

Pelagia Research Library

Advances in Applied Science Research, 2016, 7(5): 1-6



# Phytochemical compositions of some extracts used in alternative medicine in Nigeria

Tony I. Ojiezeh<sup>3</sup>, Mumini I. Adarabioyo<sup>2</sup> and Peter T. Olagbemide<sup>1</sup>\*

<sup>1</sup>Department of Biological Sciences, Afe Babbalola University, Ado-Ekiti, Nigeria <sup>2</sup>Department of Mathematics and Statistics, Afe Babalola University, Ado-Ekiti, Nigeria <sup>3</sup>Department of Medical Laboratory Sciences, Afe Babalola University, Ado-Ekiti, Nigeria

# ABSTRACT

Natural products provide many fine chemical and biochemical extracts that have medicinal values. Phytochemicals are non-nutritive plant chemicals but they are bioactive compounds found in plants that work with nutrients and dietary fibre to protect against diseases. They inhibit the growth of cancer cells, improve immune function, block carcinogens, and help clear out toxins or other damaging substances, among other actions. The aim of the study was the comparison analysis of the phytochemical compositions of some extracts used in alternative medicine. The analyses were carried out on four extracts-the extracts from earthworm, Aloe vera, Ganoderma lucidium, and snail (Archachatina) using standard methods. The means and standard error of means (SEM) were determined using SPSS version 20.The results showed that phytochemicals were majorly present in Aloe vera and Ganoderma extracts and there significant differences (P < 0.05) in the values of most of the phytochemicals between Aloe vera extract, Ganoderma extract gives credence to their usefulness by traditional herbalists in ethno medicine.

Key words: Saponins, tannins, extracts, Aloe vera, Ganoderma.

# INTRODUCTION

Alternative medicine embraces the practice of medicine that does not usually involve conventional methods. Commonly cited examples include naturopathy, chiropractic, herbalism, traditional Chinese medicine, Ayurveda, meditation, yoga, biofeedback, hypnosis, homeopathy, acupuncture, and diet-based therapies, in addition to a range of other practices. The World Health Organization (WHO) estimates that as many as 80% of the world's more than six billion people rely primarily on animal and plant-based medicines [1]. People turns to alternative methods when modern medicine fails or the side effects of medications or medical procedures outweigh the risks of living with a disease or medical condition. The philosophy of alternative medicine usually emphasizes the promotion of wellness, healing and prevention through self-awareness of the mind and body, as well as exercise, nutrition, and other forms of self-care.

Traditional human populations have a broad natural pharmacopoeia consisting of wild plant and animal species. Ingredients sourced from wild plants and animals are not only used in traditional medicines, but are also increasingly valued as raw materials in the preparation of modern medicines and herbal preparations [2]. Natural products provide many fine chemical and biochemical extracts that have medicinal values. Extracts are products of extraction, which is the separation of medicinally active portions of plant and animal tissues using selective solvents through standard procedures. Wild and domestic animals and their by-products (*e.g.*, hooves, skins, bones, feathers, tusks) are used as important ingredients in the preparation of curative, protective and preventive medicine [3], [4]. [5] reported that nearly 15–20 percent of the Ayurvedic medicine in India is based on animal-derived substances while [6] reported that in the northeast of Brazil, over 180 medicinal animals have been recorded. The use of

medicinal plants as traditional medicines is well known in rural areas of many developing countries [7]. A recent review on national pharmacopoeias from several countries reveals at least 120 distinct chemical substances from different plants that have utility as lifesaving drugs [8]. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [9].

