Chapter 3

Exploitative Beneficial Effects of Citrus Fruits

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Additional information is available at the end of the chapter

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Abstract

Citrus fruits trees have come to gain a worldwide recognition for their suiting refreshing juice, nutritious value and numerous health benefits and maintenances. Their applied health therapeutic uses have been exploited in the treatment of several health challenges as antitumor, anti-inflammatory anticancer, antiviral, antimicrobial activities, against cardiovascular diseases and macular degeneration. Lime (*Citrus aurantifolia*) juice has been shown to effectively serve as hypolipidemic, possesses the ability to interact with orthodox medicines. Obviously, citrus fruits' abilities on the exploited benefits are not far from their rich bioactive compounds and phytochemical such as minerals, vitamins, flavonoids and carotenoids. These phytochemicals may act as antioxidants, boosting the action of protective enzymes in the liver, reverse lipid peroxidation of genetic material and improve immune system. A close look at this chapter includes introduction, history and description, structures and biochemistry of phytochemicals, metabolism of phytochemicals and bioactive compounds and beneficial effects of citrus fruits.

Keywords: citrus, phytochemical, antioxidants, orthodox medicine, health benefits

1. Introduction

Citrus fruits have a worldwide spread, grown across the globe and are well-appreciated for their refreshing juice and health benefits [1]. They are fruits bearing trees, which are members of the rutaceae family. Citrus fruits have five main species according to [2], which include *Citrus sinensis* (sweet orange fruits tree), which have about 70% in the majority of the citrus family, *Citrus aurantifolia* (the lime fruits tree), *Citrus reticulata* (the tangerine fruits tree) *Citrus limonum* (the lemon fruits tree) and *Citrus vitis* (the grape fruits tree).

1.1. History and description

1.1.1. History

The origin of citrus fruits is not very clear. The trees flourish well in tropical and subtropical climates. They were thought to originate in Southeast Asia. Arab traders brought lime trees back from their journey to Asia and introduced them into Egypt and Northern Africa around the tenth century [3]. Researchers assert that Mexico, Florida, Brazil and California in America are where we currently find the largest orange orchards in the world and citrus fruits were transported by the Spaniards [4]. Citrus fruit like many other fruits and vegetables, were reported to have been brought to the Americans by Christopher Columbus, when he made his second voyage in the sixteenth century to the New World in 1493, and have been since then grown in Florida [5]. Citrus was highly appreciated such that in 1849, there was a great demand of lemon that people were willing to pay up to $1 per lemon, a price that would still be considered costly today and was extremely expensive at that time [6]. George [6] reported that the introduction of limes to the United States began in the sixteenth century when Spanish Explorers brought the West Indies lime to the Florida Keys, beginning the advent of Key limes. However, in the following century, Spanish missionaries attempted to plant lime trees in California, but the climate did not support their growth. In great demand by the miners and explorers during the California Gold Rush as a fruit that was known to prevent scurvy, limes began to be imported from Tahiti and Mexico at this time in the mid-nineteenth century. Today, Brazil, Mexico and the United States are among the leading commercial producers of limes [7].

1.1.2. Description

Citrus fruit trees are greenish and of different sizes and height, according to the species. Lime fruit trees are small in height with multiple and spiny branches and smaller green leaves [8]. Citrus fruit trees produce fruits of various sizes forms and shapes such as oblong and round shapes. The fruit is covered or protected against damage by a rough bright green or yellow color epicarp. This epicarp is composed of glands which contain essential oils, responsible for the peculiar citrus fragrance. The epicarp also houses a white, thick and spongy mesocarp which together with the epicarp forms the pericarp or peel of the fruit. Inside the fruit is the cavity which is divided into separate segments or juice sacs containing seeds or without seeds for the seedless variety. The seed is covered by a thick radical film or endocarp [9]. This inner part is rich in soluble sugars, ascorbic acid, pectin, fibers, different organic acids and potassium salt that give the fruit its characteristic citrine flavor [9]. According to the working list of all plant species, citrus species hybridize easily and that new hybrids are continuously developed by cross pollination to obtain desired qualities such as seedless, juicy and fresh taste fruits [10].

According to [11, 12], the working list of all plant species, the taxonomy of citrus plants follow the order; **Kingdom**: Plantae; **Subkingdom**: Tracheobionta; **Superdivision**: Spermatophyta; **Division**: Magnoliophyta; **Class**: Magnoliopsida; **Subclass**: Rosidae; **Order**: Sapindales; **Family**: Rutaceae; **Genus**: *Citrus.* However, citrus species/types have numerous common names depending on the country and language. Ali [10] highlighted the following common names for some species;

Citrus aurantiifolia: **Arabic**: laimon helo; **Chinese**: lai meng; **English**: Egyptian lime, Indian lime, Key lime, lime, Mexican lime, sour lime, lime; **French**: citron vert, citronnier gallet, lime

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acid, limettier, limettier des Antilles, limettier mexicain; **German**: Limette, Limettenbaum, Limone, saure Limette; **India**: kagzi nimboo, kagzi nimbu; **Italian**: lima; **Portuguese**: limão-galego, limão-tahiti; **Spanish**: limón agrio, limón ceutí, lima, lima mejicana, limero [10–13]. However, in Nigeria, Lime fruits are locally identified as follows: in Idoma, it is called Alemu Ogwuchekwo; in Igbo, it is called Oloma-oyinbo; in Hausa, it is called Lemun tsami or Babban lemu; in Yoruba, it is called Osan ghanhin-ghanhin; and in Igala, it is called Alemu inale [13].

1.2. Structures and biochemistry of phytochemicals

Phytochemicals are the numerous chemicals present in plants. They are primarily produced by the plants to serve the purpose of defense to insects and microbial attack. They are thus, called plants secondary metabolites. The following are some citrus phytochemicals.

1.2.1. Citrus flavonoids

Flavonoids are a group of extensively large class of plant phytochemical of over 5000 hydroxylated polyphenol compounds, which abound in fruits, vegetables, legumes and tea [14]. Flavonoids are divided into a major subclass of 12 based on differences in chemical structures [15]. Flavonoids that are of dietary importance include flavones, flavonols, flavanones, anthocyanidins, flavan-3-ols and isoflavones [16]. Citrus has been identified to have the following class of flavonoids; flavonols, flavans, flavones, flavanones and anthocyanins. Anthocyanins are included as citrus flavonoids because it has been isolated in blood oranges [17].

