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VERIFICATION OF THE FOLKLORIC DIURETIC CLAIM OF *HIBISCUS SABDARIFFA* L. PETAL EXTRACT

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Abstract
The folklorically acclaimed diuretic activity of the petal extract of *Hibiscus sabdariffa* was verified in saline-loaded albino rats (80 – 220 g; n=5) according to the method of De la Peurta Vazquez et al, 1989. A methanolic extract of the dried petals was prepared, and, upon lethal toxicity testing, was found to be very safe – LD_{50} >5,000 mg/kg, i.p. A metabolic assay was conducted using graded doses (5 mg/kg – 160 mg/kg) The urine produced over a period of six hours was collected per animal and its volume, density, pH, and electrolyte concentrations (Na^+, K^+ and Cl^-) were determined using standard techniques. The effect of the most active dose level was compared to those of frusemide 3 mg/kg, mannitol 200 mg/kg, hydrochlorothiazide 10 mg/kg, and spironolactone 3 mg/kg. The extract was found to cause a dose-dependent increase in urine mobilization, which peaked at a dose of 40 mg/kg. At this dose level, .the extract showed a significant ($p<0.05$) aquaretic action characterized by a 300-fold increase in urine production, a slight fall in density and a fall in urine pH, relative to the control group. Although the extract did not increase the mobilization of the urinary electrolytes assayed, as did the standard diuretics used, the observed effects somewhat justify its ethnomedicinal use in the management of oedematous conditions.

Keywords: Folk medicine; *Hibiscus sabdariffa*; Petals; Diuretics; Aquaretic; Urinary electrolytes; Rats.

INTRODUCTION

Abnormalities in fluid volume and electrolyte composition are common and important clinical problems that can become life-threatening if untreated[1]. Drugs that block the transport functions of the renal tubules - especially diuretics - are important tools in the treatment of these disorders[2]. Diuretics are among the most frequently prescribed drugs for the management of cardiovascular disorders like hypertension and congestive heart failure (CHF)[3,4]. Because of the life-long nature of these conditions, and the high cost of orthodox drugs, many affected patients often resort to ethnomedicinal practices for the management of their conditions[5, 6.] Such ethnomedicinal practices are usually passed on from generation to generation through folklores and other forms of oral tradition[7]. Many herbs with folkloric medicinal claims have been investigated scientifically with the aim of validating or invalidating their widespread use[8-10]. While many such folkloric diuretic claims have found valid[11-13], some have been found to be without scientific basis[14,15]. It is against this background that it was considered necessary to verify the claimed diuretic effects of *Hibiscus*

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sabdariffa, which is popularly used medicinally in different parts of the world as a diuretic and an emmenagogue, for the management of hypertension, for the treatment cough and wound dressing[16,17].

Hibiscus sabdariffa commonly known as yakuwa in Northern Nigeria, is a fast growing exotic, annual plant which, although originally found in tropical Africa, has become pan-tropical due to widespread cultivation[18]. This geographical spread has yielded various varieties differing in form and size[19,20]. *H. sabdariffa* has attained popularity in various parts of the world because of a refreshing, vitamin C–rich, home-made drink (called soborodo (or zobo) in Nigeria) produced from a decoction of its calyces[21-23]. Apart from its ethnomedicinal uses, the plant is cultivated widely as a vegetable[24], has found nutritional application in poultry farming[25], and has been used industrially as a food colorant and a source of fibre for paper production[26,27]. Although many of its medicinal properties have been scientifically established[28-31], not much has been done on its diuretic effects. Kirdpon et al[32] noted changes in urinary chemical composition in 36 healthy volunteers after consumption of roselle (*H. sabdariffa*) juice, but did not report any effect on urine mobilization. The purpose of this work was to verify the diuretic ethnomedicinal claim, and to attempt to characterize that effect pharmacologically (where it exists) by comparison with common standard diuretics.

