma*; AQSB = Aqueous extract of *Senecio Biafrae*; ESB = Ethanol extract of *Senecio Biafrae*; ASB = Acetone extract of *Senecio Biafrae*; PSB = Petroleum ether extract of *Senecio biafrae*; AA = Ascorbic acid

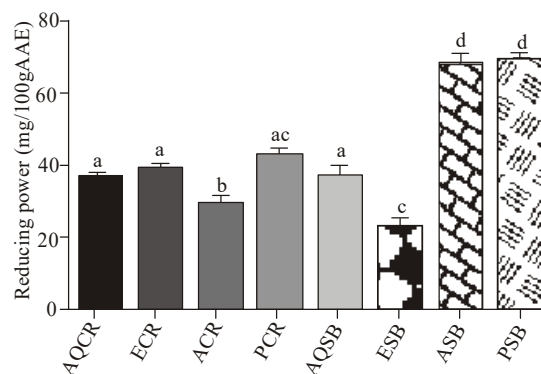


Fig. 3: *In vitro* reducing power of plant extracts; Bars with different letters differ significantly ($p < 0.05$); Where: AQCR = Aqueous Extract of *Cleome rutidosperma*; ECR = Ethanol extract of *Cleome rutidosperma*; ACR = Acetone extract of *Cleome rutidosperma*; PCR = Petroleum ether extract of *Cleome rutidosperma*; AQSB = Aqueous extract of *Senecio Biafrae*; ESB = Ethanol extract of *Senecio Biafrae*; ASB = Acetone extract of *Senecio biafrae*; PSB = Petroleum ether extract of *Senecio biafrae*; AAE = Ascorbic Acid Equivalent

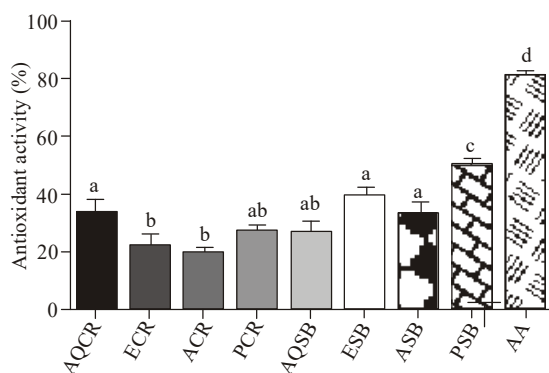


Fig. 2: *In vitro* antioxidant activity of plants extracts using the TBA method; Bars with different letters differ significantly ($p < 0.05$) Where: AQCR = Aqueous Extract of *Cleome rutidosperma*; ECR = Ethanol extract of *Cleome rutidosperma*; ACR = Acetone extract of *Cleome rutidosperma*; PCR = Petroleum ether extract of *Cleome rutidosperma*; AQSB = Aqueous extract of *Senecio biafrae*; ESB = Ethanol extract of *Senecio Biafrae*; ASB = Acetone extract of *Senecio Biafrae*; PSB = Petroleum ether extract of *Senecio Biafrae*; AA = Ascorbic Acid

From the Table, the concentrations of alkaloids for the different extracts were in the order:

ASB>ESB>AQCR>PSB>ACR>AQSB>ECR>PCR

The highest concentration of saponins was found in ACR (6.01±0.88%), followed by ECR (4.12±0.81%), AQCR (3.11±0.89%) and the least content was seen in PSB (0.18±0.02). Similarly, 0.04±0.01% and 14.15±0.73% were the lowest and highest

concentrations of tannins found in ASB and ACR, respectively. The highest concentration of glycosides was found in AQSB (0.113±0.040%), followed by ASB (0.073±0.026%) and the lowest was in PCR (0.002±0.001%), while ECR also had the highest concentration of phenol (0.21±0.02%) and lowest was seen in ASB (0.02±0.01%). Similarly, 0.433±0.170%, 0.021±0.005% and 0.020±0.003% were the highest concentrations of flavonoids and steroids found in AQCR, ESB and AQCR, respectively.