Citrus flavonols, flavones and flavanones (**Figures 1–3**) abound largely. Most flavonoids exist in their glycosylated forms or aglynol and aglycone forms. Glycosylated forms of flavonoids (**Figures 4–6**) include, naringenin, maringin, rutin and hesperidin.

The glycosylated forms have been classified into two types, these are; the neohesperidosides and the rutinosides [18].

Neohesperidosides, naringin, neohesperidin and neoeriocitrin are said to have a bitter taste [19]. While rutinosides, hesperidin, narirutin and didymin, have been found to be tasteless and

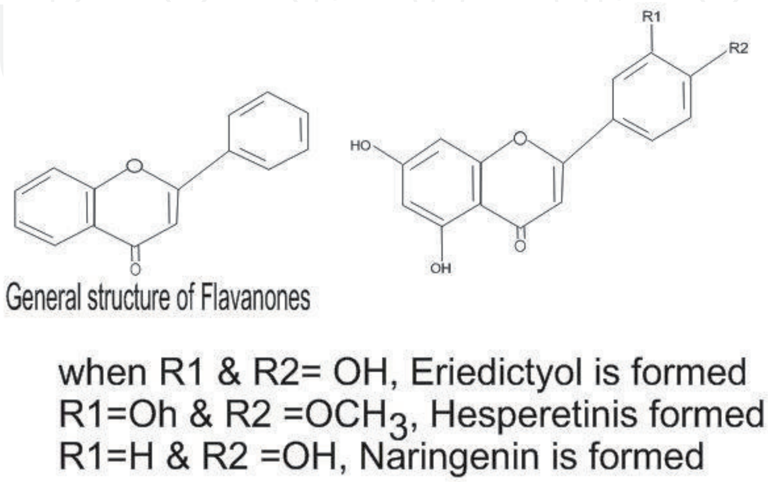

Figure 1. Structures of flavanones and derivatives.

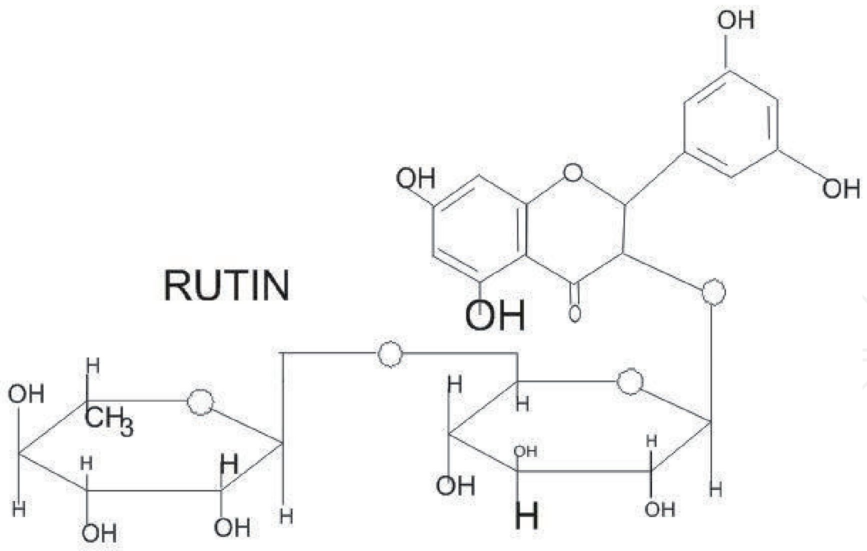

Figure 2. Structures of flavonols and derivatives.

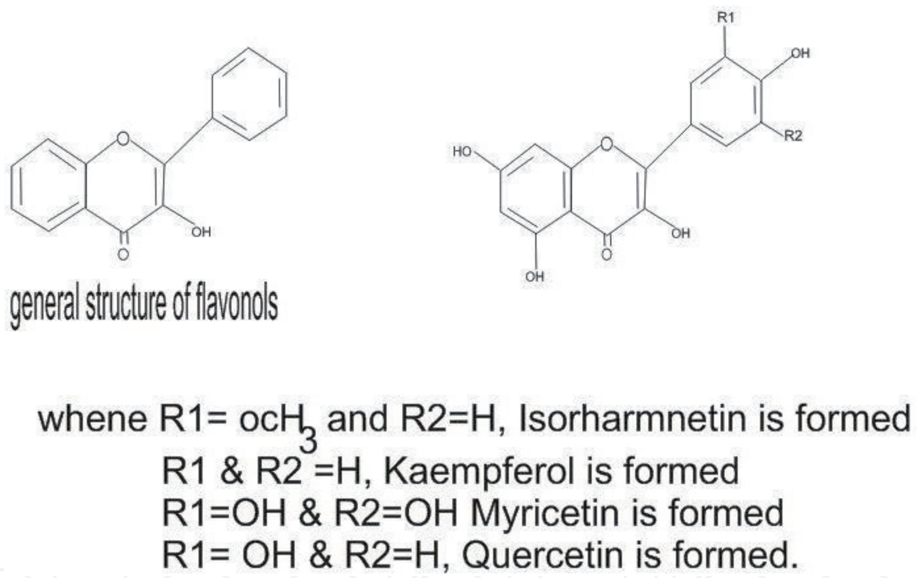

Figure 3. Structures of flavones and derivatives.

have a disaccharide residue e.g. rutinose (ramnosyl-a-1,6 glucose). Most flavanones are usually found in diglycoside forms, which confer the typical taste to Citrus fruits [19]. Using UV, IR, FABMS, 1H NMR, and 13C NMR analyses, [20] isolated two glyvones (C-glucosylflavones) from the peel of lemon fruit (*Citrus Limon* BURM. f.), and identified 6,8-di-C-b-glycosyldiosmin and 6-C-b-glycosyldiosmin. The compositions of the seed and peel of citrus fruits are not always the same. The lemon seed contains eriocitrin and hesperidin and the peel contains neoeriocitrin, naringin and neohesperidin [21]. The concentration of the glycosylated.

Flavanone in peel and seed varies. In peels, the concentrations of neoeriocitrin and naringin are similar while, in seed, the concentration of eriocitrin is reported to be 40 times higher than the concentration of naringin [22].

