Determination of Tannin Content in Various Parts of Six *Citrus* Species

Chinelo A. Ezeabara¹*, C. U. Okeke¹, Chinyere V. Ilodibia¹ and Bibian O. Aziagba¹

¹Department of Botany, Nnamdi Azikiwe University, P.M.B. 5025 Awka, Anambra State, Nigeria.

**Authors' contributions**

Author CAE designed the study, managed the literature searches and wrote the first draft of the manuscript. All authors managed the analyses of the study. Author CUO authenticated the plants and supervised the work. All authors read and approved the final manuscript.

**ABSTRACT**

Tannin content of roots, stems, stem barks, leaves and peels of six species of *Citrus* was investigated. They include *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). The study showed that tannin was present in all parts of these *Citrus* species. The highest level of tannin was contained in the leaves of *C. aurantifolia* [1.44±0.02%] and *C. limon* [1.30±0.02%] respectively; whereas the least concentrations were found in the roots of *C. reticulata* [0.06±0.02%] and *C. sinensis* [0.08±0.04%] respectively. The roots, stems, stems barks, leaves and peels extracts of these *Citrus* species could be employed in ethnomedicine as drugs, due to the therapeutic values of tannin. In addition, they could be regarded as potential rich sources of natural tannin which could be isolated and utilized in cosmetic industry as antimicrobial agents, pharmaceutical industries for production of drugs, agriculture for reduction of emission of methane gas from ruminants, tannery industry for manufacture of leather, as additives in wine production to ameliorate the astringency and in manufacture of adhesives.
Keywords: Citrus tannin; natural tannin; Citrus aurantifolia; C. grandis; C. limon; C. paradisi; C. reticulata; C. sinensis.

1. INTRODUCTION

Citrus aurantifolia (Christm.) Swingle (Lime), Citrus grandis Osbeck (Shaddock/Pummelo) Citrus limon (L.) Burm. f. (Lemon), Citrus paradisi Macf. (Grapefruit), Citrus reticulata Blanco (Mandarin/Tangerine) and Citrus sinensis (L.) Osbeck (Sweet orange) belong to the genus, Citrus of the family Rutaceae [1]. Tannins are astringent, bitter polyphenolic compounds [2,3]. Their molecular weight range from 500 to 3000 [4,5]. Two classes of tannins – condensed and hydrolysable, have been noted [6]. More numerous condensed tannins (often called proanthocyanidins) are derived from flavonoid monomers while hydrolysable tannins are based on gallic acid, usually as multiple esters with d-glucose [5]. In addition, they may be formed by condensations of flavan derivatives which have been transported to woody tissues of plants [5]. They are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation [7].

Plants are vital sources of drugs and raw materials from the ancient time. Modern pharmacopeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants [8]. Many indigenous plants are used in herbal medicine to cure a variety of ailments in Nigeria. The medicinal attributes of plants is as a result of bioactive compounds in them. Active substances that have phenolic groups in their structure have reported to have great pharmacological potential.

In addition to pharmaceutical usefulness of plant-derived tannins, they have a variety of industrial applications. They have been reported to form the basis of the tanning industry for many years [6]. Leather which is the end product has a variety of uses. It is used in making shoes, straps, bags, belts and all kinds of upholstered seats. Furthermore, the use of tannins for reduction of methane (a greenhouse gas) production from ruminants is being considered [9]. Greenhouse gases have been of serious global concern to environmentalists because they are seen to be responsible for global warming [10]. In the US, total agricultural greenhouse – gas emission, with quarter each coming from ruminant enteric fermentation and animal waste has been reported [10][11]. Research noted that ingestion of small amount of naturally – occurring condensed tannin by ruminants can provide benefits including potential reduction of ammonia and nitrous oxide emission over the long – term by reducing their urine excretion [12].

Although these species of Citrus have long been regarded as food, medicinal plants and in the manufacture of household products, only the juices and the essential oils are being properly utilized. This suggested that they are under – utilized; hence, Citrus tannins from the roots, stems, stem barks, leaves and peels of these six species could be isolated and used as natural tannins. Thus, it becomes absolutely necessary to investigate these parts for presence of tannin. The objective of this work, therefore, was to evaluate the different parts of Citrus species for presence of tannin which appear to have many industrial and therapeutic values; with the view of providing abundant sources for natural tannin.
2. MATERIALS AND METHODS

2.1 Sources of Materials

Roots, stems, stem barks, leaves and fruits of *Citrus aurantifolia*, *C. grandis*, *C. limon*, *C. paradisi*, *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, Southeastern 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.

2.2 Preparation of Plant Materials

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).

2.3 Qualitative Determination of Tannin

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 tannin according to the methods described by [13]. The performed qualitative tests were briefly described as:

**Test for Tannins:** To 0.5g of prepared extract, 1ml of distilled water and few drops of ferric chloride were added respectively. A blue-black, green or blue-green precipitate indicates the presence of tannins.