Antioxidants activity in different extracts: Results for the *in vitro* antioxidant activity of the extracts by the FTC method are shown in Fig. 1. There was significantly ($p > 0.05$) higher activity observed in AQCR and ESB compared to ECR, ACR, PCR, ASB and PSB, while ACR significantly ($p < 0.05$) displayed lower activity compared to all other extracts. The antioxidant activity of AA was significantly ($p < 0.05$) higher than all extracts.

Similarly, results for the antioxidant activity by the TBA method shown in Fig. 2 reveals significantly ($p < 0.05$) higher activity by PSB when compared to all other extracts. Also, ACR was significantly ($p < 0.05$) lower in activity compared to all other extracts. Significantly higher activity ($p < 0.05$) was observed in AA compared to all extracts.

Reducing power measurement: The reducing power of different extracts of the two plants is shown in Fig. 3. The petroleum ether extract of *Senecio biafrae* (PSB) had the highest value among the extracts of the plants examined, followed by ASB in the order:

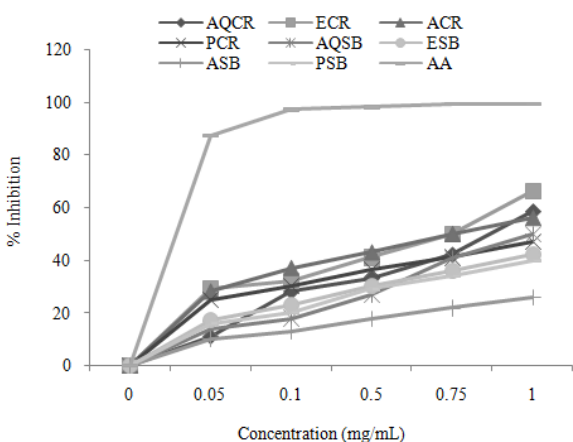


Fig. 4: *In vitro* DPPH Radical scavenging activities of plants extracts; Where: AQCR= Aqueous Extract of *Cleome rutidosperma*; ECR = Ethanol extract of *Cleome rutidosperma*; ACR = Acetone extract of *Cleome rutidosperma*; PCR = Petroleum ether extract of *Cleome rutidosperma*; AQSB = Aqueous extract of *Senecio Biafrae*; ESB = Ethanol extract of *Senecio Biafrae*; ASB = Acetone extract of *Senecio Biafrae*; PSB = Petroleum ether extract of *Senecio Biafrae*; AA = Ascorbic Acid

PSB>ASB>PCR>ECR>AQSB>AQCR>ACR>ESB

DPPH radical scavenging activity: To evaluate the antioxidant activity of the different extracts of the plants, the radical scavenging capacity based on DPPH assay was determined and the results are shown in Fig. 4. The percentage of scavenging effect on the DPPH radical was increased with the increase in the concentrations of the extract from 0.05 -1 mg/mL. The percentage of inhibition of the DPPH radical was varied from 11.61±1.08 % to 58.58±1.58% (in 0.05mg/mL to 1 mg/mL of the aqueous extract of *Cleome*

rutidosperma -AQCR), 29.23±1.33% to 66.12±1.41% for ECR, 28.75±1.32% to 56.78±1.19% for ACR and 25.4±1.67% to 47.83±1.30% for PCR, while percentage inhibition for *Senecio biafrae* extracts were: 14.05±0.78% to 50.88±1.08% for AQSB, 17.72±1.29% to 42.15±1.30% for ESB, 10.43±1.71% to 26.23±0.94% for ASB and 16.09±0.74% to 39.47±0.68% for PSB. The extracts at all concentrations showed lower percentage of inhibition of free radicals than the standard drug, Ascorbic acid (87.21±0.82%-99.46±2.54% in the concentration of 0.05-1 mg/mL).

Acute effect of oral administration of 500 mg/kg body weight of different extracts of *Cleome rutidosperma* or *Senecio biafrae* on Fasting Blood Glucose (FBG) in streptozotocin-induced diabetic mice: To investigate the antidiabetic potential of the plant extracts-*Cleome rutidosperma* (leaves) and *Senecio biafrae* (roots), different solvent extracts were administered to STZ-induced diabetic mice and their blood glucose lowering effects was monitored for (6) h.