MATERIALS AND METHODS

Plant Material: Dried samples *H. sabdariffa* petals were purchased from Kaduna main market in December 2000, and were identified by Mr. A. Ozioko of the Bioresources Development & Conservation Programme (BDCP) Laboratory, Nsukka.

Extraction: 1kg of dried samples were pulverized with mortar and pestle and then subjected to continuous cold maceration with 95% methanol (BDH chemicals, England) for 72 hours, with fresh solvent replacement after each 24-hours period, to ensure complete extraction. The extracts were pooled together and lyophilized using a Labconco® Freezone 6 Freeze-Dry/ Shell-Dry System at the BDCP laboratory, Nsukka. The extract was reconstituted in normal saline (prepared in the laboratory of the department of Pharmacology and Toxicology of the University of Nigeria, Nsukka) before use.

Drugs: The following standard diuretics were used in the work, viz.; spironolactone (Aldactone®), hydrochlorothiazide (Esidrex®), frusemide (Lasix®) and mannitol (mannitol-D®). They were all purchased from local pharmacies within Nsukka, and administered dissolved in appropriate volumes of normal saline.

Animals: A total of 12 albino mice (18g-35g) and 70 rats (80g –220g) were used for the work. The mice were obtained from the animal house of the department of Pharmacology and Toxicology, UNN, and were of uniformly mixed sexes. The rats were obtained from the department of Veterinary Reproduction, U.N.N. and were made up of 50 females and 20 males. All the animals were housed in the animal house of the department of Pharmacology and Toxicology before use, and had free access to food and water. They were, however, fasted for 18 hours before use.

Acute Toxicity Test (LD_{50}): An acute toxicity test based on the method of Lorke[33] was carried out on the extract using 12 mice and the intra-peritoneal route.

Diuretic Assay Experiment: The diuretic assay experiment was carried out according to the method of de la Peurta Vazquez et al[35]. The experiment was carried out in two parts. The first part was aimed at determining the diuretic effects of graded doses of the extract, while the second was aimed at comparing the action of the dose found most effective in part 1 with those of the standard diuretics. In the

first part, 6 doses ranging in geometrical progression from 5 –160 mg/kg of the extract were administered to 6 groups of rats (n= 5), and the urine mobilization over a period of 6 hours was noted. The doses were administered intra-peritoneally in dose-volumes not exceeding 0.5ml using 10 mg|ml or 100 mg|ml concentrations of the extract. As much volume of normal saline as was needed to make up to a total volume of 1ml in each case was used to rinse the syringe after each administration, and thereafter administered to the animal. To facilitate urine collection, the individual animals were placed in metabolic cages in the animal house of the department of Home Science & Nutrition, U.N.N. throughout the duration of the experiment. At the end, the total urine produced by each animal over the period was pooled together and measured using suitable syringes, and mean values for each group were determined. The effects of mannitol (200 mg/kg) and normal saline (1ml) were also determined in the same way and served as controls for comparison.

In the second part of the experiment, 40 mg/kg of the extract was compared with 3 mg/kg spironolactone, 10 mg/kg hydrochlorothiazide, 200 mg/kg mannitol and 3 mg/kg frusemide. The doses were administered as earlier described for the extract, and normal saline (1 ml) was used as the control. 6 groups (n =5) of rats were therefore used for this part of the experiment, which also lasted for 6 hours. At the end, the total amount of urine obtained for each animal in the period was collected and used for the determination of urinary pH, density, and electrolytes (Na^+, K^+, Cl^-), and mean values for each index were calculated for each group. The urinary pH was determined using a Consort® pH Electrode Meter (K120) obtained from the Service Training Centre (STC), U.N.N.; the urinary electrolytes were determined using a Gallen Kamp® Flame Analyzer obtained from the laboratory of the department of Soil Science, U.N.N.; while the urinary density was calculated using the formula: Density= Weight/Volume.

