Determination of saponin content of various parts of six *Citrus* species

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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: *Saponin, Citrus aurantifolia*, *C. grandis*, *C. limon*, *C. paradisii*, *C. reticulata, C. sinensis*

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 Nyam *et al*. [12]. The performed qualitative tests were briefly described as:

In a test tube, 0.5g of the extract was shaken with water. A stable frothing was taken as evidence for the presence of saponin.

The following ranking was used:

+ = Present

2.5 Quantitative Determination of Saponin

The saponin content of the samples 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:

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

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 (Tables 1 and 2). It was noted that the chemical compositions of herbal extracts can vary widely with the plant variety [14]. Saponins have been reported to be common in a variety of higher plants and usually found in roots, tubers, leaves, blooms and seeds [8]. The saponins present in these species of *Citrus* were most likely to be the triterpenes; since they are cultivated crops and dicotyledonous species. The available literature reported that triterpenes are found principally in dicotyledonous species, while many of the major steroidal saponins are synthesized by monocotyledons [5]. In addition, the triterpenoid saponins are generally predominant in cultivated crops, while steroid saponins are common in plants used as herbs or for their health-promoting properties [15] 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. It has been reported that saponins are bitter-tasting triterpene glycosides found in many dicotyledonous plants [8]. 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 [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].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 [18]. It has been revealed that saponin have both hypertensive and cardiac depressant properties [19]. 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

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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].It has been 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 [4]. 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,*

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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.

AUTHORS' CONTRIBUTIONS

Chinelo A. Ezeabara designed the study, managed the literature searches and wrote the first draft of the manuscript. All authors managed the analyses of the study. Okeke C.U. authenticated the plants and supervised the work. All authors read and approved the final manuscript.

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