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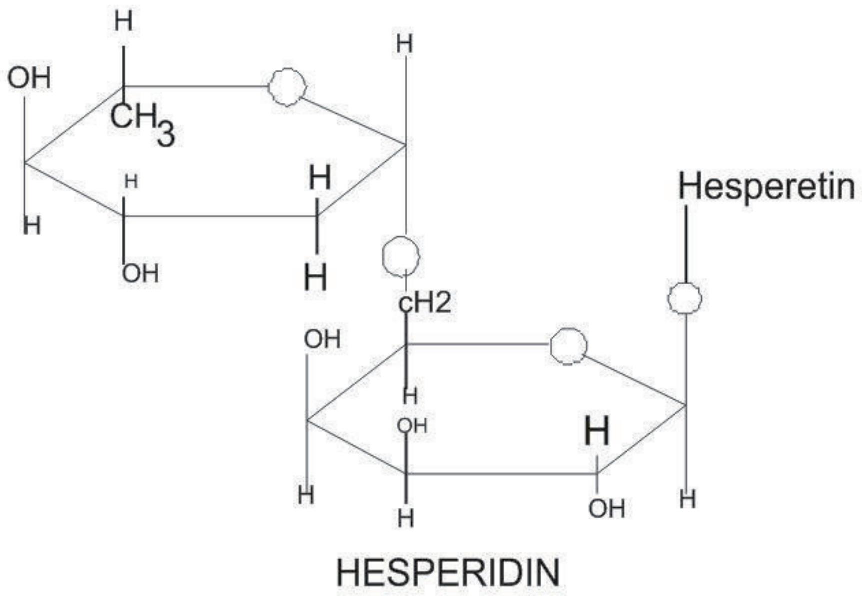

Figure 4. Structure of rutin, a glycosylated flavonoid.

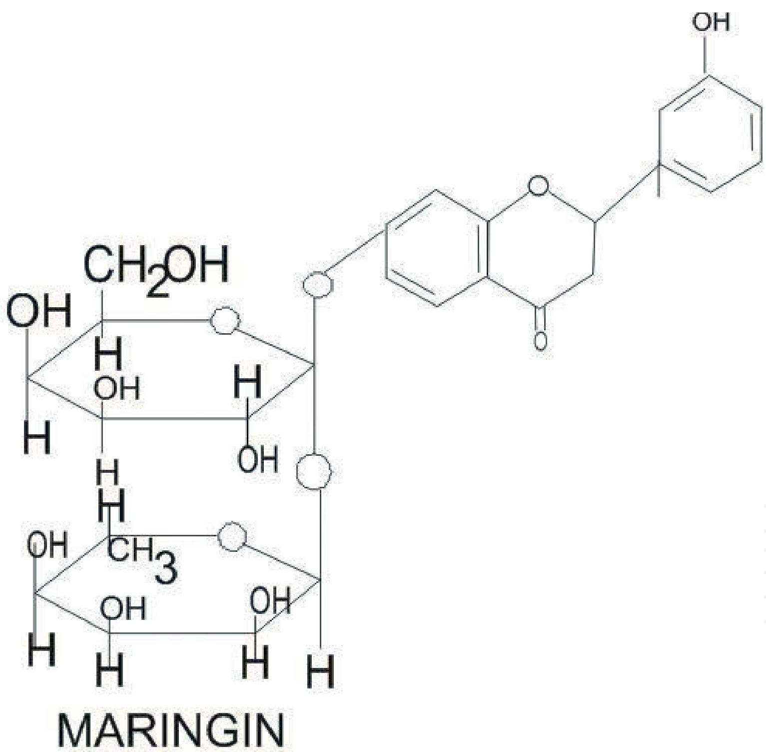

Figure 5. Structure of hesperidin, a glycosylated flavonoid.

Neohesperidin, naringin and neoeriocitrin are extracted from peel in great amounts [23]. It has been reported that bitter orange is a marvelous source of neohesperidin and naringin which are very significant in the industry for the production of sweeteners [19]. Generally, most citrus fruits are said to possess little quantity of glycosylated naringin [24].

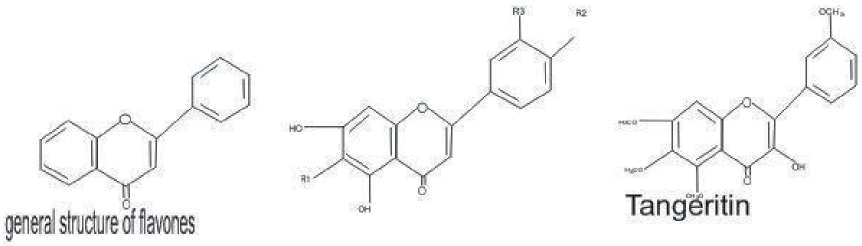

Figure 6. Structure of naringin, a glycosylated flavonoid.

It is reported that naringin is found in lemon peel and seed, in mandarin seed and absent in the juices [25]. Mouly et al. [26] found that glycosylated flavanones, responsible for bitterness, cannot be in sweet orange juice, thus their presence will mean the fruit is aldulterated or spoit.

1.2.2. Citrus carotenoids

It has been reported that pink grapefruit has a higher content of carotene than other citrus fruits such as tangerines and oranges, which contain high levels of other carotenoids, including lutein, zeaxanthin, cryptoxanthin that have significant anti-oxidant activity [27].

Carotenoids are hydrocarbon of the class of carotene and their oxygenated derivatives, the xanthophyls. Carotenoids are composed of eight isoprenoid units linked in a reversed isoprenoid units at the center of the molecule, making the two central methyl groups to have 1,5-position relationship, and are the pigments responsible for the colors of many plants [28]. **Figure 7** shows structures of some selected (including lutein, zeaxanthin, lineal, epoxy carotenoid, lycopene and β-carotene) carotenoids. There are more than 800 carotenoids and their derivatives identified and isolated and are divided into two main groups, called carotenes and xanthophylls. Carotenes are composed of hydrocarbon structure and xanthophylls that contain oxygen atoms in their structure [29]. The pink grapefruit also has been found to be very rich in the red pigment, lycopene, with a potent anti-tumor activity [30]. They serve as light harvesting complexes in photosynthesis [1]. Carotenoids (β-carotene and lycopene clarified in the carotenes) are known to be responsible for the orange-red colors found in orange, tomatoes and carrots fruits as well as the yellow colors of many flowers [31] and in xanthophylls, lutein in spinach and broccoli and β-cryptoxanthin in Satsuma mandarin are well-known [29]. Yokayama and White [32] reported that the flavedo of the fruit of the trigeneric hybrid, Sinton citrangequat contains new carotenoid ketones (apocarotenones) pigments that are unique in the carotenoid series in that they contain the terminal methyl ketone group in the side chain responsible for the rich red color of the flavedo. Carotenoids in plants are a very important component of photosynthesis and prevent disastrous photo oxidation [31]. They isolated and characterized these methyl ketone carotenoids with nonaeneone and decaeneone chromophores to include; sintaxanthin, citranaxanthin, 3-OH-sintaxanthin, reticulataxanthin