Statistical Analyses: The difference between the each of the mean urinary indices of the extract and those of each of the standard diuretics were tested for statistical significance at $p< 0.05$ (student's t–test, two–tailed).

RESULTS

Extraction: 1 kg of dried pulverized calyces gave a yield of 345.68g of the extract equivalent to approximately 35 % of the dried plant material**.**

Acute Toxicity Test (LD_{50}): At the end of the two–stage experiment, no death was observed among the 12 animals used within 24 hours. The LD_{50} was, therefore, taken to be >5,000 mg|kg**.**

Verification of diuretic activity: The results of the first part of the diuretic assay experiments are shown in Figure 1. As can be observed, the extract at all dose levels used except 160 mg|kg elicited more urine mobilization than normal saline. This effect was, however, noticeably lower than that of mannitol 200 mg|kg at all dose-levels used except 40 mg|kg, at which dose the extract had its peak effect. This dose was, therefore, used for the second part of the experiments.

Comparison of Pharmacologic Actions of Extract (40 mg|kg) and Standard Diuretics:. The results of the second part of the diuretic assay experiments are shown in Table 1 and Figure 2. From Table 1, the extract at 40 mg|kg can be noted to have elicited more urine after 6 hours than all the other standard diuretics except frusemide. From Figure 2, it can be observed that the extract demonstrated a peak effect in the 4th hour, after which the effect gradually dropped. This effect was similar but not identical to that of mannitol, but differing mainly in the fact that the drop in effect after the 4th hour was not as gradual. From Table 2 it can be observed that the

extract caused a fall in urine density as well as urinary pH, and also caused reduced excretion of Na^+ and Cl^- , while slightly increasing that of K^+ . Upon comparison with the other diuretics, the extract can be observed to be definitely unlike spironolactone and frusemide in all parameters studied, and also observed to share various features with the others, albeit in different parameters and to varying degrees.

Statistical Analyses: The results of the statistical analysis are also indicated in Table 2, and are most pertinent as they concern the urinary electrolytes. Even though mannitol (200 mg|kg) did not affect electrolyte excretion to the same extent as did the extract, the differences between them were not found to be statistically significant. Also, the only statistically significant difference between the extract and hydrochlorothiazide was with respect to K^+ excretion.

TABLE 1: URINE PRODUCTION BY EXTRACT AND STANDARD DIURETICS

	VOLUME OF URINE PRODUCED (ml)					
TIME (HOUR)	NORMAL SALINE (1ml)	EXTRACT (40mg/kg)	MANNITOL (200mg/kg)	FUROSEMIDE (3mg/kg)	SPIRONO-LACTONE (3mg/kg)	HYDRO-CHLOROTHIAZIDE (10mg/kg)
1	0.01	0.15	-	2.85	-	0.50
2	0.10	-	0.26	0.75	0.20	-
3	-	0.40	-	0.10	-	-
4	-	0.58	1.21	0.47	-	0.65
5	0.36	0.40	0.01	0.20	-	-
6	-	0.30	0.25	0.45	0.10	-
TOTAL	0.47	1.83	1.73	4.82	0.30	1.15

TABLE 2: COMPARISON OF EXTRACT (40mg/kg) WITH STANDARD DIURETICS

SUBSTANCE ADMINISTERED (Dose)	MEAN VOLUME (ml)	MEAN DENSITY (kg/l)	MEAN PH	MEAN ELECTROLYTE CONCENTRATION (mg/l)		
				Na^+	K^+	Cl^-
NORMAL SALINE (1ml)	0.094±0.01	0.030±0.005	8.34±0.09	10.00±3.58	24.20±6.33	0.44±0.15
EXTRACT (40mg/kg)	0.31±0.03	0.021±0.000	7.20±0.38	9.40±1.33	27.60±2.42	0.16±0.05
FUROSEMIDE (3mg/Kg)	0.96±0.01	0.028±0.004	8.16±0.20	23.00±5.39*	35.00±6.20	0.72±0.23
SPIRONO-LACTONE (3mg/kg)	0.06±0.01*	0.038±0.002	8.34±0.07*	8.40±1.57	17.80±3.82*	1.32±1.17
MANNITOL (200mg/kg)	0.34±0.01	0.028±0.003	8.07±0.35	11.60±2.93	27.80±9.39	0.47±0.24
HYDRO-CHLORO THIAZIDE (10mg/kg)	0.23±0.04	0.035±0.001	7.70±0.45	4.00±0.89*	48.60±11.83*	0.25±0.03