Acute toxicity was tested up to a high concentration of 5 g/kg. Extracts tested did not cause any mortality up to a dose of 5 g/kg body weight. Even at this high dose there were no gross behavioral changes and the rats did not exhibit any sign of toxicity.

Changes in the levels of blood glucose in normal, diabetic control and experimental groups after oral administration of 500 mg/kg body weight of different extracts of *Cleome rutidosperma* are shown in Fig. 5. Diabetic control mice showed a significant increase ($p<0.05$) in blood glucose at 60 min after treatment. In animals treated with petroleum ether, acetone, ethanol and water extracts, the blood glucose level were significantly ($p<0.05$) decreased from the 60 min up 360 min post treatment when compared with the FBG

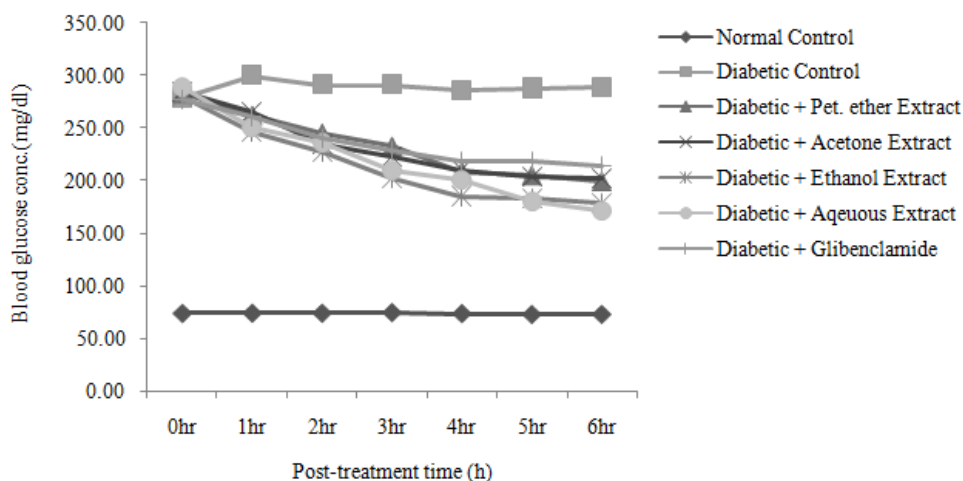


Fig. 5: Acute antihyperglycemic activity of different extracts of *Cleome rutidosperma* and glibenclamide on blood glucose level of STZ-induced diabetic mice

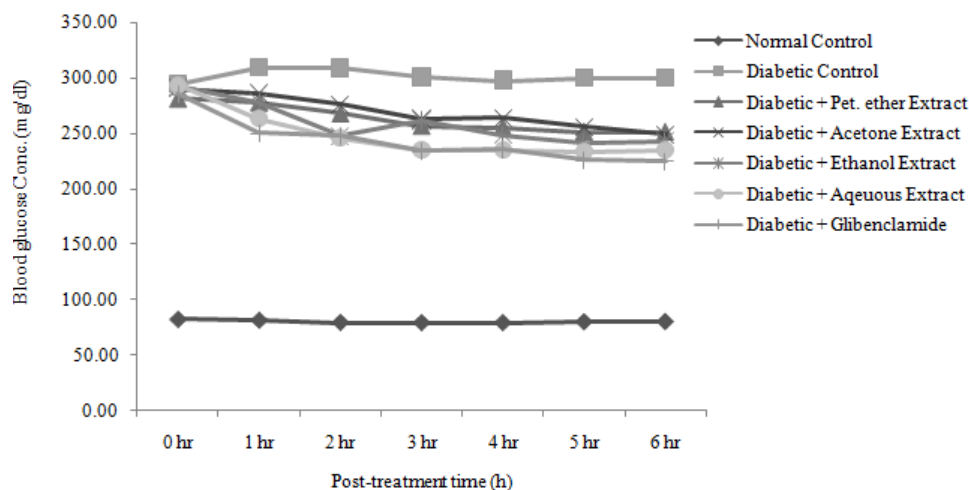


Fig. 6: Acute ant hyperglycemic activity of different extracts of *Senecio biafrae* and glibenclamide on blood glucose level of STZ-induced diabetic mice

