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Qualitative and quantitative phytochemical properties of some selected herbal plants used in the treatment of snake bite in the vicinity of Billiri and Kaltungo Local Government areas of Gombe State, Nigeria.

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ABSTRACT

The Qualitative and quantitative phytochemical properties of *Gardenia terniforlia, Annona senegalens, Combretum*, *Balanite aegyptiaca and Borreria verticillata* herbal plants used in treatment of snake bite in the vicinity of Billiri and Kaltungo Local Government areas of Gombe State were analyzed. Phytochemical analyses conducted on the plants revealed the presence of constituents which are known to exhibit medicinal, as well as physiological activities. In these screening processes, alkaloids, saponins, steroids, terpenoids, phenols and volatile oils were present in all plant samples. Tannins, flavonoids, and Cardiac Glycoside were absent in root bark of *Balanite aegyptiaca*, root bark of *Annona Senegalensis* and leaves of *Gardenia Ternifolia epiphyte* respectively. The results of the quantitative analysis of plant parts extract revealed that for alkaloids, leaves of *combretum* epiphyte have the highest yield of 28.4% while root bark of *Balanite aegyptiaca* recorded the least yield of 0.8%. For flavonoids, root bark *Balanite aegyptica* has the highest yield of 70.4% while root of *Boreria verticillata* have least yield of 12%. Stem bark of *Gardenia ternifolia* epiphyte have the highest yield for saponins which is 63.2% while stem bark of *Combretum* epiphyte recorded the least of 21.6%. Leaves of *Combretum* epiphyte have the highest yield of tannins which is 30.1%, while the stem bark of *Combretum* epiphyte records the least of 10.3%. Leaves of combretum epiphyte have the highest for phenols 35.3%, while root bark of *Annona senegalensis* recorded the least yield which is 5.1%. These phytochemical suggest the medicinal functionality and hence the bioprospect of this anti-snake venom medicinal plants, because phytochemicals are known to exhibits medicinal, as well as physiological activities. Scientific research should be carried out on these plants to confirm their anti-venom efficacy and activity

Keywords: Phytochemicals, herbal plants, snake bite, Gombe state, Nigeria.

INTRODUCTION

Medicinal plants are plants that have at least one of their parts (leaves, stem, bark or roots) used for therapeutic purposes [1]. [2] defined medicinal plants as any plant which contains substances that can be used for therapeutic purposes in one or more of its organ or substances which are precursors for the synthesis of useful drugs. Furthermore, [3] noted that a plant become a medicinal plant only when its biological activity has been ethno-botanically or scientifically established. Plants are great sources of medicines,

especially in traditional medicine, which are useful in the treatment various diseases [4,5]. Generally, the use of herbs to treat disease is almost universal among non-industrialized societies, and is often more affordable than purchasing expensive modern pharmaceuticals. The plant constituents have identified which are used to neutralize the effects of snake venoms [6,7,8]. The way of management of snake bites through herbals are by treating with single herbal drugs or in combination applications, because of its design to control infection, stop pain, improve symptoms, correct imbalance, adjust immune system and boost energy for better health and quality of life [9]. With reflection to that area, an attempt is being made to collect available information about some medicinal plants advancement against snakebites and to present in the form of a comprehensive article. Recent scientific investigations have confirmed the efficacy of many of these preparations, some of which are remarkably effective [10]. The herbs which appear most effective as per the symptoms of snake bites are relatively non-toxic and have substantial documented efficacy, among them some are *Aristolochia* species, *Cissus assamica, Echinacea* species, *Guiera senegalensis, Hemidesmus indicus, Parkia biglobosa, Securidaca longipedunculata, Tamarindus indica, Trianosperma tayuya, Thea sinensis, Withania somnifera* [11]. ***Annona senegalensis:*** Commonly known as African custard-apple, wild custard apple, and wild soursopas, is a species of flowering plant in the custard apple family, *Annonaceae*. A traditional food plant in Africa, the fruits of *A. Senegalensis* have the potential to improve nutrition, boost food security, foster rural development and support sustainable land care. It takes the form of either a shrub or small tree, growing between two and six meters tall. Occasionally, it may become as tall as 11 m [12]. ***Gardenia:*** This is a genus of flowering plants in the coffee family, *Rubiaceae*as native to the land subtropical regions of Africa, southern Asia, Australasia and Oceania. ***Combretum:*** The bushwillows orcombretums make up the type genus of the family Combretaceae. The genus comprises about 370 species of trees and shrubs, roughly 300 of which are native to tropical and southern Africa, about 5 to Madagascar, some 25 to tropical Asia and approximately 40 to tropical America. The genus is absent from Australia. ***Narcissus pseudonarcissus:*** This is a bulbous perennial with upright, strap-like, grey-green leaves. The leaves arise from the base of the stem and are up to 35 cm long and 12 mm wide, with rounded tips. ***Balanite aegyptiaca:*** It is a multibranched, spiny shrub or tree up to l0 m tall, crown spherical in one or several distinct masses. ***Borreria verticillata:*** This is a woody, bushy shrub, it is a perennial reaching 1 m in height, glabrous and erect with many ramifications. It is very common in humid areas. The stem is obscurely angled, glabrous with stipuler sheaths which are glabrous or scabrid. The leaves are oblanceolate, 3-5cm long, 5-10 mm broad, glabrous with faint lateral nerves [13].