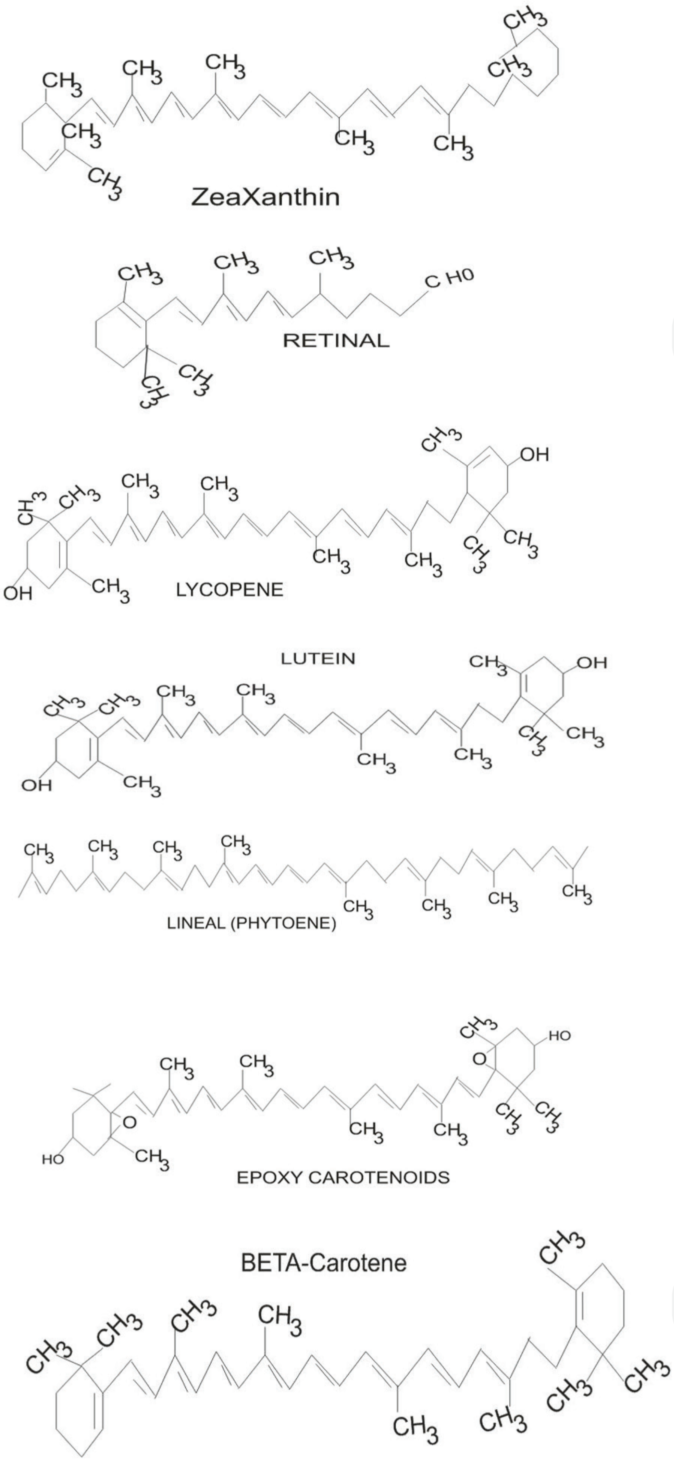

Figure 7. Structures of citrus carotenoids.

and an in-chain hydroxyl group 8′-OH-7′8′-dihydrocitranaxanthin methyl ketone carotenoid [32]. For the first time, other carbonyl carotenoids consisting of β-apo-10′-carotenal, β-apo-8′carotenal, β-citraurin, Neurosporene, γ-carotene, β-carotene and probably 3-OH-β-apo-10′-carotenal. β-Zeacarotene were detected and isolated from citrus in minor amounts [32].

1.2.3. Citrus limonoids

In citrus, there are more than 30 limonoids with limonin and nomilin being the most identified. Limonoids are compounds which have high concentration in grapefruit (*C. vitis*) and orange juice (*C. sinensis*), which are partly associated with the bitter taste in citrus [33]. Shin and Masaki [34] reported the potentials of some limonoids to include antifeedant activity against insects, suggesting that one of the biological functions of limonoids in plants is pest control and that citrus limonoids are unique for many species and varieties of citrus, which make them excellent taxonomic markers.

1.2.4. Citrus terpene

Citrus terpenes are clear, colorless and liquid cyclic hydrocarbons called monoterpenes or D-limonene and are produced as oil from the rind of citrus through the process of distillation [35]. Terpenes are not derived from isoprene rather they are found as isopentenyl pyrophosphate in nature. Isopentenyl pyrophosphate is derived in a series of complex metabolic reaction steps in the mevalonate pathway [36]. Limonene is a terpene, which get its name from lemons and is the main constituent extracted from the citrus fruit rind. Limonene preparations in the laboratory have been reported to employ Diels-Alder reaction, an addition reaction which involves the joining of two isoprene molecule without librating nothing [37]. Limonene is reported to be the source of citrus flavor and fragrance—whether in desserts as a food-grade chemical or in cleansers—or to produce other flavors and fragrances via the use of chemical reactions [37]. Limonene is a terpene which is relatively stable and can be distilled without decomposition. However, it is reported to crack at elevated temperatures to form isoprene [38]. Limonene reacts with sulfur by dehydrogenation to produce p-cymene and limonene oxide, carveol and carvone are formed as oxidation products when exposed to moist air to form carveol, carvone, and limonene oxide [39]. With sulfur, it undergoes dehydrogenation to p-cymene [40].

1.2.5. Citrus alkaloids

Ref. [41] developed a method for the analysis of adrenergic amines in tangerine juice. The method is sensitive, fast, simple and reproducible when using Cogent Diamond Hydride HPLC column and an Agilent MS TOF instrument. The alkaloids analyzed were tyramine, N-methyltyramine and synephrine (**Figure 8**).