Phytochemicals are a large group of plant-derived compounds hypothesized to be responsible for much of the disease protection conferred from diets high in fruits, vegetables, beans, cereals, and plant-based beverages such as tea and wine [10]. Phytochemicals are non-nutritive plant chemicals but they are bioactive compounds found in plants that work with nutrients and dietary fibre to protect against diseases. They are non-essential nutrients, meaning that they are not required by the human body for sustaining life. Plants produce these chemicals to protect themselves but recent research demonstrates that they can also protect humans against diseases. One of the most important ways phytochemicals promote health is as antioxidants. While oxygen is needed by the body, it is also a very unstable molecule if it is damaged in any way. When oxygen is an unstable molecule, it is called a free radical. These free radicals act as oxidants that can damage cells and tissues. Phytochemicals help to repair damage or prevent the damage of these oxidants by acting as antioxidants. Phytochemicals may also inhibit the growth of cancer cells, improve immune function, block carcinogens, and help clear out toxins or other damaging substances, among other actions. Some medicinal plants used in Nigeria include Garcina kola, used in the treatment of asthma, Carica papaya, used as a remedy for hypertension, Ocimum basilicum, a cure for typhoid fever, and Cola nitida, for treatment of pile [11]. [12] reported that various parts of Vitex doniana, commonly called black plum are used by traditional medicine practitioners in the eastern and western parts of Nigeria in the management and treatment of several disorders which include rheumatism, hypertension, cancer, and inflammatory diseases.

The aim of this study is the comparison analysis of the phytochemical compositions of some extracts used in alternative medicine.

### MATERIALS AND METHODS

Samples of earthworm were procured from riverside of Okitipupa, Ondo State. They were washed with water and transported in a clean plastic bucket with moist sand to the laboratory for processing. The extraction of the samples was carried out according to the method described by [13]. Samples of succulent leaves of *Aloe vera* plant were procured from the neighbourhood, washed with distilled water and taken to the laboratory for processing. Extraction of *Aloe vera* juice was done according to the method described by [14]. Samples of *Ganoderma lucidium* were obtained from a farmland in Owo Local Government Area, Ondo State, Nigeria. Aqueous extraction was carried out on the samples according to the method of [15]. Samples of matured giant land snails were obtained from Oje market, Ibadan, Oyo State and were transported to the laboratory for processing. The samples were thoroughly cleansed with distilled water. The bluish supernaut got after the shell is carefully removed and the resultant fluid from the snails was centrifuged at 500 rpm for 15 minutes.

The phytochemical analyses for the presence of saponins, tannins, alkaloids, and cyanogenic glycosides, phytates and oxalates in the samples were carried out according to the methods described by [16] and Trease and [17].

All assays were carried out in triplicate, and the means and standard error of means (SEM) were determined using SPSS version 20. Analysis of variance was performed to determine significant differences between the paired samples. Differences in paired samples performance for the nutritional and chemical compositions were tested by the Student's t-test. <0.05 implies significance.

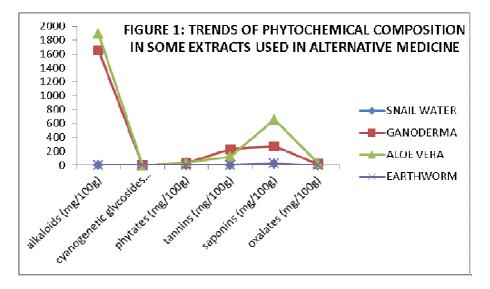
#### RESULTS

Table 1 shows the results of phytochemical analyses of the four extracts. Saponins and tannins were found in all the extracts. The range of tannins in the extracts was from 1.67 to 228.33 mg/100g with *Ganoderma* having the highest value and snail water and earthworm extract having the lowest value. Saponins in the extracts ranged from 16.67 to 658.33 mg/100g with *Aloe vera* extract having the highest value and snail water having the lowest value. The values of phytates ranged from 2.17 to 41.67 mg/100g with highest value found in *Aloe* vera and the lowest value found in snail water. Phytate was not detected in earthworm extract. Oxalates and alkaloids were found only in *Ganoderma* extract and *Aloe vera* extract with the higher value found in *Aloe vera* and lower in *Ganoderma* in both cases. Cynogenic glycosides was detected in extract of *Aloe vera* only with a value of 0.07 mg/100g. Figure 1 shows the trends in the phytochemical composition of the extracts.