The Nigerian Ministry of Health reports that snake bites kill every year. Research in recent years has provided critical data on the conditions that promote snake-human contact, snake bite patient profile, and the necessity of in-state production of large quantities of affordable antivenin. At this time, development of anti-venom facilities in Nigeria is in place due to the EchiTab Study Group of Liverpool, England, and novel research with herbal medicine is being conducted at the University of Nigeria Nsukka [14,15]. According to data presented to a WHO study group in January, 2007 by a project director from the Nigerian Ministry of Health, globally over 1 million humans are bitten annually by venomous snakes, resulting in 20,000 deaths. Venomous

snake species which inhabit Nigeria include *Bitis gabonica* (gaboon viper), *Bitis arietans* (puff adder) and *Naja nigricollis* (black necked spitting cobra). However, *Echis ocellatus*, the West African carpet viper, is the most common. It accounts for 90% of bites and 60% of the fatalities in the country, which add up to 20% of all African cases [16]. Since pre-historical time, snake bite has been an issue in the areas of Billiri and Kaltungo local government areas of Gombe State. Many researchers have worked on different snake bite medicinal plants. But certain essential elemental pollutants in these medicinal plants were normally not part of the results presented in literature. Hence there is need to carry out the phytochemical screening of some medicinal plants so as to establish scientific data, with respect to safety and benefits derived from them [17].

Phytochemicals are chemical compounds produced by plants, generally to help them thrive or thwart competitors, predators, or pathogens [18]. Some phytochemicals have been used as poisons and others as traditional medicine [19]. The ground samples of *Gardenia terniforlia, Annona senegalens, Combretum*, *Balanite aegyptiaca and Borreria verticillata* herbal plants used in treatment of snake bite in the vicinity of Billiri and Kaltungo Local Government areas of Gombe State were examined for the its' phytochemical properties. **Glycosides** are used medicinally. Scientifically, a glycoside is formed when the hydroxyl group on the anomeric carbon atom of pyranose or fructose is replaced by a neuleophilic atom eg S-glycoside and N-glycoside [20]. **Saponins:** Are sterols occurring naturally. Saponins are detergents and disintegrate bacteria membrane and blood cells. It allows the level of plasma, cholesterol, impact bitter taste and psychological disturbance and toxicity to the human system [21]. **Tanins** are naturally occurring polyphenolic compounds with astringent taste. They are lustrous, faintly yellow, amorphous powder; glistering scales darken on exposure to air, soluble in benzene, chloroform, and ether [22, 23,24]. They have antiseptic properties of the ability to react with protein forming insoluble co-polymers. They have bitter astringent taste [25]. **Alkaloids:** Alkaloids are basic nitrogen containing heterocyclic compounds, which are derived from high plants with physiological activities. Alkaloids are regarded as a group of organic compounds that occur chiefly in seed bearing plants [26]. **Flavonoids:** These are large family of polyphenolic synthesized plants, it has anti-inflammatory, anti-oxidants and metal chelating properties. Consumptions of black tea, apples, and onions, which are high in flavonoids, are associated with significant reduction in the risk of coronary heart disease [27]. **Phenols:** These protect plants from chemical damage and perform the same function in humans. Found in tea, is a class of aromatic organic compounds in which one or more hydroxyl groups are attached to the benzene ring [28]. **Terpenoids:** Are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical functions [29].

