Research Article

Evaluation of Saponin Content of Extracts of Six *Citrus* Species in relation to their Medicinal Potentials.

ABSTRACT

Different parts of *Citrus aurantifolia* (Christm.) Swingle (Lime), *C. grandis* Osbeck (Shaddock/Pummelo), *C. limon* (L.) Burm. f. (Lemon), *C. paradisii* Macf. (Grapefruit), *C. reticulata* Blanco (Mandarin/Tangerine) and *C. sinensis* (L.) Osbeck (Sweet orange) commonly cultivated in Southeastern Nigeria were investigated for presence of saponin. All the parts of these *Citrus* species were found to contain saponin in varying levels. The highest level of saponin was contained in the leaves of *C. sinensis* and peels of *C. aurantifolia* respectively. High level of saponin was also contained in the leaves of *C. paradisii, C. grandis, C. reticulata* and *C. aurantifolia* respectively. These indicated that these parts of these *Citrus* species contained high level of saponin and could be regarded as possible sources of it; which could be used for a variety of commercial purposes. In addition, they can be used in ethnomedicine as drugs.

Keywords: antifungal, anti-inflammatory, antiviral, anti-ulcer, anti-protozoa, herbal drugs, insectcides

1. INTRODUCTION

Citrus aurantifolia (Christm.) Swingle (Lime), *C. grandis* Osbeck (Shaddock/ Pummelo), *C. limon* (L.) Burm. f.(Lemon), *C. paradisii* Macf. (Grapefruit), *C. reticulata* Blanco (Mandarin/Tangerine) and *C. sinensis* (L.) Osbeck (Sweet orange) are *Citrus* species commonly cultivated in Southeastern Nigeria. They belong to the genus, *Citrus* of the family, Rutaceae [1]

Saponins which are glycosides of both triterpenes and steroids are widely spread and have been reported to have been found in over seventy plant families[2]; both wild plants and cultivated crops [3][4]. Triterpenes are found principally in dicotyledonous species, while many of the major steroidal saponins are synthesized by monocotyledons [5].Traditionally, they have been extensively used as detergents [6][7].They have been reported to have a variety of beneficial health effects. The therapeutic effects of a large number of folk medicines are thought to be associated with their saponin content [8]; in several of the more familiar examples was liquorice extract, used in the treatment of stomach ulcers [9].

Knowledge of the chemical constituent of plants is desirable because such information will be of value for the synthesis of complex chemical substances [10]. Despite the wealth of human experience and folklore concerning the medicinal uses of plants, proper scientific investigation has only been applied to a small fraction of the world's plant [11].These made the investigation of more plants for presence of bioactive compounds, a necessity. The objective of this research, therefore, was to evaluate different parts of *Citrus* plants for saponin content; of which when found in high concentration would present them as

rich sources of it, which can be exploited for commercial uses and in ethnomedicine as drugs.

2. MATERIALS AND METHODS

2.1 Sources of Materials

The roots, stems, stem barks, leaves and fruits of *Citrus aurantifolia, C. grandis*, *C. limon*, C. *paradisii*, *C. reticulata* and *C. sinensis* were collected in the months of November – December at optimum maturity, from Agricultural and Natural Resources Department Market Garden, Amawbia, Awka South Local Government Area, Anambra State, Nigeria.

The *Citrus* species were authenticated by Prof. C.U. Okeke, a plant taxonomist in Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, where the voucher specimens were deposited.

2.3 Preparation of Plant Materials for Saponin Determination

The rinds of healthy ripe fruits of the six *Citrus* species were peeled off with a knife. The roots, stems, stem barks and peels were sun dried for seven days whereas the leaves were air dried in the laboratory at room temperature for ten days. The dried samples were then crushed with mortar and pestle before grinding into fine powder using a manual grinder (Corona, USA.).

2.4 Qualitative Determination of Saponin

The homogenous sample of each of the samples of the roots, stem, stem barks, leaves, and peels of the six species of *Citrus* was subjected to phytochemical analysis for qualitative determination of saponin according to the methods described by [12]. The performed qualitative tests were briefly described as:

In a test tube, 0.5g of the extract was shaken with water. Frothing which persists on warning was taken as evidence for the presence of saponin.