Table 2: Percentage decrease in FBG 6 hours after treatment with 500 mg/kg of different extracts and 600 µg/kg of Glibenclamide

| Group | % Decrease in FBG at 6 h post-treatment | |
|--------------------|-----------------------------------------|--------------------------|
| | <i>Cleome rutidosperma</i> | <i>Senecio biafrae</i> |
| Normal control | 1.27±3.09 ^a | 2.406±1.68 ^a |
| Diabetic control | -3.736±1.82 ^b | -2.024±2.62 ^b |
| Pet. Ether Extract | 29.706±16.27 ^c | 10.652±6.36 ^c |
| Acetone extract | 28.784±15.46 ^c | 14.07±6.94 ^c |
| Ethanol extract | 35.928±15.33 ^d | 16.568±7.22 ^c |
| Aqueous extract | 40.866±15.61 ^d | 19.858±7.65 ^d |
| Glibenclamide | 22.646±14.53 ^e | 21.34±7.95 ^e |

Values are mean of five determinations±SD; *: Values with different superscripts down the column differ significantly (p<0.05); Negative value means an increase in FBG

at 0 h. The maximum blood glucose lowering effect on diabetic mice, as compared to the diabetic control group of animals was observed with water extract at the 6 h. The percentage blood glucose reduction (Table 2) of the different extracts at the 6 h were 29.73, 28.80, 35.95 and 40.88% for petroleum ether, acetone, ethanol and water, respectively, while standard drug, glibenclamide reduced the blood glucose level by 22.68%. The extracts had greater blood glucose lowering effect than the standard drug, glibenclamide.

Shown in Fig. 6 are the changes in levels of blood glucose in normal, diabetic control and experimental groups after oral administration of 500 mg/kg body weight of different extracts of *Senecio biafrae* and 600 µg/kg body weight of glibenclamide. There was significant (p<0.05) increase in diabetic control group (p<0.05) at the 60 min and 120 min, while significant decreases (p<0.05) were observed in all treated groups when compared with the FBG of each group at 0 h. The percentage blood glucose reduction (Table 2) of the different extracts and glibenclamide at the 6 h were 10.68, 14.14, 16.64 19.90 and 21.39% for petroleum ether, acetone, ethanol, water and glibenclamide, respectively.

DISCUSSION

The use of herbal medicine is a common practice in many countries, particularly in Asia (Chacko, 2003) and Africa (Shapiro and Gong, 2002). The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need to find safer and more effective antidiabetic drugs (Grover *et al.*, 2002; Rajagopal and Sasikala, 2008).

The quantitative estimation of the percentage crude yields of chemical constituents of the plant extracts showed that the leaves of *Cleome rutidosperma* and roots of *Senecio biafrae* were rich in alkaloids, saponins, tannins, glycosides, phenols, flavonoids and steroids. Mondal *et al.* (2009a) had reported from preliminary phytochemical screening, the presence of terpenoids, flavonoids, tannins and saponins in ethanolic extract of the root of *Cleome rutidosperma*. Also, Edeoga *et al.* (2005) reported the quantitative phytoconstituents(alkaloids, phenols, tannin, flavonoid and saponin) for aqueous leaves extract of *Cleome rutidosperma*. Similarly, the quantitative phytochemical concentration reported for the ethanolic root extracts for *Senecio biafrae* by Gbadamosi *et al.* (2012) were alkaloids, tannins, β Carotene, saponins, flavonoids, steroids, cardenolides, anthraquinones and glycosides. Ajiboye *et al.* (2013) also found alkaloids, saponin, tannin, phenol, flavonoids and glycosides from quantitative estimation of aqueous leaves extracts of *Senecio biafrae*. However, there has not been comparative study on the different extracts evaluated in this report for the two plants. There were slight differences in this study from previous reports in proportion of phytochemicals of the extracts. This may be due to environmental conditions which are mostly based on the nature of the soil and nutrient level of the plant and environmental pollutants (Ubani *et al.*, 2012).