MATERIALS AND METHODS

The plant samples were collected from Gombe State of Nigeria, in southern part of Gombe precisely at Lawampe village near Ayaba off Filiya road of Billiri local Government Area, and Kalargu of Kaltungo Local Government Area. Random sampling method as described by FAO/WHO (1986) was adopted for collection of all the plant samples. The plant parts used were leaves and stem bark of *Gardenia terniforlia* epiphyte, root bark of *Annona senegalens,* leaves and stem bark of *Combretum*, *root bark of Balanite aegyptiaca*

and Borreria verticillata plant. These sampled plants were identified in their fresh state and were authenticated by a traditional herbalist named Mr. Molta Kwana.

Sample preparation was done by air drying of the plants sample separately, grinding to have a fine powder which is then reduced to a manageable size, before analyzing them differently for phyto-chemical. Chemical tests were carried out on the plant samples using standard procedure to identify the presence of phytochemical constituents; alkaloids, saponins, tannins, terpenoids, glycosides, flavonoids, phenols, steroids and volatile oils as described by Edeoga (2005) [8].

Qualitative phytochemical screening

Determination of alkaloids: one ml of 1% HCL was added to 3ml of the extract in a test tube. The mixture was heated for 20 minutes cooled and filtered. About two drops of Mayer's reagent was added to 1ml of the filterate. A creamy precipitate indicates the presence of alkaloids.

Determination of saponins: A volume of 5cm^3 of extract was vigorously shaken with 10cm^3 of water in a test tube. Persistent frothing was taken as an evidence for the presence of saponins.

Determination of tannins: About 0.5g 0f the dried powder sample was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration [8].

Determination of terpenoids (salkowski test): About 5ml of each extract was mixed in 2ml 0f chloroform, and conc H_2SO_4 (3ml) was carefully added to form a layer. A redish bron colouration of the inter-face was formed to show positive result for the presence of terpenoids.

Determination of cardiac glycosides (keller-killani test): About 5ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of conc sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring appeared below the brown ring, while in the acetic layer, a greenish ring formed just gradually throughout the layer.

Determination of flavonoids: one ml of 10% sodium hydroxide was added to 3ml of the extract, yellow colouration was observed for the presence of flavonoids.

Determination of phenols: Equal volume extract plus equal volume of ferric chloride, a deep bluish green solution was taken as a positive test for the presence of phenols.

Determination of steroids (salkowski test): five drops of conc. sulphuric acid was added to 1ml of the extract in a test tube. Red colouration was observed to indicate the presence of steroid.

Determination of volatile oils: The extract was dissolved with 90% ethanol and a drop of ferric chloride was added. A green colouration was taken as an indicative for the presence of volatile oils.

Quantitative screening

Alkaloid determination: 5g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoid determination: 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through

whalman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Saponin determination: The method used was that of Obadoni and Ochuko (2001) [18]. 20 g of samples powder was put into a conical flask and 100ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^0c. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combine extracts were reduced to 40 ml over water bath at about 90^0c. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was removed while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combine n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

Determination of total phenolic content: The total phenolic content was estimated using the modified Folin-Ciocalteu photometric method. The appropriate amount of filtered methanol extracts were oxidized with Folin-Ciocalteu's reagents and after 5 mins was the reaction neutralized with saturated sodium carbonate. The solution was then immediately diluted to the volume of 50 ml with distilled water. The absorbance was measured at 750 mm after 90 mins of incubation at room temperature against the blank/ as the standard was used gallic acid equivalents (GAE) per 100 of dry weight (dw).

Determination of tannins: Tannin content was determined using Follin-Denis reagent. 1$cm^3$of the sample extract was pipette into 100 cm^3 volumetric flask containing 75 cm^3 distilled water. 5 cm^3 of Follin-Denis reagent and 10 cm^3 of saturated sodium carbonate solution were added. The solution was diluted to mark with distilled water and mixed properly by shaking. The solution was left to stand for 30 mins then poured into cuvettes and the absorbance read at 760 nm in a spectrophotometer. 0-10 cm^3 a liquot of standard tannins acid solution were pipette into 100 cm^3 volumetric flasks, each containing 75 cm^3 distilled water. The standard was treated as the sample extracts and the absorbance read. A standard calibration curve was constructed and the concentrations of the sample extracts extracted from the curve.