The following rankings were used:

+ = Present

++ = Deeply present

+++ = Very deeply present

2.5 Quantitative Determination of Saponin

The saponin content of the sample was determined by double extraction gravimetric method described by [13].

A measured weight (5g) of the powdered sample was mixed with 50 ml of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55^0C; it was then filtered through Whatman filter paper [No 42]. The residue was extracted with 50 ml of 20% ethanol and both extract were poured together and the combined extract was reduced to about 40 ml at 90^0C and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Re extraction by partitioning was done repeatedly until the aqueous layer become clear in colour. The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre weighed evaporation dish. It was dried at 60^0C in the oven and reweighed after cooling in a dessicator. The process was repeated two more times to get an average. Saponin content was determined by difference and calculated as a percentage of the original sample thus:

$$\text{Percentage (\%) Saponin} = \frac{W2 - W1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:-

W_1 = Weight of evaporating dish

W_2 = Weight of evaporating dish + sample

2.6 Statistical Analysis

The quantitative data obtained were statistically analyzed by calculating the mean of three replicates followed by calculation of the Sum of Square, Variance, Standard Deviation and Standard error. The results were presented as mean ± standard error.

3. RESULTS AND DISCUSSION

The result showed that saponin was present in all the parts of *Citrus* species investigated, but in varied levels. Saponin have been reported to be common in a variety of higher plants and usually found in roots, tubers, leaves, blooms and seeds[8].[14] Noted that the chemical compositions of herbal extracts can vary widely with the plant variety. The highest level of saponin was contained in the leaves of *C. sinensis* [0.98±0.03%] and peels [0.96±0.01%] of *C. aurantifolia* respectively (Tables 1 and 2). High level of saponin was also contained in the leaves of *C. paradisii* [0.89±0.01%]; *C. grandis* [0.87±0.06%]; *C. reticulata* [0.81±0.01%]; and *C. aurantifolia* [0.64±0.01%] respectively (Tables 1and 2). High level of saponin was observed in the roots of *C. limon* [0.73±0.03%]; *Citrus aurantifolia* [0.72±0.01%]; *C. paradisii* [0.67±0.01%] and *C. grandis* [0.63±0.01%] respectively.

The high content of saponin in the roots of these *Citrus* species might be attributed to the bitterness and sourness of their fruits. [8] Reported that saponins are bitter-tasting triterpene glycosides found in many dicotyledonous plants. In addition, the high level of saponin in the roots might be as a result of the need to protect plants against soil pathogen attacks. It has been noted that many saponins are present in healthy plants in high concentrations, because of their antifungal properties [15][16]. Low saponin content was observed in the stem and stem bark of all the species (Tables 1 and 2). It has been reported that any part of the plant may contain active components [17]. Its presence in the stem and stem bark might be to serve as a natural defense mechanism. Plants need to protect themselves against herbivory and diseases; they can be eaten by vertebrate herbivores or molluscs, or suffer from viral, bacterial or fungal infections [4].

Saponin has been reported to have a wide range of pharmacological and medicinal activities. Interestingly, it has been indicated to usually have low oral toxicity in humans [8].[18] Noted that the presence of saponin in plants have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs. [19] Revealed that saponin have both hypertensive and cardiac depressant properties. They have been found to be potentially useful for the treatment of hypercholesterolemia which suggested that saponin might be acting by interfering with intestinal absorption of cholesterol, thus have antidiabetic effects [20]. In addition, they have been reported to have antinematicidal, molluscicidal, insecticidal and antioxidant properties [3][21]; anti-cancer agents[6][8]; aphrodisiac properties [22][23]; anti-protozoal effects [24]; antibiotic, antifungal, antiviral, hepatoprotective, anti-inflammatory and anti-ulcer effects [25][26][27][28][29]. The use of these parts of *Citrus* plants in ethnomedicine as drugs is thus suggested, due to the high concentration of saponin in them and the several health beneficial effects reported to be associated with saponin.