1.3. Metabolism of phytochemicals and bioactive compounds

The retinoids (vitamin A and retinal), are a very important metabolites of carotenoids in mammals, including humans and monkeys [42, 43]. The report on the synthesis of vitamin A from β-carotene showed that it could be formed by central or eccentric cleavage of β-carotene [30].

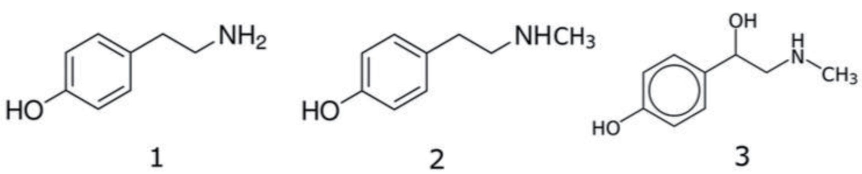

Figure 8. Structures found in citrus alkaloids (1 = tyramine; 2 = N-methyltyramine, 3 = synephrine).

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It was demonstrated that the enzyme 15–15[1] β-carotenoid dioxygenase in the intestine and liver, convert α-carotene, β-carotene and β-cryptoxanthin to vitamin A (retinal) [30]. However, [28] reported that such in vivo formation of retinal is homeastatically controlled, so that the conversion to retinol is limited in individuals with adequate vitamin A. Currently, there is an increase production of carotenoids by biotechnology due to its demand in industry, added as colorants to many manufactured food drinks, fruit juice and animal feeds either in the form of natural extracts or as pure compounds manufactured by chemical synthesis [30].

The importance of dietary citrus flavonoids becomes appreciated only when they are absorbed and become available to target tissues within the body. In the intestine and liver, absorption and metabolism of flavonoids is rapidly carried out. In the liver phase II reaction, flavonoids metabolized to intermediate metabolites and transported in the bloodstream and excreted as urine [44]. It appears that the biological activities of flavonoid metabolites are different from their parent compounds [45] and these metabolites (xenobiotics) must first be modified in the mucosa of intenstine and then in the liver [46]. Enzymatic transformation of flavonoids by the gut microbial enzymes of the large intestine is done through deglycosylation, ring fission, dehydroxylation, demethylation into metabolites that can then be absorbed or excreted [46, 47]. Different metabolites are produced after transformation but production depends on the diverse activity of the colon bacteria, resulting from an individual's dietary intake of flavonoids rich diet [47, 48]. The bioavailability of flavonoids in the system increases the beneficial exploits of the nutrients which in turn depend on the composition of the colon bacteria [49]. When polyphenols are administered orally, only small quantities of these compounds appear in systemic circulation because of very high levels of uridine diphospho (UDP)-glucuronosyltransferases and sulfotransferases in the small intestine and liver, thus resulting in very low oral bioavailability [50]. Quercetin was originally assumed to be absorbed from the small intestine following cleavage of the β-glucoside linkage by colonic microflora [51].

1.3.1. Metabolism of minerals and vitamins

Metabolism is a dual process involving catabolism (breaking down or oxidation) and anabolism (biosynthesis). It a biochemical process which makes energy available to an organism following the conversion of ingested food. Catabolism involves hydrolyses, digestion, absorption and excretion of ingested food. Most vitamins and mineral are absorbed by the intestinal cells of the body. Magnesium is absorbed by the intestinal cells through a specific carrier system; zinc is absorbed mainly in the duodenum, dietary Mn is normally absorbed in the small intestine, however, iron inhibits the absorption of Mn. Metallothionein is the transport protein that facilitates copper absorption mainly in the duodenum. Iron in the ferous form is soluble and readily absorbed in the stomach and duodenum [52]. About 90% of K^+ is absorbed from the gastrointestinal tract. Sodium is readily absorbed in the gastrointestinal tract. Phosphate absorption takes place at the jejunum. However, calcitriol promotes phosphate uptake along with calcium. By an energy dependent active process, calcium is mostly absorbed in the duodenum [53].

1.3.2. Biosynthesis of phytochemical in citrus plant

Generally, the biosynthesis of phytochemicals in citrus and other plants has been reported to be organ, cell or development specific in almost all higher plant species [54]. The pathways, and genes involved in their synthesis are most tightly regulated and may be linked to environmental, seasonal or external triggers. Cellular sites of synthesis are compartmentalized in the plant cell, with the majority of pathways being at least partially active in the cytoplasm [54]. There are evidences that compounds such as alkaloids, quinolizidines, caffeine and some terpenes are synthesized in the chloroplast [55–57]. Most often, phytochemicals are detected throughout the plant, however, they are initially synthesized in single organ such as roots, fruits or leaves and later transported by the phloem or xylem tissues around the plant or via symplastic or apoplastic transport and stored in a number of different tissues [54]. The site of storage often depends on the polarity of the compounds, with hydrophilic compounds such as alkaloids, glucosinolates and tannins being stored in vacuoles or idioblasts, while lipophilic compounds such as the terpene-based essential oils are stored in trichomes, glandular hairs, resin ducts, thylakoid membranes or on the cuticle [56]. The storage of some compounds such as alkaloids, flavonoids, cyanogenic glycosides, coumarins that are present in the plant and serve defense purpose are in the epidermis [56–60]. Shin and Masaki [34] reported that nomilin, a limonoid is biosynthesized from acetate through the terpenoid biosynthetic pathway in the phloem region of stems and then transported to the leaves, fruit tissues, peels, and seeds where it is further metabolized to other limonoids. The citrus limonoid aglycones are then glucosidated by limonoid UDP-ᴅ-glucose transferase in maturing fruit tissues and seeds. These limonoid glucosides are accumulated in such high concentrations that they are one of major secondary metabolites in citrus fruit tissues [34].

The enzymatic biosynthesis of terpenes is understood to be divided into four enzyme catalyzed steps to include; (1) biosynthesis of two precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) [36]. This is done through two different pathways; (2) repetitive addition of the precursors to form a series of homologs of prenyl diphosphate, which are the immediate precursors of the different classes of terpenes; (3) enlargement of the terpenes backbones by the activity of specific synthases and (4) secondary enzymatic modification of these backbones resulting in the functional properties and family diversity [61]. The two pathways involved in the synthesis of IPP and DMAPP are called the acetate-mevalonate pathway, located in the cytoplasm and non-mevalonate pathway located in the plastids of the cell [36, 61]. The acetate-mevalonate pathway is reported to be involved in the synthesis of sesquiterpenes and sterols while the non-mevalonate pathway is responsible for the synthesis of monoterpenes, diterpenes, tetraterpenes and polyterpenes [35]. Marco et al. [35] also reported that terpene synthases enzymes need geranyl diphosphate, farnesyl diphosphate and geranylgeranyl diphosphate as substrates for the production of different terpenes. Monoterpenes are derived from geranyl diphosphate, sesquiterpenes from farnesyl diphosphate, and diterpenes from geranylgeranyl diphosphate by the action of terpene synthases or cyclases [35]. Biosynthesis of limonene involves a cyclization of a neryl carbon from geranyl pyrophosphate [62].