PARAMETERS (mg/100g)	SNAIL WATER	GANODERMA	ALOE VERA	EARTHWORM
ALKALOIDS	ND	1655.00±10.41	1893.33±10.14	ND
PHYTATES	2.17±0.33	30.00±2.89	41.67±3.33	ND
TANNINS	1.67±0.33	228.33±1.67	125.00±2.89	1.67±0.17
SAPONINS	16.67±1.67	271.67±6.01	658.33±1.67	23.33±1.67
OXALATES	ND	15.00±0.00	28.33±3.33	ND
CYNOGENIC GLYCOSIDES	ND	ND	0.07±0.17	ND

Table 1. Phytochemical compositions of some of the extracts used in alternative medicine

Values are means ±SEM (Standard error of means) of triplicate sample; ND=Not Detected



#### Table 2. Paired samples test phytochemical compositions of some extracts used in alternative medicine

Parameters (mg/100g)	Paired samples	Diff. Mean	Sig. (2-Tailed)
Alkaloids	snail_water- Ganoderma	ND	ND
	snail_water- Aloe_vera	ND	ND
	snail_water- Earthworm	ND	0.06
	Ganoderma- Aloe_vera	-238.33±19.22	ND
	Ganoderma- Earthworm	ND	ND
	Aloe_vera- Earthworm	ND	ND
	snail_water- Ganoderma	-27.83±2.91	0.011
Phytates	snail_water- Aloe_vera	-39.50±3.51	0.008
	snail_water- Earthworm	ND	ND
	Ganoderma- Aloe_vera	$-11.67 \pm 6.01$	0.192
	Ganoderma- Earthworm	ND	ND
	Aloe_vera- Earthworm	ND	ND
	snail_water- Ganoderma	-226.67±1.86	0.000
Tannins	snail_water- Aloe_vera	-123.33±3.18	0.001
	snail_water- Earthworm	0.00±0.29	1.000
Tammis	Ganoderma- Aloe_vera	103.33±33	0.001
	Ganoderma- Earthworm	226.67±1.83	0.000
	Aloe_vera- Earthworm	123.33±2.89	0.001
Saponins	snail_water- Ganoderma	$-255.00\pm5.00$	0.000
	snail_water- Aloe_vera	-641.67±1.67	0.000
	snail_water- Earthworm	-6.67±1.67	0.057
	Ganoderma- Aloe_vera	-386.67±6.67	0.000
	Ganoderma- Earthworm	248.33±4.41	0.000
	Aloe_vera- Earthworm	635.00±2.89	0.000
	snail_water- Ganoderma	635.00±2.89 ND	ND
Oxalates	snail_water- Aloe_vera	ND	ND
	snail_water- Earthworm	ND	ND
	Ganoderma- Aloe_vera	13.33±3.33	0.057
	Ganoderma- Earthworm	ND	ND
	Aloe_vera- Earthworm	ND	ND
	snail_water- Ganoderma	ND	ND
Cynogenic Glycosides	snail_water- Aloe_vera	ND	ND
	snail_water- Earthworm	ND	ND
	Ganoderma- Aloe_vera	ND	ND
	Ganoderma- Earthworm	ND	ND
	Aloe_vera- Earthworm	ND	ND

Significant difference (P < 0.05); ND= Not Detected

Table 2 shows the paired samples test of the phytochemical compositions of the extracts. There was no significant difference in alkaloid composition between *Ganoderma* extract and *Aloe vera* extract. Alkaloids was not detected in the two others extracts. In phytate composition, there were significant differences between snail water and *Ganoderma* extract; snail water and *Aloe vera* extracts but there was no significant difference between *Ganoderma* extract and *Aloe vera* extract. Phytate was not detected in earthworm. Saponins and tannins showed similar trends. There were significant differences in their compositions between the extracts: snail water and *Ganoderma* extract; snail water and *Aloe vera* extract and *Aloe vera* extract and *Aloe vera* extract and *Earthworm* extract and *Aloe vera* extract. There was no significant differences in their compositions between snail water and *Ganoderma* extract; snail water and *Aloe vera* extract; *Ganoderma* extract; *Ganoderma* extract and *Earthworm* extract except between snail water and earthworm extract. There was no significant difference in oxalates composition between *Ganoderma* extract and *Aloe vera* extract. Oxalate was not detected in other two extracts. Cynogenic glycoside was found in *Aloe vera* extract and thus, there are no paired samples.