***** - Significant difference relative to extract at $p < 0.05$

FIG 1: TOTAL URINE VOLUME FOLLOWING GRADED DOSES OF EXTRACT

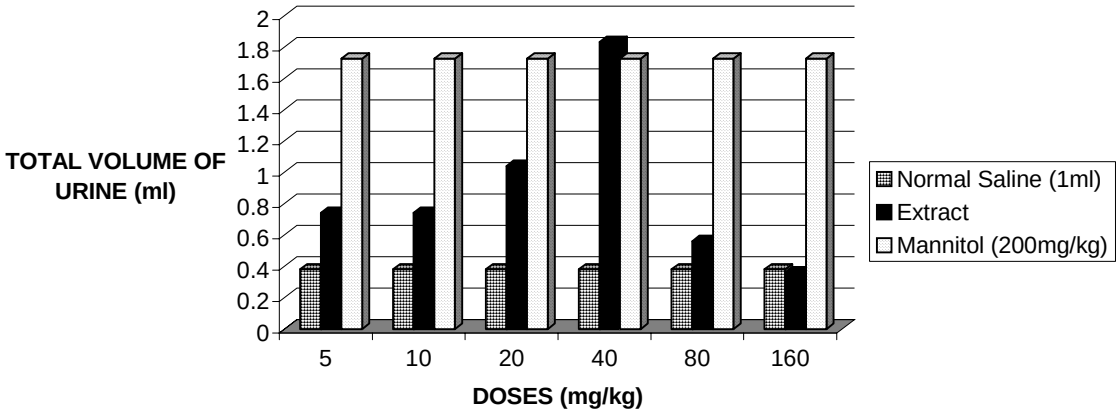
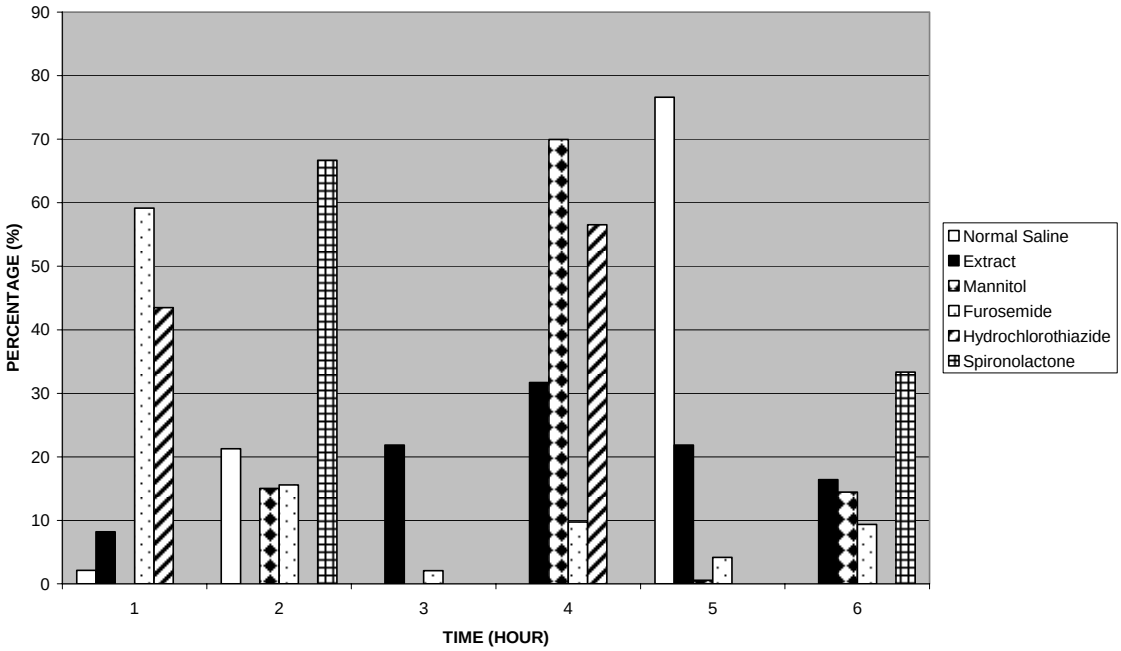