Masaya [29] reviewed and reported the biochemical pathway of the first committed step in carotenoids biosynthesis in plant as demonstrated by [63, 64] to involve a head-to-head condensation of two molecules of a 20 caborn molecule of geranylgeranyl pyrophosphate (GGPP) to form a colorless 40 carbon molecule of phytoene catalyzed by phytoene synthase (PSY). Carotenoid biosynthesis and its regulation have been studied in tomato fruit during fruit ripening and development [65]. Carotenoid concentration and composition are influenced by growing conditions and fruit maturity. They also differ among geographical origins [66]. Kato et al. [66] reported their investigation on the relationship between carotenoid accumulation and the gene responsible for the expression of carotenoid biosynthetic during fruit maturation in three citrus varieties, Satsuma mandarin (*Citrus unshiu* Marc.), Valencia orange (*Citrus sinensis* Osbeck), and Lisbon lemon (*Citrus limon* Burm.f.). After successful cloning of the genes, in the flavedo of Lisbon lemon and Satsuma mandarin, massive accumulation of phytoene was observed with a decrease in the transcript level for *CitPDS* and concluded that the carotenoid accumulation during citrus fruit maturation was highly regulated by the coordination of the expression among carotenoid biosynthetic genes [66]. Massive accumulations of carotenoids have been shown to occurred concomitantly with the degradation of chlorophyll during citrus fruit development in mandarin varieties, such as Satsuma mandarin [67]. β-Cryptoxanthin accumulated predominantly in the flavedo and juice sacs in mature fruit [68].

1.4. Beneficial effects of citrus fruits

1.4.1. Citrus phytochemicals and bioactive compounds in disease prevention

The anti-oxidant activities of carotenoids are said to be associated with a lower incidence of age-related macular degeneration, which happen to be the leading cause of blindness in human after the age 65 [28]. The role of citrus carotenoids in disease prevention and in human health management cannot be over emphasized. Carotene plays an essential role as sources of vitamin A. The most active role is protection against serious disorders such as cancer, heart diseases and degenerative eye diseases. An inference can be deduced from an epidemiological data provided that diets which are rich in carotenoids containing fruits are associated with pronounced decreased risks for a variety of degenerative diseases [31]. Similarly reports from several epidemiological studies have shown decrease in cataract onset with high blood content of carotenoids [69]. It was reported that the combination of vitamin C and β-cryptoxanthin intakes might provide benefit to bone health in post-menopausal Japanese female subjects [70]. The stimulating ability of limonoids on the enzyme glutathione S-transferase (GST) to inhibit tumor is reported [33]. Glutathione S-transferase is a detoxifying enzyme that catalyzes the reaction of glutathione with dangerous electrophiles to form less toxic and more importantly water soluble compounds that can be easily excreted from the body [71]. Craig and Okwu [33, 72] reported that orange and lemon oil contain substantial amounts of GST that also possesses anti-cancer activity. The potentials of citrus pulp and the albedo (the white of the orange) are extensively being studied to be rich in glucarates and in preventing breast cancer and to lower the risk and symptoms of premenstrual syndrome [33]. Flavonoids have reported to poccess strong inherent ability to modify the body's reaction to allergens, viruses and carcinogens as they have demonstrated effective anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity [73].

1.4.2. Domestic and industrial benefits of citrus

D-Limonene has been reported to be used as botanical insecticide [74] and in the production of the organic herbicide "Avenger" [75]. It is an important additive to cleaning products in the preparation of hand cleansers to give a lemon-orange fragrance. The ability of citrus oil byproduct of orange juice manufacture produced from a renewable source as an organic solvent in dissolving oils is also known, as it has been used for the removal of oil from machine parts. Limonene is reported to be used as a paint stripper, as a constituent of some paints and used as an alternative fragrance to turpentine [75]. Limonene uses as a solvent in some model airplane glues and commercial air fresheners is documented. It was also shown that Philatelists used air propellants containing limonene to remove self-adhesive postage stamp from envelope paper [75].

1.4.3. Antimicrobial potentials of citrus

D-limonene has been reported to be used by researchers in preparing tissues for histopathological analysis as a less toxic substitute for the chemical, xylene when clearing dehydrated specimens [76, 77]. The often used clearing agents are liquids which are miscible with alcohols such as ethanol or isopropanol and with melted paraffin wax, in which specimens are embedded to facilitate cutting of thin sections for microscopy [78]. The use of ᴅ-limonene in traditional medicine has been reported to manage heartburn, gallstones and gastroesophageal reflux disease. However, high quality and robust clinical research is yet to support such claim [79]. The antibiotic effects of citrus have been shown from a research carried out in several villages in West Africa where cholera epidemics had occurred, the inclusion of lime juice during the main meal of the day showed that lime juice has protective effects against the contraction of cholera. This gave birth to the use of lime juice as a sauce eaten with rice and was also found to have a strong protective effect against cholera [80, 81]. The antibacterial and antioxidant potential of the essential oil of *Citrus aurantifolia* leaf was reported by [82]. Results shows the essential oil in *Citrus aurantifolia* leaf exhibited pronounced activity against Gram-positive and Gram-negative bacteria and their activity was quite comparable with the standard antibiotics such as tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin screened under similar conditions [82]. Another antibacterial study in Malaysia, involving five different Malaysian citrus varieties using *Citrus aurantifolia*, *Citrus reticulata*, *Citrus microcarpa*, *Citrus limon* and *Citrus sinensis* against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was carried out by [83] to evaluate the antibacterial potentials of these five species. Results showed that the methanol extract of the five varieties of citrus exerted no inhibition at 5 and 10 mg/ml. The methanol extract of *Citrus microcarpa*, *Citrus reticulata* and *Citrus sinensis* at 20 mg/ml showed better inhibition compare to *Citrus aurantifolia* and *Citrus limon* against *Staphylococcus aureus* and *Escherichia coli* [83].