RESULTS

Table 1. Qualitative Screening of the phytochemicals

Plants	A	T	S	St	Fl	CGs	Te	Ph	VO
Leaves of *Combretum* epiphyte	+	++	+	+	+	+	+	+	+
Stem bark of *Combretum* epiphyte	+	+	+	+	+	+	+	+	+
Leaves of *Gardenia ternifolia* epiphyte	+	+	++	+	+	_	+	+	++
Stem bark of *Gardenia ternifolia* epiphyte	+	+	+	+	+	+	+	+	+
Root bark of *Annona senegalensis*	+	+	+	+	_	+	+	+	+
Root bark of *Balanite aegyptiaca*	+	_	++	+	+	+	+	+	+
Root bark of *Boreria verticillata*	+	++	+	++	+	+	++	+	+

Key: + Presence of constituent, - Absence of constituent, A-Alkaloids, T-Tannins, S-Saponins, St-Steroids, Fl- Flavonoids, CGs-Glycosides, Te- Terpenoids, Ph- Phenols, VO-Volatile Oils.

Table 2: Quantitative Analysis of the Phytochemicals

Plant	A (%w/w)	F (%w/w)	S (%w/w)	T (%w/w)	Ph (%w/w)
Leaves of *Combretum* epiphyte	28.4	58.8	50.4	30.1	35.3
Stem bark of *Combretum* epiphyte	13.6	38.4	21.6	10.3	30.1
Leaves of *Gardenia ternifolia* epiphyte	10.4	16.8	42.8	25.0	35.2
Stem bark of *Gardenia ternifolia* epiphyte	9.2	25.6	63.2	20.4	30.0
Root bark of *Annona senegalensis*	4.0	0.00	25.2	15.2	5.1
Root bark of *Balanite aegyptiaca*	0.8	70.4	24.4	0.0	10.3
Root of *Boreria verticillata*	1.6	12.0	25.2	25.2	10.0

Key: A-Alkaloids, F- Flavonoids, S-Saponins,T-Tannins, Ph- Phenols.

DISCUSSION

Phytochemical analyses conducted on the plants revealed the presence of constituents which are known to exhibit medicinal, as well as physiological activities. The phytochemicals include alkaloids, tannins, terpenoids, phenols, saponnins, cardiac glycosides, steroids, flavonoids and volatile oils [30,31]. The present study on these anti-snake venom medicinal plants, revealed the presence of medicinal active constituents. The phytochemical active compounds of these plant samples were qualitatively analyzed separately. In these screening processes, alkaloids, saponins, steroids, terpenoids, phenols and volatile oils were present in all plant samples. Tannins, flavonoids, and Cardiac Glycoside were absent in root bark of *Balanite aegyptiaca*, root bark of *Annona Senegalensis* and leaves of *Gardenia Ternifolia epiphyte* respectively. The various classes of compounds identified in the phytochemical study of extract [1,2] could be closely studied. Akunyili and Akubue (1986) [5] had earlier related anti venom activity to alkaloidal glycosides they traced in the plant they studied. Tannins are also known to unspecifically inactivate proteins [31]. The activity of extract may therefore, be linked to its tannin contents. Present study supports its traditional use against snake bite.

Tannins were observed in the entire samples screened except in root bark of *Balanite aegyptiaca* which was absent. Tannins are widely distributed in many plants species, they have astringent properties which are important anti-oxidant and in wound healing [14]. Alkaloids were found in the entire samples. Alkaloids are produced by large variety of organisms, plants and animals. They almost uniformly invoke bitter taste [13]. They have pharmacological effects and often used as medications and recreational drugs. The root bark of *Annona Senegalenses* showed the absence of flavonoids in the samples screened. Flavonoids are the most common group of