Saponin has also been reported to have an insecticidal effect, which gave them the potency to be used as natural insecticides. The use of *Taraxacum vulgare* for killing scabies, fleas and lice as practiced in Mongolia which was attributed to the presence of saponin was reported [24][30]. [4] Documented that saponin possesses clear insecticidal activities; they exert a strong, immediate impact and rapid-working action against a broad range of pest insects and stages, which is different from neurotoxicity. Furthermore, they observed that

saponin lowered the food intake of the insects at sub lethal concentrations, thereby reducing the damage done to the crops. It could be applied exogenic, by spraying it on fields as presently commercially utilized as natural insecticides in China in the form of saponin powder and solutions [4].This indicated that it could be extracted from these parts of *Citrus* species as natural saponin and made available for farmers.

Table 1. Qualitative saponin content of roots, stem, stem bark, leaves and peels of *Citrus* species.

Species	Root	Stem	Stem bark	Leaves	Peels
Citrus aurantifolia	++	+	++	++	+++
C. grandis	++	+	+	+++	+
C. limon	++	+	++	+	+
C. paradisii	++	+	+	+++	+
C. reticulata	+	+	+	+++	+
C. sinensis	+	+	+	+++	++

Table 2. Quantitative saponin content of roots, stem, stem bark, leaves and peels of *Citrus* species (%).

Species	Root	Stem	Stem bark	Leaves	Peels
Citrus aurantifolia	0.72±0.01	0.38±0.04	0.52±0.01	0.64±0.01	0.96±0.01
C. grandis	0.63±0.01	0.32±0.01	0.44±0.02	0.87±0.06	0.31±0.03
C. limon	0.73±0.03	0.30±0.03	0.61±0.02	0.34±0.02	0.57±0.03
C. paradisii	0.67±0.01	0.34±0.01	0.45±0.02	0.89±0.01	0.41±0.01
C. reticulata	0.52±0.01	0.24±0.02	0.35±0.01	0.81±0.01	0.57±0.02
C. sinensis	0.43±0.01	0.26±0.01	0.38±0.03	0.98±0.03	0.70±0.04

*Data are mean obtained from analysis of three replicates ± standard error.

4. CONCLUSION

The concentration of saponin in these species of *Citrus* was high, which makes them good sources of saponin, from which it could be isolated for variety of commercial uses. In addition, farmers could also employ the use of natural saponin obtained from these *Citrus,* for protection of their plants against insect, mollusc and nematode attacks. In order to enhance animal health and production, the inclusion of these parts of *Citrus* in animal feed as additives is also suggested. They could also be potentially used in ethnomedicine, as anti-cancer, antibiotics, antidiabetic, antifungal, anti-inflammatory, antioxidant, antiprotozoal, anti-ulcer and antiviral agents. Clinical studies is recommended to determine at what level saponin becomes toxic to human and farm animals; and ascertain side effects, if any.

REFERENCES

[1]Nyananyo BL. *Plants from the Niger Delta.* Onyoma Research Publications Port Harcourt, Rivers State. 2006; 403pp.

[2]Shibata S. *Chemistry and Cancer Preventing Activities of Saponins and Some Related Triterpenoid Compounds.* Medical Sciences Press, Korea; 2001;430pp.

[3]Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: a review. *British Journal of Nutrition*, 2002; 88: 587–605.

[4]Geyter ED, Lambert E, Geelen D, Smagghe G. Advances of saponins as natural insecticides. *Pest Technology*, 2007;1(2): 96-105.

[5]Osbourn AE. Saponins in cereals. *Phytochemistry,* 2003; 62:1-4.

[6]Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y. Saponins from edible legumes: Chemistry, processing and health benefits. *Journal of Medical Food*, 2004;7:67-78.

[7]Humphrey AJ, Beale MH. Terpenes. In: Crozier A, Clifford MN, Ashihara H, Editors. Plant *Secondary Metabolites: Occurrence, Structure and Role in the Human Diet.* Blackwell Publishing Limited, Oxford, UK; 2006. 47 – 101pp.

[8]Sparg SG, Light ME, van Staden J. Biological activities and distribution of plant saponins. *Journal of Ethno pharmacology*, 2004; 94: 219–243.

[9]Castleman M. *The New Healing Herbs.* Hinkler Books Pty Ltd., Dingley, Australia; 2003.

[10] Savithramma N, Linga M, Suhrulatha D. Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research*, 2011; 8 (3): 579-584.