1.4.4. Citrus effects on immunity

Owing to the antioxidant ability citrus vitamin C, its function in boosting a strong immune system has been reported [84]. The potentials of lemon, *Citrus medica* L. (citrus), and *Cydonia oblonga* as immunomodulators and antiallergic substances were investigated on an in vitro human mast cells, IL-8 and TNF-α with results which showed reduction of degranulation of basophil cells and inhibition of IL-8 and TNF-α of human mast cells [85].

1.4.5. Anti-rheumatoid arthritis and cardiovascular effects of citrus

A human study documented in the Annals of the Rheumatic with more than 20,000 subjects showed that subject who maintained high consumption of citrus food rich in vitamin C, had protection against inflammatory polyarthritis, a form of rheumatoid arthritis involving two or more joints. Subjects who consumed the lowest amounts of vitamin C-rich foods were more than three times more likely to develop arthritis than those who consumed the highest amounts [86].

An experimental study of the effects of *Citrus aurantifolia* on cardiovascular parameters was carried out on Spargue Dawely rats by checking the anti-hypertensive effect on three experimental hypertensive models. The models include cadmium induced hypertensive model, glucose induced hypertensive model, Egg feed diet induced hypertensive model, and normotensive model. Result obtained after 0.75 mg oral administration of *Citrus aurantifolia* methanol extract revealed a significantly ($p < 0.01$) reduced blood pressure parameters of the test groups compared to control groups [87]. The diastolic blood pressure of healthy middle-aged, normal-weight men was reported to be reduced after consuming orange juice for 4 weeks [88]. A study on the protective effect of the ethanolic extracts of Otroj, *Citrus medica* (EEOT) against isoproterenol (ISO)-induced cardiotoxicity was evaluated in rats. Results obtained from histopathological examination and myocardial biochemical assay demonstrated cardioprotective potential of EEOT [89]. It was reported recently that citrus fruits offer protection against cardiovascular diseases by reducing levels of homocysteine [90]. Homocysteine is a toxic agent for the vascular wall and, when plasma levels rise above normal, there is an increased risk of cardiovascular disease. It was reported that a low dietary intake of citrus folate contributes to the decrease of plasma folate and the raising of plasma homocysteine levels [91]. A recent study from the juice of freshly squeezed oranges, with high intakes of vitamin C (500 mg/day) showed that a rise in the levels of oxidized LDL was prevented, even in the presence of a high-saturated fat diet [92].

1.4.6. Anticancer potentials of citrus

In a laboratory test carried on human cells and animal studies, limonoids from different species and category of citrus fruits, including lemons and limes, have been reported to posses' anticancer ability against cancers of the mouth, skin, lung, breast, stomach and colon [3]. Do-Hoon et al. [93] reported that the numerous phytochemical contents in citrus including terpernoids, alkaloids, flavonoids, limonoids, and coumarins are found to be associated with a reduced risk of gastric cancer, breast cancer, lung tumorigenesis, colonic tumorigenesis, hepatocarcinogenesis, and hematopoietic malignancies [94, 95]. The flavedo extract of Ougan (*Citrus reticulata cv. Suavissima*) was found to exhibit potential anti-tumor effects by its inhibitory effect on epithelial-to-mesenchymal transition and interfering with the canonical TGF-β1-SMAD-Snail/Slug axis [96]. Purified bioactive compounds isolated from seeds and peels of *Citrus aurantifolia* have been reported to have inhibiting and suppressing effects on pancreatic cancer and colon cancer cells respectively [97]. *Citrus aurantifolia* potentials as anticancer were reported to be due to apoptosis-mediated proliferation inhibition of human colon cancer cells by volatile principles [98]. Human colon cancer has been reported to have 78% inhibition and induction of apoptosis confirmed by isolated volatile oil of *Citrus aurantifolia* fruit [99]. Effects of volatile oils from fresh *Citrus limon* fruit peels have been shown to possess a genotoxic effects on human lymphocytes by measurement of mitotic and blast indexes [100].

1.4.7. Citrus hypoglycemia and antidiabetic effect

The hypoglycemic potential of citrus flavonoids including hesperidin, naringin, neohesperidin, and nobiletin, were reported to significantly inhibit amylase-catalyzed starch digestion, where naringin and neohesperidin specifically inhibited amylose digestion, hesperidin and nobiletin inhibited both amylose and amylopectin digestion. Results showed the potential of citrus flavonoids in preventing the progression of hyperglycemia, partly by binding to starch, increasing hepatic glycolysis and the glycogen concentration, and lowering hepatic gluconeogenesis [101]. Also, the dietary hesperidin, was reported to have exhibited antidiabetic activities, partly by lowering hepatic gluconeogenesis or improving insulin sensitivity in diabetic animals [102]. Annadurai et al. [103] demonstrated in a study the antihyperglycemic and antioxidant effects of a flavanone, naringenin, in streptozotocin-nicotinamide-induced experimental diabetic rats and showed that naringenin conferred protection against experimental diabetes through its antihyperglycemic and anti-oxidant properties in streptozotocin-nicotinamide-induced diabetic rats. In another study, it was shown that in vivo chronic treatment of diabetic rats with naringenin could prevent the functional changes in vascular reactivity in diabetic rats through a NO-dependent and prostaglandin-independent pathway [104]. Another study evaluated the antihyperglycemic activity of *Citrus limetta* fruit peel in streptozotocin-induced diabetic rats and the results showed that hexane extract exerted significant hypoglycemic activity and the activity of extract was comparable to that of standard drug [105].

1.4.8. Citrus effect on body weight

Asnaashari et al. [106] investigated and reported that essential oil from *Citrus aurantifolia* prevents ketotifen (an antihistaminic drug that causes weight-gain) induced weight-gain in mice. Groups treated with *Citrus aurantifolia* essential oil showed decrease in body weight and food consumption, possibly through promoting anorexia which might have played a role in weight loss. The results reveal the potential of *Citrus aurantifolia* essential oil in weight loss and could be useful in treatment of drug-induced obesity and related diseases. Similarly, the effect of *Citrus aurantifolia* (fresh lime fruit juice) and honey on lipid profile fed different concentrations of cholesterol enriched diet, using rat model were investigated. During the experiment, groups were administered with lime alone, honey alone and mixture of lime and honey. Administration of lime alone resulted in significant decrease ($p < 0.05$) of LDL, TAG and TC and a significant increase ($p < 0.05$) in HDL and a corresponding weight loss compared to other groups [13].