FIG 2: PATTERN OF URINE MOBILIZATION (HOURLY PERCENTAGE MOBILIZED) BY EXTRACT (40mg/kg) AND STANDARD DIURETICS



DISCUSSION

Based on Lorke's recommendations[33], the extract can be assumed to be safe. This result is in agreement with literature[35-36]. Although Jaiyesimi et al[36] noted significant toxic effects on vital organs, this observation was made at a dose of 1 g/kg, which was obviously very much higher than the dose found effective in this study. If an extrapolation of the results of the present study was to be made, it can be predicted that the extract would be definitely antidiuretic at much higher doses. The extract

was also discovered to cause a dose-dependent increase in urinary excretion at doses ≤ 80 mg\kg, with the best action at a dose of 40 mg\kg. It was, however, found to be much less effective than mannitol at all doses used except 40 mg\kg, at which dose it was also not significantly more effective. Since mannitol is not a high efficacy diuretic, these observations point to a mild effect. Unlike manintol or any of the diuretics used, however, the extract did not significantly increase or decrease the excretion of any of the electrolytes studied. These results seem to suggest an aquaretic action, since there was excretion of water in excess of electrolytes[37]. Although further studies are required to confirm this suggestion, these observations understandably account for the extract's documented folkloric claim.

It is noteworthy that the extract, albeit non-significantly, decreases urinary Na^+ excretion, a result which is in accord with the findings of Kirdpon et al[32]. This would not seem to encourage use of the extract for management of hypertension. However, since this study was not carried out on hypertensive animals, and in the light of the positive effect reported by Faraji and Tarkhani[17] in a clinical trial on hypertensive patients, it would be wise to draw no negative conclusions from the result of this study. The present results may, however, indicate the possible use of the extract in the management of dilutional hyponatraemia[35]. The tendency of the extract to reduce the urinary excretion of Cl^- as well as the urinary pH suggests the possibility of development of metabolic acidosis with continued use, which would antagonize the effects of certain diuretics like carbonic anhydrase inhibitors[38], and possibly complicate the management of diabetes[39,40]. The use of the extract in such clinical conditions should, therefore, be monitored or completely avoided.

CONCLUSION

Nearly all the physical characteristics that favour densification and therefore easier rate of packing in capsule filling and tablet compression such as lower Carr's index, low angle of repose and larger mean particle size were found to be more inherent in pregelatinized maize starch then in maize starch. The move porous PGS and amorphous form favours shorten disintegration time which usually preceeds access to faster dissolution of active ingredients and therefore makes the medication more bioavailable the simple nature of PGS production favours its industrial production even locally.