polyphenolic compounds in the human diet and are found in plants [11]. The widespread distribution of flavonoids and their low toxicity compared to other active plant compounds means that many animals, including humans, ingest significance quantities in their diet. Flavonoids have anti allergic, anti-inflammatory, anti-bacterial, anti-cancer, anti-diarrhea and anti-oxidant properties [10]. Glycosides were not recorded in leaves of *Gardenia Ternifolia* epiphyte. Glycosides play important roles in living organisms. They are used as medications for treatment of congestive heart failure and cardiac arrhymia [12]. The entire samples showed the presence of steroids and are pharmalogically active compounds and show the analgesic properties Malairajan*et al.(2006).* The steroids also exhibit central nervous system activities. [10] reported the terpenoids to decrease blood sugar level in animals. Cardiac glycosides are also of medicinal importance and used in the treatment of congestive heart failure and cardiac arrhythmia. Phenols compounds have tremendous antimicrobial potential. They have been extensively used in disinfections and remained the standards with which other bactericides are compared [16]. They have been reported to exhibit cellular defense mechanism in atherogenesis and cancer. A wide range of phenolic substances show strong antioxidant and antimutagenic activities. As per recent evidences, phenolic compounds could also play an essential health promoting role [14]. Phenols were recorded present in the entire samples. The entire samples showed the presence of saponins. Saponnins is being promoted commercially as dietary supplements and nutraceuticals in traditional medicine preparations. They also possess hypocholesterolemic and antidiabetic properties [15]. Certain tannins (ellagitannins from *Lagerstroemia speciosa*) stimulate glucose uptake. They exhibit insulin like activity acting as glucose transport activators of fat cells [17].

The results of the quantitative analysis of plant parts extract revealed that for alkaloids, leaves of *combretum* epiphyte have the highest yield of 28.4% while root bark of *Balanite aegyptiaca* recorded the least yield of 0.8%. For flavonoids, root bark *Balanite aegyptica* has the highest yield of 70.4% while root of *Boreria verticillata* have least yield of 12%. Stem bark of *Gardenia ternifolia* epiphyte have the highest yield for saponins which is 63.2% while stem bark of *Combretum* epiphyte recorded the least of 21.6%. Leaves of *Combretum* epiphyte have the highest yield of tannins which is 30.1%, while the stem bark of *Combretum* epiphyte records the least of 10.3%. Leaves of combretum epiphyte have the highest for phenols 35.3%, while root bark of *Annona senegalensis* recorded the least yield which is 5.1% [13]. This study has comprehensively confirmed the medicinal functionality and hence the bioprospect of this anti snake venom medicinal plants, because phytochemicals are known to exhibits medicinal, as well as physiological activities [19]. Therefore these plants are safe to be used as snake bite remedy in neutralizing the activity of the venom thereby reducing severity of toxic effect. Scientific research should be carried out on these plants to confirm their anti venom efficacy and activity. Also, further research should be carry out to isolate the active components using chromatographic seperations and characterized their structures using FTIR, MS or NMR.

REFERENCES

1. Abubakar M.S., Sule, M. I., Pateh U. U., Abdurahaman, E. M., Haruna, A. K, Jahun, B.M. (2000) In vitro snake venom detoxifying action of the leave

extract of Guierasenegalensis, *Journal Ethnophamacology* **69:** 253-257

2. Adzu B., Amos S., Adamu M., Gamaniel K, (2003) Anti-nociceptive and Anti-inflammatory effects of methanol extract of Annonasenegalensis root bark, *Journal of Natural Remedies* **3(7):** 63-67

3. Adzu, M. S. Abubakar K. S., Izebe D. D., Akumka K. S. Gamaniel (2005), Neuropharmacological evaluation of Annonasenegaalenes leaves, *Journal of Ethnopharmacol*, **96:**507-513

4. Afshin A. and Masoud A. Z. (2008) Investigation of minimization of excess sludge reduction by sequencing batch reactors,*Asian Journal of Tropical Medicine and Public Health* **39(2):** 335-340

5. Akunyili D. N. Akubue P. I. (1986) the anti-snake venom from the stem bark of Schumannin-phytonmagnificum Harms, *Journal of Ethnopharmacology***18(9):** 167-172

6. Berlow, Burton and Routh (1974) Introduction to the Chemistry of Life, Concepts of Biology **1:**109-128

7. Brian, F. H; Thomas-Bigger, J and Goodman G. (1985)the Pharmacological Basis of Theuraupeutic, Macmillan **7:** 123-124