## DISCUSSION

The results in Tables 1 and 2 showed that the phytochemicals were majorly present in *Ganoderma* extract and *Aloe vera* extract which are plant extracts and were insignificantly present in snail water and earthworm extract which are animal extracts. This indicates that phytochemicals are produced mainly by plants. [18] and [19] reported that saponnins are mainly produced by plants, but also by lower marine animals and some bacteria. Other studies demonstrated that InsP6 (phytate) found in all animal cells [20], [21] and in biological fluids [22] has a dietary origin and is not a consequence of endogenous synthesis [23], [24]. However, the reasons for the occurrence of InsP6 in animal cells and fluids are not totally understood. The results also revealed the presence a variety of secondary metabolites (alkaloids, saponnins, oxalates, cynogenic glycosides, phytates and tannins) in *Aloe vera* and *Ganoderma* extracts and this is in agreement with the report of [25], [26], [27] and [28] who reported that plants generally have variety of secondary metabolites. Phytochemicals are the natural plant substances that play a key role in natural defense system in plants against numerous diseases and stress condition [29], [30], [31], [32] and are well recognized as having therapeutic potential to human suffering and disease [33], [34]. The medicinal value of plants depends upon chemical compound that produce a specific physiological action on human body [35], [36], [37], [38], [39].

Phytochemicals affect the availability of nutrients required by the body and interfere with metabolic process so that growth and development of the body is negatively influenced [40], [41], [42]. However, their medicinal values cannot be over-emphasized. Saponins have been shown to have a range of biological activities and potential health benefits such as hypocholesterolemic, anti-coagulant, anticarcinogenic, hepato-protective, hypoglycemic, immunomodulatory, neuroprotective, anti-inflammatory anti-oxidant activity, inhibition of dental caries, and platelet aggregation [43], [44]. Saponins were also reported to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic; permeabilization of the intestine) properties [45], [46]. Powdered oxalate is used as a pesticide in beekeeping to combat the bee mite [47]. Tannins are in effective in curbing hemorrhages as well as restrict bare swellings. In addition to haemostatic property of tannins, they are also beneficial when applied on mucosal coating in mouth. Hence, herbs possessing tannins are widely used as mouthwashes, eyewashes, snuff and even as vaginal douches and also treat rectal disorders [48]. Furthermore, the antimicrobial property of tannic acid can also be used in food processing to increase the shelf-life of certain foods, such as catfish fillets [49]. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immunoresponses. Phytate (InsP6) is an important antioxidant [50], [51], protects against cancer [52], [53], [54], [55], [56], [57], [58] and prevents pathological calcifications such as renal calculi [59], [60] and tissue calcifications [61]. Alkaloids have physiological effect that renders them valuable medicine against various diseases including malaria, diabetics, cancer, cardiac dysfunction etc. These are also used in local anesthesia and relief of pain. The use of alkaloid-containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization [62]. Alkaloids are well known for their central nervous system activities [63], [64] and their neurotoxic and analgesic activity [65]. They have antibacterial and antihelminthic activities [66], raise serum testosterone levels in men [67] and are possible solutions for erectile dysfunctions [68]. Antibiotic activities are common for alkaloids and some are even used as antiseptics in medicine, e.g. berberine in ophthalmics and sanguinarine in toothpastes [62]. Cynogenic glycosides were detected only in Aloe vera extract and this class of compound has been found useful in the treatment of asthma [69] [70]. Glycoside has been used for over two centuries as stimulant in cases of cardiac failure and diseases [71]. This perhaps justifies the already locally established function of the plant in the treatment and management of hypertension [72]. The low cyanide contents in Aloe vera extracts is another significant finding in this study. Cyanide as an effective cytochrome oxidase inhibitor in the electron transport chain interferes with aerobic respiration [73]. The lethal dose of cyanogenic glucoside for an adult man is 50-60 mg kg<sup>-1</sup> body weight as reported by [74]. Hence, the cyanogenic glucoside content recorded in this study is quite too low to cause any deleterious effects.