REFERENCES

1. Adrogue, H. J. and Madius, N. E. (1998). Mnagement of life-threatening acid –base disorder N. Engl. J. Med, 338; 26.

2. Anderson, K. E. (1993). Pharmocology of lower urinary tract smooth muscles and penile erectile tissue, *Pharmacol. Rev*. 45=253

3. Smith, T. W. and Braunawald, E. (987). The management of heart failure. In: Braunwald, E.(Ed): Heart Disease 3rd ed Saunders.p.485

4. Benowitz, N. L. (2001). Antihypertensive Agents. In: Katzung, B.G. (Ed): Basic aid Clinical Pharmacology. 8th ed. New York: McGraff & Hill, pp.155

5. Ballero, M., Poli, E. and Santus, M. (1998). Plants and folk medicine of Monteleone (Northern Sardinia). *Fitoterapia* 69 (1): 52

6. Ziyyat, A., Legssyer, A., Mekhfi, H., Dissouli, A., Serhrouchni, M, and Monteleone, W. (1997). Phytotherapy of hypertension and diabetes in oriental Morocco. *J. Ethnopharmacol*. 58:45.

7. Raja, D., Blanche, C. and Xirau, J. V. (1997). Contribution to the knowledge of the pharmaceutical ethnobotany of La Sagarra region (Catalonia, Iberian penisula). *J. Ethnopharmacol*. 57:149

8. Alkofahi, A., Batshoun, R., Owais, W and Najib, N. (1997). Bidogical activity of some Jordanian medicinal plants extracts Part II. *Fitoterapia* 68 (2): 163

9. Duncan, A.C., Jazer, A.K. and Van Staden, J. (1999). Screening of Zulu Medicinal plants for

angiotensin converting enzyme(ACE) inhibitors *J. Ethnopharmacol.* 68: 63

10. Njmen, U., Joshi, P., Madsen, L. B., Pedson, T. B., Pinstrup, M., RajaseKharan, S. et al (1998). Ethnomedical information and in vitro screening for angiotensin-converting enzyme inhibition of plants utilized as traditional medicines in Gujarat, Rajasthan and Kerala (India). *J. Ethnopharmacol.* 60:247

11. Calderon, C.P., Garcia-Aseff, S. B. and Fuentes, L. B. (1997). Evaluation of diuretic activity of *Alternanthera pungens* extract in Rats. *Phytother. Res*. 11(8): 606

12. Ahmed, M., Sadhu, S. K., Datta, B. K., Keenu, J. K., Bachar, S.C.. (1997). Preliminary studies on the anti inflammatory, analgesic and diuretic activities of stagninol, a sesquiterpene isolated from Persicaria stagnina. *Pharmazie* 52 (6): 472

13. Adeboye, J. O., Asije, W. and Awe, S. O. (1997). Diuretic and antidiuretic activity of the leaf extracts of *Vernonia cinerea* (Less) (Fam: Compositae). *Phytother. Res*. 11(6): 454

14. Howell, R. V. and Montero, G. U. (1996). Diuretic effects of *Gecropia obtusifolia* (Moraceae) in albino rats. *Revista de Biolugie Tropical* 44 (1): 93

15. Kavimani, S., Ilango, R., Thangadurai, J. G., Jaykar, B., Majumdar, U.K. (1997) Diuretic activity of aqueous extract of *Orthosiphon thymiflorus* in rats. *Indian J. Pharm. Sci*. 59 (2): 96

16. Obiefuna, P. C. M., Owolabi, O. A., Adegunloye, B. J., Obiefuna, I.P., and Sofola, O. A. (1994). The petal extract of Hibiscus sabdariffa produces relaxation of isolated rat aorta. *Intl. J. Pharnacog.* 32 (1) :69

17. Faraji, H. M. and Tarkhani, A. H. (1999) The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. *J. Ethnopharmacol*. 65: 231.

18. Hutchinson, J. and Dalziel, J. M. (1958). Flora of West Tropical Africa 2nd ed. Vol. 1 part 2 London: Crown Agents. p 335

19. Shchori, Y. (1989) A new variety for the the development of a new ornamental crop, *Hibiscus sabdriffa. Acta Horiticult*. 252: 263.

20. Omer, E. A., Khattab, M. E. and Ibrahim, M. E. (1997) Effect of pinching and foliar application of some growth regulators on two new early mature varieties of *Hibiscus sabdariffa* L. *Egypt. J. Horticult*. 4 (2): 117.