1.4.9. Citrus effect on hypolipidemia

The effects of Lime Juice and Honey on Lipid Profile of Cholesterol Enriched Diet Fed Rat Model were investigated by [13]. The research investigated the effects of lime juice and honey on lipid profile of albino Wistar rats fed varying concentrations of cholesterol enriched diet. Results obtained showed that fresh undiluted lime juice, honey and mixture of lime juice and honey possess anti-inflammatory ability in preventing hypercholesterolemia, with effect greater in administration of fresh lime juice alone than in mixture of lime juice and honey. Another study using *Citrus medica* cv Diamante peel extract, showed a lowered plasma cholesterol and triglycerides in mice [107]. Demonty et al. [108] reported that tangeretin and nobiletin, with the optimal molecular structure, may lower blood cholesterol and triacylglycerol concentrations, whereas other citrus flavonoids without a fully methoxylated A-ring such as hesperidin and naringin may have virtually no or only weak lipid-lowering effects in humans. The effect of *Citrus aurantifolia* peel essential oil was studied on serum triglyceride and cholesterols in thirty Wistar rats of five groups. The results of experimental groups treated with peel essential oil in 50 and 100 μl/kg doses demonstrated a significant reduction in triglyceride, cholesterol, and LDL ($p < 0.01$) [109]. In a study of a high-fat fed $Ldlr^{-/-}$ mice, the addition of nobiletin resulted in a dramatic reduction in both hepatic and intestinal triacylglycerol accumulation, attenuation of very low-density lipoprotein(LDL)-triacylglycerol secretion and normalization of insulin sensitivity [110].

1.5. Citrus mineral, nutrients and vitamin contents

Citrus is loaded with appreciable mineral, nutrients and vitamins, especially the antioxidant vitamins contents. The nutritional content of carbohydrate, protein and fats in citrus fruits were reported to varied from 4.60 to 8.50, 5.80 to 7.90 and 2.50 to 9.50 g, respectively [111]. Katrine [112] reported some mineral value of citrus fruits as follows; calcium in citrus fruits ranges between 20 and 30 mg calcium/100 g and the iron content of citrus ranges from 0.2 to 0.4 mg/100 g. Obviously, citrus is generally not a good source of iron, however, iron is concomitantly released from other source of food owing to the high level of vitamin C content in citrus and citrus juices and therefore maintaining iron status [113, 114]. Consumption of orange juice or citrus foods with iron containing foods has been recommended by nutritionists for optimum iron absorption [115]. Low iron status has been reported to be one of the major deficiency challenges in Australia, particularly for adolescent girls and young women [112]. Citrus was reported to have a magnesium value of ranges between 8 and 11 mg/100 g, phosphorus value from 16 to 24 mg/100 g, a very low sodium content between 0 and 2 mg/100 g, a very low zinc content ranging from 0.1 to 0.2 g/100 g in citrus, copper value of citrus also very low to be between 0.03 and 0.05 mg/100 g, manganese value in citrus to be 0.01–0.03 mg/100 g, the content of the antioxidant element, selenium, ranges from 0.4 to 1.4 mg/100 g in citrus and the value of potassium in citrus fruits ranges between 120 and 145 mg/100 g potassium [116]. It is reported that fruits currently provide about 10% of potassium in the Australian diet daily [112].

The nutrients and non-nutrients contents of citrus fruits and juices products are wide spread. An assessment carried out in Australia by [112] on the composition of oranges, lemons, mandarins and grapefruit in relation to other common fruits and the composition of orange juice in comparison to soft drinks and sports drinks shows that the carbohydrate (sugar) content of citrus fruits ranges from 1.8 g/100 g for lemons to 4.8 g/100 g for grapefruit and about 8 g/100 g for oranges and mandarin. The values of carbohydrate in citrus and many other fruits assessed show a low glycemic index [117]. Protein content of citrus fruits ranges from 0.6 g/100 g for lemon to about 1 g/100 g for other citrus and generally, protein is low for all fruits assessed, ranging from 0.3 to 1.7 g /100 g [118]. While citrus fruits assessed for dietary fiber, ranged from 0.6 g/100 g (grapefruit) to 2.5 g/100 g (lemons) [112].

Assessment of citrus fruits vitamins reveals that citrus fruits have vitamin A value from 2 to 20 μg and vitamin A retinol equivalents of 10–130 μg betacarotene [112]. Citrus fruits vitamin C content ranges from 36 to 52 mg/100 g. Essentially, fruits are not known to be a rich source of vitamin E, a fat-soluble vitamin. However, the US data base, states that the vitamin E content of citrus is about 0.25 mg/100 g. Fruits are generally not a major contributor to the B vitamins, other than folate [118]. For vitamin B, citrus fruits content of thiamin range from 0.03 to 0.11 mg thiamin/100 g, riboflavin content in citrus is between 0.02 and 0.03 mg/100 g, niacin content in citrus ranges from 0.3 to 0.6 mg, vitamin B6 values in citrus was assessed to be between 0.04 and 0.08 mg and citrus seem to be a rich source of folate with the value ranging from 11 mg/100 g in lemons to 30 mg/100 g in oranges [112, 118]. Folate anticancer and protective effects against heart disease and spinal tube defects and its role in maintaining mental function have been reported [119, 120]. It was reported that a glass of orange juice of 225 ml provides about 75 mcg of folic acid [121].

2. Conclusions

Numerous therapeutic properties have been attributed to citrus fruits, like anticancer, antiviral, anti-tumor, anti-inflammatory activities, and effects on capillary fragility as well as an ability to inhabit platelet aggregation. It is therefore established that the exploitative benefits of this plant are not unconnected to the active biochemical substances present in the plant in abundance. These bioactive substances (vitamins, phytochemicals, minerals and other nutrients) may act as antioxidants, which stimulate the immune systems; induce protective enzymes in the liver or block the damage of the genetic materials. From the review, it may be concluded that fresh citrus fruits juice offer better advantage thus, the best way to exploit citrus, especially the fruits parts is using it freshly **Table 1**.

	Orange	Grapefruit	Tangerine
Weight (g)	131	236	84
Energy (kcal)	62	78	37
Fiber content (g)	3.1	2.5	1.7
Ascorbic acid (mg)	70	79	26
Folate (mcg)	40	24	17
Potassium (mg)	237	350	132

Source: [122].

Table 1. Nutritional facts about citrus fruit.

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