[11]Hamilton AC. Threats to plants: an analysis of Centers of Plant Diversity. In: Proceedings of the 4th International Botanic Gardens Conservation Congress. Kings Park and Garden, Perth, Australia; 1997; 309 - 322pp.

[12]Nyam MA, Wonang DL, Akueshi CO. Phytochemical screening and antimicrobial studies on *Canarium schiveinfurthii* Linn ("Atili") fruits and oil. *Nigerian Journal of Botany,* 2009; 22 (2): 247-253.

[13]Harborne JB. *Phytochemical Methods*.1st ed. Chapman and Hall, London;1973; 273pp.

[14]Wang Y, Tang H, Nicholson JK. Metabolomic strategy for the classification and quality control of phytomedicine: a case study of chamomile flower (*Matricaria recutita* L.) *Planta Medica*, 2004; 70: 250–255.

[15] Hostettman KA, Marston A. *Saponins.* Cambridge University, Press, Cambridge, U.K; 1991.

[16]Papadopoulou K, Melton RE, Leggett M, Daniels MJ, Osbourn AE. Compromised disease resistance in saponin-deficient plants. *Proceedings of the National Academy of Sciences,* 1999**;** 96(22):12923 – 12928.

[17]Cragg GM, David JN. Natural product drug discovery in the next millennium. *Journal of Pharmacy and Biology*, 2001; 39: 8-17.

[18]Alinnor IJ. Preliminary phytochemical and antibacterial activity screening of leaves of *Venonia amygdalina*, *Journal of Chemical Society of Nigeria*, 2008; 33(1): 172-177.

[19]Olaleye MT. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa.* Antioxidant effect of *Cysticus scoparius* against carbon tetrachloride treated liver injury in rats. *Journal of Ethonopharmacology,* 2007; 109: 41-47.

[20]Soetan KO, Aiyelaagbe OO. The need for bioactivity–safety evaluation and conservation of medicinal plant – A review. *Journal of Medicinal Plant Research,* 2009; 3 (5): 324-328.

[21]Argentieri MP, D´Addobbo TA, Agostinelli A, Jurzysta M, Avato P. Evaluation of nematicidal properties of saponins from Medicago species. *European Journal of Plant Pathology*, 2007;120: 189-197.

[22]Gauthaman K, Adaikan PG, Prasad RN. Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in normal and castrated rats. *Life Science*, 2002; 71: 1385–96.

[23]Guardian. Herbal cocktail better than Viagra in humans, say researchers. April 19, 2009; Lagos, Nigeria.

[24]Makkar HPS, Norvsambuu T, Lkhagvatseren S, Becker K. Plant Secondary Metabolites in some Medicinal Plants of Mongolia Used for Enhancing Animal Health and Production. *Tropicultura*, 2009; 27(3): 159-167.

[25]Jun, HK, Park KY, Jo JB. Inhibitory effects of Ginseng saponins on Aflatoxin production in culture. *Chemical Abstracts*, 1989; 106: 116-199.

[26]Price KR, Fenwick GR. The Chemistry and Biological significance of saponins in foods and feeding stuffs. *International Review in Food Science and Nutrition*, 1990; 157: 62.

[27]Arao T, Udayama M, Kinjo J, Nohara T. Preventive effects of Saponins from the *Pueraria lobata* root on in – vitro immunological liver injury of rat primary hepatocyte cultures. *Planta Medica,* 1998; 64(5): 413-416.

[28]Chao AC, Nguyen JV, Broughall M, Recchia J, Kensil CR, Daddona PE, Fix JA. Enhancement of intestinal model compound transport by DS-1, a modified Quillaia saponin. *Journal Pharmaceutical Science*, 1998; 87(11): 1395-1399.

[29]Just MJ, Recsio, MG, Gner RM, Cuellar MJ, Marez S, Bilia AR, Rios J. Anti-inflammatory activity of unusual lupane saponins from *Buleurum fruiticescens. Planta Medica*, 1998; 64(5): 404-407.

[30]Wina E, Muetzel S, Becker K. The impact of saponins or saponin containing plant materials on ruminant production - a review. *Journal of Agricultural and Food Chemistry,* 2005; 53: 8093-8105.