Phytochemical compounds in plants have been reported to be responsible for antidiabetic activities of medicinal plants (Bnouham *et al.*, 2006; Kumar *et al.*, 2011). Flavonoid and tannins isolated from antidiabetic medicinal plants have been found to stimulate secretion of or possess an insulin like-effect (Marles and Farnsworth, 1995).

The present study indicated high *in vitro* antioxidant activities of the plant extracts, which may be responsible for the biological properties manifested by them. Although, the comparative study of antioxidant activity between ethanolic and aqueous extract of *Cleome rutidosperma* has been reported (Chakraborty and Roy, 2010) and aqueous leaves extracts of *Senecio bialfrae* by Adefegha and Oboh (2011), no comparative analysis has been done on the extracts reported in this study for the plants.

Various methods have been used to monitor and compare the antioxidant activity of plants. These methods differ in terms of their assay principles and experimental conditions. In different methods particular antioxidants have varying contributions to total antioxidant potential (Kataliniæ *et al.*, 2006). The antioxidant activity of plants has also been attributed to the presence of phenolic hydroxyl or methoxyl groups, flavones hydroxyl, keto groups, free carboxylic groups and other structure features (Patt and Hudson, 1990).

Increased oxidative stress associated with reduction in the antioxidant status has been postulated: Oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes and play a role in the long term complication of diabetes (Sabu and Kuttan, 2002; Boynes, 1991; Collier *et al.*, 1990).

The oral minimum lethal dose (LD₅₀) of the aqueous extracts of both *Cleome rutidosperma* and *Senecio bialfrae* leaves and roots respectively was estimated and were found to be >5000 mg/kg, which suggest that the extracts may have low toxicity. It has been established that any substance with LD₅₀ estimate greater than 2000 mg/kg body weight by oral route may be considered of low toxicity and safe in humans (Bruce, 1987). Mondal *et al.* (2009b) reported the LD₅₀ ethanolic extract of the roots of *Cleome rutidosperma* to be >3000 mg/kg body weight and Bose *et al.* (2012) found the the LD₅₀ of the aqueous extract of the aerial parts of *Cleome rutidosperma* to be >2000 mg/kg. b.w. So far, there has been no report on the LD₅₀ of *Senecio bialfrae*.

The study revealed that the leaves extract of *Cleome rutidosperma* and root extracts of *Senecio bialfrae* caused significant reduction in the blood glucose level for all extracts tested in STZ-induced diabetic mice, this observation is in line with the work of Mondal *et al.* (2009b) who reported the hypoglycaemic activity from the ethanolic extract of

Cleome rutidosperma roots. To the best of our knowledge, no report exist for antidiabetic activity of *Senecio bialfrae*.

Comparatively, the most significant reduction of blood glucose level was observed in the aqueous extract of *Cleome rutidosperma* leaf, which was followed closely by the ethanolic extract. Similar trend was also observed in the extracts of *Senecio bialfrae* root. Interestingly, the phytoconstituent and *in vitro* antioxidant evaluations reveals higher concentrations of flavonoids, alkaloids and glycosides for the aqueous extract of *Cleome rutidosperma*, while glycosides, saponins, phenols and flavonoids were highest for the aqueous extract of *Senecio bialfrae*. Also, high antioxidant activities were displayed by the aqueous extracts of both plants compared to other extracts.

These variation (s) in concentration and composition of phytochemicals in different extracts of the plants could be responsible for their differences in blood glucose lowering effect. The antihyperglycemic activities of plant extracts have been attributed to the presence of tannins, phenols, steroids, terpenoids and alkaloids (Manickam *et al.*, 1997; Akowuah *et al.*, 2002). The plausible mechanism of action of these plants are still unclear but may be elucidated in ongoing research on these plants.

CONCLUSION

In conclusion, the present results confirmed that the different extracts of *Cleome rutidosperma* (leaves) and *Senecio bialfrae* (roots) are rich in phytochemicals and highly valuable source of natural antioxidants and free radical scavengers. Also, the results confirm the antihyperglycemic effect of *Cleome rutidosperma* and *Senecio bialfrae* extracts when administered to STZ-induced diabetic animals which suggest the presence of biologically active components which may be worth further investigation and elucidation. Further studies are in fact currently under way to determine the chronic toxic effects of the crude extracts.

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