‘+’ was used to denote presence of tannin

2.4 Quantitative Determination of Tannin

The Folin-Denis spectrophotometric method described by [14] was used.

A measured weight of each sample (1.0g) was dispersed in 10ml distilled water and agitated. This was left to stand for 30minutes at room temperature, being shaken every 5mins. At the end of the 30mins, it was centrifuged and the extract gotten. Into a 50 ml volumetric flask, 2.5ml of the supernatant (extract) was dispersed. Similarly, 2.5ml of standard tannic acid solution was dispersed into a separate 50ml flask. One milliliter (1.0ml) of folin-denis reagent was measured into each flask, followed by 2.5ml of saturated Na₂CO₃ solution. The mixture was diluted to mark in the flask (50ml) and incubated for 90mins at room temperature. The absorbance was measured at 250nm in a Genway model 6000i electronic spectrophotometer. Readings were taken with the reagent blank at zero. The tannin content was given as;
Percentage (%) Tannin = \( \frac{An \times C \times 100 \times Vf}{As \times W \times Va} \)

Where:
- \( An \) = Absorbance of test sample
- \( As \) = Absorbance of standard solution
- \( C \) = Concentration of standard solution
- \( W \) = Weight of sample used
- \( Vf \) = Total volume of extract
- \( Va \) = Volume of extract analysed

2.5 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 results showed that tannin was present in all the parts of Citrus species investigated. The highest level of tannin was contained in the leaves of \( C. aurantifolia \) [1.44±0.02%] and \( C. limon \) [1.30±0.02%] respectively (Tables 1 and 2). In addition, high level of tannin was observed in the roots of \( C. aurantifolia \) [0.82±0.04%] and \( C. limon \) [0.84±0.04%]; leaves [0.83±0.05%] and the root [0.78±0.04%] of \( C. paradisii \) (Tables 1 and 2). The high level of tannin in these species of Citrus might be responsible for the astringency of their fruits. It is reported that tannin has astringent properties [15,7]; which arises as a result of binding to salivary proteins, thereby producing a taste which humans recognize as astringency [6]. Leaf extractions of \( C. paradisii \) have been reported to show antibiotic activity [16]. This might be as a result of the high level of tannin in the leaves. High level of tannin was also observed in the peels of Citrus sinensis [0.90±0.02%] (Tables 1 and 2).

Many therapeutic actions of tannin have been reported. Report by [15] showed that tannin could be used for healing of wounds and inflamed mucous membranes. Tannins are reported to exhibit anti-diuretic [16], anti-inflammatory and anti-bacterial [17,18], antiulcer [19,20,21], antidiarrhoeal [22,23,24,25], antiviral and anti-tumor [26] activities. It is also reported to be able to inhibit HIV replication selectively [26]. These therapeutic properties of tannin indicated that tannin extracts from these parts of Citrus could give the human body a resistance against parasites. A number of uses of different parts of Citrus in medicine, especially in traditional Chinese medicine have been reported; and this might be attributed to the presence of tannin in them. An infusion of the immature fruit of Citrus sinensis is taken to relieve stomach and intestinal complaints [26]. The dried peels of sweet orange was reported to be used in the treatment of anorexia, colds and cough [27]. These medicinal uses of the peels of Citrus sinensis is probably as a result of high tannin content in them and the antibacterial, antiulcer and antiviral actions of tannin. Furthermore, for hundred years, herbalists trained in traditional Chinese medicine have used mature Citrus reticulata peel in intestinal gas and bloating; and to resolve phlegm [27]. In addition, the immature peel of Citrus reticulata known as “qing pi” in Chinese medicine, acts primarily on the liver and stomach to promote digestion, relieve food retention and abdominal digestion; and promote good liver function [27]. The pericarp of Citrus reticulata has been clinically used in the treatment of lung related diseases in traditional Chinese medicines for a long time [28]. The
rind of *Citrus aurantifolia* is reported to be used to suppress stomach ache [29]. These probably indicated that the bioactive compounds in the peels of these species of *Citrus* act on digestive and respiratory systems. Many developing countries use plant materials in primary health care for treatment of a variety of diseases. These parts of these *Citrus* species could therefore be used in ethnomedicine as drugs. Their extracts could also be used in cosmetic industries as antimicrobial agents. In addition, they could be used in the treatment of animal diseases. It is noted that tannin containing plants could possibly be used to prevent diarrhoea in pigs [30]. This is probably as a result of the antidiarrhoeal property of tannin. Study has shown that this is attributed to its ability to stimulate the reabsorption of water from the intestinal lumen as well as significantly reducing the intestinal transit time and intestinal motility [31]. The use of extracts of these parts of *Citrus* as antidiarrhoeal and antimicrobial agents in animal feeds is thus suggested.