21. Haruna, A. K. (1997). Carthartic activity of the aqueous extract of the calyx of *Hibiscus sabdariffa* L. *Phytother. Res*. 11 (4): 307.

22. Duh, P. D and Yen, G. C. (1997). Antioxidative activity of three herbal water extracts. *Food Chem.* 60 (4): 639.

23. Abu-Tarboush, H. M. Alumed, S. A. B. and Al-kalitane, H.A. (1997). Some nutritional and functional properties of Karkade *(Hibiscus sabdariffa)* seed products. *Cereal Chem*. 74 (3): 352.

24. Lucas, E. O. (1988). The potentials of leafy vegetables in Nigeria. *Outlook on Agricult* 17 (4):163

25. Soriano-Torres, T. and Tajada-de-Harmandes, I. (1995). Preliminary study of the nutritive value of *Hibiscus sabdariffa* for fattening chickens, *Tecnica-Pecuaria-en-Mexico* 33 (1): 48.

26. Bridle, F. and Timberlake, C. F. (1997). Anthocyanins as natural food colours: selrcted aspects. *Food Chem*. 58 (1-2): 103.

27. Saakia, C. N., Goswani, T and Ali, F. (1997). Evaluation of pulp and paper-making characteristics of certain fast-growing plants *Sci. Tech*. 31(6): 467

28. Ali, M. B., Salih, W. M., Mahamed, A. H. and Humeida, A. M. (1991). Invetigation of the antipasmodic potemtial of *Hibisus sabdariffa* calyces *J. Ehopharmacol* 31 (2): 249

29. Abu-Tarboush, H. M. (1996). Antibactoral effect of Roselle (Hibiscus sabdariffs. L.)calyces and its relation to pH. *Egypt J. food Sc*i 22 (2): 317.

30. Dafallah, A.A. and Al-Mustafa, Z (1996). Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa.* Am. J. Clin. Med. 24 (3-4): 263

31. Onyenekwe and P.C. Ajani E.O. (1999) Antihpersive effect of rosselle *(Hibiscus sabdariffa)* calyx infusion in spontaneously hypertensive rats, and a comparison of its toxicity with that in wister rats. *Cell Biochem. Funct.* Sept 17(3): 199

32.Kirdpon, S, Nakorn, S.N. and Kirdpon, W.(1994). Changes in Urinary chemical composition in healthy volunteers after consuming roselle (*Hibiscus sabdariffa.* L.). Juice *J. Med. Assoc. Thai.* 177 (6): 314.

33.Lorke, D. (1983). A new approach to practical acute toxicity testing. *Arch. Toxicol*. 54: 275.

34. De la Peurta Vazquez, R., Gimenez, G. and Saenz Radriguenz, M. T. (1990) Effects sur la diurèse de

differents espèces du genre Helichrysum. *Fitoterapia* 61 (4): 350.

35. Askari, A., Nirza, M and Solangi, M.S.P. (1996). Toxicological studies of herbal beverages and seed extract of *Hibiscus sabdariffa* L. (Roselle). *Paki. Sci. Ind. Res.* 391(1-4): 38

36. Jaiyesinmi, A.E.A., Adeyemi, A.A., Ogunleye, D.S., Ogundahusi. O. and Sanyaolu O. (2002) *Nig. J. Pharm. Res.* 1 (1): 41

37. Gines, P. and Jimenez, W. (1996). Aquaretic agents: a new potential treatment for dilutional hyponatraemia in cirrhosis. *J. Hepatol.* 24: 506

38. Brates, D. C. (1998). Diuretic therapy. *N. Engl. J. Med*. 339: 387

39. McFarland, K. F. (1997). Type II diabetes: stepped care approach to patient management *Geriatics* 52:22

40. Short, R. I. (1997). Incidence and risk factors for serious hypoglycaemia in older persons using insulin or sulfonylureas. *Arch. Intern. Med*. 157:1681