8. Edeoga H. O, Okwu D.E and Mbaebie B.O (2005) phytochemical constituents of some Nigerian medicinal plants, *African Journal of BioTechnology***4 (7):** 685-688

9. Edgar J. DaSilva, Elias Baydoun, Adnan Badran (2002) Biotechnology and the Developing World, *Electronic Journal of Biotechnology* **5 (1):** 204-230

10. Fabricant D. S., Farnsworth NR (2001) the value of plants used in traditional medicine for drug discovery, *Journalof* Environmental *Health Perspect*ive **13(5):** 389-399

11. Godwin T. W. and Mercer E. I. (1983) Introduction to plant biochemistry **2**: 677

12. Hayashi T.; H Maruyama; Hatton K.; Hazeki O; Yamasaki K.; Tanaka T., (2002)Comprehensive Natural Products, *International Journal of Chemistry and Biology* **68(2):** 173-175

13. Mahadeswaraswamy Y. N., Devaraja S., Kumar M.S., Goutham Y. N. J., Kemparaju K. (2009) Effects of anticoagulants on the plasma hyaluronidaseactivities,*Indian Journal of Biochemistry and Biophysics* **46:** 154-160.

14. Meenatchisundaram S., Parameswari G., Michael A. (2009)Chiken egg yolk as an alternative to mammalian antibodies, *Indian Journal of Science and Technology***2(4):** 76-79

15. Mino Y, Ota N, (1984) Pharmacology in the Middle Ages,*Chemical and Pharmaceutical Bulletin***32**: 591

16. Mohammed N. K and Khamis, F.O. (2012) Assesment of Heavy Metal Contamination in Vegetables consumed in Zanzibars, *Journal of Natural Science***4(8):** 588-604

17. Ndoye M, (2004) Reproductive biology in *Balanitesaegyptiaca (L). Del, a Semi-Arid Forest Tree, African Journal of Biotechnology **8(7):** 109-110*

18. Obdoni B. O., Ochuko P. O. (2001) Phytochemical studies and comparative

efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. *Global Journal of pure and Applied Sci*ences **8**: 203-208.

19. Okonogi T., Hattori Z., Ogiso, A., Mitsui, S. (1979) Detoxification of Persimon Tannin of Snake Venom and Bacterial Toxins. *British Medical Journal***17:** 524–527

20. Otten EJ (2009). Venomous animal injuries In: Marx JA, ed. Rosen's Emergency Medicine: Concepts and Clinical Practice **7:**500-530

21. Pandey CN. Gujarat, India: (2005) Gujarat Ecological Education and Research Foundation; Medicinal plants of Gujarat **14:** 100-150

22. Paramonte, B. (2007). Snake Bites in Nigeria, *Medical Journal of Therapeutics in Africa***3(6):** 222

23. Sofowara A (1993) A Textbook of Medicinal Plants and Traditional Medicine in Africa **5**:289

24. Spencer, P.E. (2008)Biomarkers of the intake of dietary polyphenols,*British Journal of Nutrition* **99:** 60 – 77

25. Stepp, John R. &Moerman, Daniel E. (2001) the Importance of Weeds in Ethnopharmacology, *Journal of Ethnopharmacology* **75 (1):**19–23

26. Stepp, John R. (June 2004) the role of weeds as sources of pharmaceuticals, *Journal of Ethnopharmacology* **92 (2&3):** 163–166

27. Stohlgren T. J, Falkner M. B, Shell L. D (1994) A Modified-Whitetaker Nested Quadrant for Vegetation Sampling Method, *Medical Journal of Therapeutics***17:** 113-121

28. Stojanoski, N. (1999) Development of health culture in Veles and its region from the past to the end of the 20th century, *Vales society of science and art Journal* **10 (2):** 50-56

29. World Health Oganization, Quality Control Method for Medicinal plants materials, WHO, Geneva, Switzerland, 1998

30. World Health Oganization, Quality Control Method for Medicinal plants materials, WHO, Geneva, Switzerland, 2005

31. Yamauochi, M., Tsuruma, K., Imai, S., Nakanishi, T., Umigai, N., Shimazawa, M., Hara, H. (2011). "Croceting prevent retinal degeneration induced by oxidative and endoplasmic reticulum stresses via inhibition of caspase activity, *European Journal of Parmacology***650 (1):** 110