Though, *Aloe vera* extract and *Ganderma* extract are both plant extracts, there were significant differences in the values of the phytochemicals between them except in phytates and oxalates. A number of factors, such as physiological age, environmental and agronomic factors, have been shown to affect phytochemical content of plants [19] while [75], [76] reported that variation in agronomic conditions (plant species, cultivar, developmental stage, plant organ, plant competition, fertilization, pH), season, climatic factors, water availability, light (intensity, quality, duration) and  $CO_2$  are known to significantly affect content and profile of phytochemicals.

## CONCLUSION

There are varieties of phytochemicals present in plants that though may interfere with metabolic processes in animals, so that growth and development of the body is negatively influenced, but they also have great medicinal values. The present study of the phytochemical constituents in *Aloe vera* extract, *Ganoderma* extract gives credence to their usefulness by traditional herbalists in ethnomedicine.

#### REFERENCES

[1] EBAA, Encyclopedia Britannica advocacy for animals. *Traditional Chinese medicine and endangered animals*, **2007.** 

[2] Kang S, Phipps M, A question of attitude: South Korea's Traditional Medicine Practitioners and Wildlife Conservation TRAFFIC East Asia, Hong Kong, 2003.

- [3] Adeola MO, Environmental Conservation, 1992, 19(2): 125-134.
- [4] Anageletti LR, Agrimi U, Curia C, French D, Mariani-Costantini R, Lancet, 1992, 340:223-225.
- [5] Unnikrishnan PM, Amruth, 1998, 1(Supl):1-15.
- [6] Costa-Neto EM, Sust Dev., 2004, 12:161-174.
- [7] Sandhu DS, Heinrich M, Phytotherapy Res., 2005, 19:633-42.
- [8] Goswami A, Barooch PK, Sandhu, JS, J. Sci. Ind. Res., 2002, 61: 423-443
- [9] Ncube NS, Afolayan AJ, Okoh AI, African Journal of Biotechnology, 2008, 7 (12): 1797-1806.
- [10] Arts IC, Hollman PC, Am J Clin Nutr., 2005, 81(1 Suppl): 317S-325S.
- [11] FAO, Journal of Science, 1996, vol. 67: 75–78.

[12] Sofowora AE, The State of Medicinal Plants in Nigeria, 1993, University of Ibadan, Ibadan, Nigeria.

[13] Ang Lopez J, Realm A, Indigenous uses of the native *L. rubellus* extract and its fatty acid profile. Paper presented at International Symposium-workshop on vermin-technology for the developing countries (ISWVT) at Los Baños, Laguna, Phils. *Philippine Fisheries Association, Inc.*, **2005**, 135p.

[14] Wu JH, Xu C, Shan CY, Tan RX, Life Sci., 2006, 78: 622–630.

[15] Oluba MO, Onyeneke EC, Ojieh GC, Idonije, BO, Ojiezeh TI, Der pharmacia letter, 2010, 2(4): 432-439.

[16] Harborne JB, *Phytochemical methods: a guide to modern techniques of plant analysis*. Chapman and Hall Ltd. London, UK, **1973, pp** 279

[17] Trease GE, Evans WC, *Textbook of pharmacognosy*. 12th Ed. Balliese Tindall and Company, **1983**, pp: 343-383.

[18] Riguera R, Journal of Marine Biotechnology, 1997, 5: 187–193.

[19] Yoshiki Y, Kudou S, Okubo K, Bioscience Biotechnology and Biochemistry, 1988, 62: 2291–2299.

[20] Bunce CM, French PJ, Allen P, Mountford JC, Moor B, Greaves MF, Michell RH, Brown G, *Biochem J*, **1993**, 289: 667–673.

[21] Stephens LR, Hawkins PT, Stanley AF, Moore T, Poyner DR, Morris PJ, Hanley MR, Kay RR, Irvine RF, *Biochem J.*, **1991**, 275, 485–499.