| Table 1. Qualitative Tannin 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. paradisi* | + | + | + | + | + |
| *C. reticulata* | + | + | + | + | + |
| *C. sinensis* | + | + | + | + | + |

| Table 2. Quantitative Tannin content of roots, stem, stem bark, leaves and peels of *Citrus* species (%) |
|------------------------------------------|
| Species | Root | Stem | Stem bark | Leaves | Peels |
| *Citrus aurantifolia* | 0.82±0.04 | 0.35±0.04 | 0.49±0.01 | 1.44±0.02 | 0.64±0.03 |
| *C. grandis* | 0.75±0.01 | 0.27±0.02 | 0.35±0.01 | 0.77±0.03 | 0.53±0.02 |
| *C. limon* | 0.84±0.04 | 0.36±0.03 | 0.50±0.07 | 1.30±0.02 | 0.58±0.05 |
| *C. paradisi* | 0.78±0.04 | 0.30±0.05 | 0.41±0.03 | 0.83±0.05 | 0.58±0.03 |
| *C. reticulata* | 0.06±0.02 | 0.22±0.02 | 0.29±0.02 | 0.22±0.01 | 0.43±0.02 |
| *C. sinensis* | 0.08±0.04 | 0.27±0.07 | 0.31±0.02 | 0.53±0.05 | 0.90±0.02 |

*Data are means of triplicate determinations ± standard error.*

Moreover, many types of forages known to contain condensed tannins have both been shown to decrease methane production both *in vivo* and *in vitro* [10]. Studies have shown that there was a 16% reduction in methane production in lamps fed on *Lolium pedunculatus*, which is due to the presence of condensed tannins [32,10]. The condensed tannins contained in the *Sericea lespedeza* and crabgrass/tall fescue (17.7 and 0.5%) respectively, decreased methane emission (7.4 vs. 10.6 g/d and 6.9 vs. 16.2 g/kg Dry Matter Intake) for *Sericea lespedeza* and crabgrass/tall fescue respectively, in Angora goats [33]. Ingestion of water containing condensed tannin (CT) by ruminants can produce valuable outcome such as improved nitrogen use and reduced bloating, methane output and gastrointestinal parasitism [34]. The use of these parts of *Citrus* as feeds, either as supplements, whole meal or additives in drinking water of ruminants could be practicable means of reducing emission of greenhouse gases from ruminants based on the action of tannin on ruminant digestion.
In addition, *Citrus* could serve as source of natural tannin which could be used for a variety of industrial purposes. It can be utilized as additives in wine production to create a more astringent feel to the taste. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine [35]. Tannins are generally known as important ingredient in the tannery industry for tanning of leather. Condensed and hydrolysable tannins have been reported to be capable of binding to and precipitating the collagen proteins in animal hides [6]. This changes the hide into leather making it resistant to putrefaction. Oak bark, mimosa, chestnut and quebracho tree have traditionally been the primary source of tannery tannin, though inorganic tanning agents are also in use today and account for 90% of the world's leather production [36]. This indicated that natural tannin from *Citrus* species could be isolated and utilized as local source of raw material in leather industries in Nigeria. Most of these tannery industries in Nigeria are located in the Kano metropolis in Kano State. It could also be used as a local source of raw material for manufacture of adhesives. Report has it that tannin is a component in a type of industrial particle board adhesive developed jointly by the Tanzania Industrial Research and Development Organization and Forintek Labs Canada [37].

4. CONCLUSION AND RECOMMENDATIONS

All the parts of these *Citrus* plants contained tannin which has a variety of therapeutic properties, therefore, their use in ethnomedicine as drugs is recommended. The decoction of the root, stem and stem bark; and the infusion of the leaves and peels could be taken as herbal tea to serve as resistant against parasites and for treatment of digestive and respiratory systems, but *in vivo* animal and human studies are needed. Their extracts could also be regarded as potential raw materials in pharmaceutical industries for production of drugs. In addition, they can be used in treatment of diarrhoea and as antimicrobial agents in animals. The use of these *Citrus* species would yield huge benefits because plants - derived drugs are natural, easily available, less expensive, efficient, rarely have side effects and environmentally friendly.

Moreover, they could be regarded as rich and cheap sources of natural tannin which could be isolated and utilized in industries for a variety of applications. It could be used in tannin industry for manufacture of leather, cosmetic industries as antimicrobial agents, and as additives in wine production to improve the astringency of wine. These Tannery industries: Challawa, Great Northern, Hafawa Enterprise, Tannorth Limited and Unique Leather Finishing in Bompai, Sharanda and Challawa Industrial Estate, Kano State, Nigeria [38]; could exploit the natural tannin extracted from these *Citrus* species as readily available and affordable raw material.

In addition, the use of these parts of these *Citrus* species and the inclusion of their tannin extracts as additives in the feeds and drinking water of ruminants is recommended; because they could reduce the agricultural greenhouse gases emission from ruminants, thereby eliminating the hazardous effects these gases pose to the environment. Further research however, is needed to confirm these possibilities, especially to determine if cattle, goat and sheep would take water containing *Citrus* tannin.

COMPETING INTERESTS

Authors have declared that no competing interests exist.
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