- [22] Grases F, Simonet BM, March JG, Prieto RM, BJU International, 2000<sup>a</sup>, 85: 138-142
- [23] Grases F, Simonet BM,, Prieto RM, March JG, British Journal of Nutrition, 2001<sup>a</sup>, 86: 225–231.
- [24] Grases F, Simonet BM, Prieto RM, March JG, Journal of Nutritional Biochemistry, 2001<sup>b</sup>, 12: 595-601.

[25] Geyid A, Abebe D, Debella A, Makonnen Z, Aberra F, Teka F, Kebede T, Urga K, Yersaw K, Biza T, Mariam

BH, Guta M, J. Ethnopharmacol., 2005, 97: 421–427.

[26] Tedong L, Dimo T, Dzeufiet PDD, Asongalem AE, Sokeng DSP, Callard P, Flejou JF, Kamtchouing P, African Journal of Traditional medicine, **2006**, 3(1): 23-35

- [27] Agrawal B, Singh J, J. Indian Chem. Soc., 2010, 87: 1143-1144.
- [28] Pandey A, Shukla YN, *Indian Drug*, **2001**, 38: 51-51.
- [29] Sparg SG, Light ME, Stadan JV, J. Ethnoph. 2004, 94: 219-243.
- [30] Hashmi N, Muhammad F, Javed I, Khan JA, Khan MZ, Khaliq T, Aslam B, Pak. Vet. J., 2013, 33: 330-334.
- [31] Rahim G, Qureshi R, Arshad M, Gulfraz M, Int. J. Agric. Biol., 2013, 15: 377-381.
- [32] Tupe SB, Patil PD, Thoke RB, Aparadh VT, Int. Res. J. Pharm. App. Sci., 2013, 3: 49-51.
- [33] Okwu DE, Journal of sustainable Agriculture and Environment, 2004, 6: 30-34.
- [34] Ivanova D, Gerova D, Chervenkov T, Yankova T, J. Ethnopharmacol., 2005, 96: 145-150.

[35] Edeoga HO, Okwu DE, Mbaebie BO, Afr. J. Biotechnol., 2005, 4: 685-688.

- [36] Akinmoladun AC, Ibukun EO, Afor E, Obuotor EM, Farombi, EO, Sci. Res. Essay, 2007, 2: 163-166.
- [37] Canigueral S, Tschopp R, Ambrosetti L, Vignutelli A, Scaglione F, Petrini O, Pharm. Med., 2008, 22: 107-118.
- [38] Varadarajan P, Rathinaswamy G, Asirvatahm D, Ethnobotanical Leaflet, 2008, 12: 841–845.
- [39] Kaur R, Arora S, J. Med. Plants Res., 2009, 3: 196-216.
- [40] Singh M, Krikorian AD, Journal of Agricultural and Food Chemistry, 1982, 30(4):799-800.
- [47] Wikipedia, Pesticide. Wikipedia, the free encyclopedia. 2015.
- [41] Maynard LA, Animal nutrition. McGraw Hill book company Ltd. New York, 1997, P. 47.
- [42] Richard W, Katie E, Ferrell T, seeds. Pakistan J. Nut. 2006, 6: 40-43.
- [43] Rao AV, Gurfinkel DM, Drug Metabol.Drug Interact, 2000, 17: 211-235.
- [44] Güçlü-Üstündag Ö, Mazza G, Cr. Rev. Food Sci. Nutr., 2007, 47: 231-258.
- [45] Price KR, Johnson IT, Fenwick GR, CRC Crit. Rev. Food Sci. Nutr., 1987, 26: 127-135.
- [46] Oakenful D, Sidhu GS, Saponins. In: Cheeke, P.R. (Ed.), *Toxicants of Plant Origin*. Academic Press, New York, **1989**, P. 78-113.
- [48] Elvin-Lewis P, Memory FL, Walter H, *Medical Botany: Plants Affecting Man's Health.* Wiley, New York. ISBN: 0-471-53320-3. **1977.**
- [49] Chung KT, Wong TY, Wei CI, Huang YW, Lin Y, Crit. Rev. Food Sci. Nutr. 1998, 38(6): 421-64.
- [50] Graf E, Eaton JW, Free Radic Biol Med, 1990, 8: 61-69.
- [51] Hawkins PT, Poyner DR, Jackson TR, Letcher AJ, Lander DA, Irvine RF, Biochem J., 1993, 294: 929–934.
- [52] Graf E, Eaton JW, Cancer, 1985, 56: 717-718.
- [53] Shamsuddin AM, J Nutr., 1995, 125: 7258–7328.
- [54] Shamsuddin AM, Vucenik I, Cole KE, Life Sci., 1997, 61: 343-354.
- [55] Shamsuddin AM, International Journal of Food Science and Technology, 2003, 37(7):769–782.
- [56] Hirose M, Fukushima S, Imaida K, Ito N, Shirai T, Carcinogenesis, 1999, 19: 3665-3670.
- [57] Singh JP, Selvendiran K., Banu M, Padmavathia R, Sakthisekaran D, Phytomedicine, 2004, 11(4): 309-314.
- [58] Agarwal R, Mumtaz H, Ali N, Molecular and Cellular Biochemistry, 2009, 328(1-2): 155-165.
- [59] Conte A, Pizá P, García-Raja A, Grases F, Costa-Bauzá A, Prieto RM, Arch Esp Urol., 1999, 52: 305-310.
- [60] Grases F, Garcı´a-Gonzalez R, Torres JJ, Llobera A, Scand J Urol Nephrol., 1988, 32: 262–265.
- [61] Grases F, Prieto RM, Simonet BM, March, JG, *Biofactors* 2000<sup>b</sup>, 11: 171-177.
- [62] Roberts MF, Wink M, Alkaloids: Biochemistry, Ecology and Medicinal Applications. Plenum Press, New York. **1998.**
- [63] Lewis WH, Elvin-Lewis PF, *Medical botany: Plants affecting human health*. 2<sup>nd</sup> edition. John Wiley & Sons, Washington. 2003.
- [64] Carlini EA, Plants and the central nervous system. *Pharmacology Biochemistry and Behavior*, **2003**, 75: 501-512.
- [65] Van Wyk BE, Gericke N, People's plants. A guide to useful plants of South Africa. Briza Publications, Pretoria. 2000.
- [66] Oliver-Bever B, *Medicinal plants in tropical West Africa*. Cambridge University Press, Cambridge, Great Britain. **1986**, 375pp
- [67] Maggi M, Filippi S, Ledda F, Magini A, Forti G, European Journal of Endocrinology 2000, 143:143-154.
- [68] Drewes SE, George J, Khan F, Phytochemistry, 2003, 62: 1019-1025.
- [69] Trease MT, Evans SE, Chem. Sci. Nig., 1989, 26:57-58.
- [70] Evans WC, *Pharmacognosy*, (15th edition), London W.B. Saunders Company Ltd., 2002, pp. 191-393.
- [71] Taiwo A, Abidemi C, Oyedepo J, Adebayo B, Oluwadare I, Agboto D, *Afr. J. Biotech.* 2009, 8(21): 5888-5890.
  [72] Lans CA, *J. Ethnobiol Ethnomed.*, 2006, 2: 45–55.
- [73] Onwuka GI, Food Analysis and Instrumentation: Theory and Practice. 1st Edn., Naphthali Prints, Lagos, Nigeria, 2005, pp: 1-219
- [74] Bolhuis GG, Neth. J. Agric. Sci., 1954, 2: 176-185.
- [75] Björkman M, Klingen I, Birch AN, Bones AM, Bruce TJ, Johansen TJ, Meadow R, Mølmann J, Seljåsen, R., Smart LE Stewart D, *Phytochemistry*, **2011**, 72(7): 538-56.
- [76] Rahman MM, Kawamura O, Asian-Aust. J. Anim. Sci., 2011, 24 (3): 439 448.