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Carotenoids, Phenolics, Hydroxycinnamic Acids and Tannin Composition of *Salacia senegalensis* (Lam) DC Leaves

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Abstract

Salacia senegalensis leaves with established antimalarials activities were assayed (dry weight) for its carotenoids, phenolics, hydroxycinnamic acids and tannin composition using gas chromatography analysis. The results showed the presence of carotenoids, phenolic acids, hydroxycinnamic acids and tannic acids. Ten known carotenoids were detected, mainly lutein (6.4370100 mg/100 g), carotene (4.0964900 mg/100 g), viola-xanthin (3.3858800 mg/100 g), zeaxanthin (2.8979100 mg/100 g), asta-xanthin (1.3826700 mg/100 g), beta-cryptoxanthin (0.3527960 mg/100 g), neo-xanthin (0.3501600 mg/100 g), malvidin (0.3150850 mg/100 g), anthera-xanthin (0.0063927 mg/100 g) and lycopene (0.0010883 mg/100 g). The phenolic acid constituents found were ferulic acid (0.1370260 mg/100 g), vanillic acid (0.1168070 mg/100 g), ellagic acid (0.0049325 mg/100 g), piperic acid (0.0015819 mg/100 g), syringic acid (0.0005970 mg/100 g) and rosmarinio acid (0.0005967 mg/100 g). Hydroxycinnamic acids detected were caffeic acid (3.3525900 mg/100 g), p-coumaric acid (2.2189400 mg/100 g), o-coumaric acid (0.0042574 mg/100 g), coumarin (0.0027936 mg/100 g), cinnamic acid (0.0012745 mg/100 g), and sinapinic acid (0.0003049 mg/100 g). While tannic acid (162.86 mg/100 g) was, the only tannin found in the leaves of *Salacia senegalensis*.

Keywords: *Salacia senegalensis*; Carotenoid; Phenolic; Hydroxycinnamic; Dry weight

Introduction

Carotenoids are very popular family of terpenoids that have been widely accepted as safe chemicals for food supplementation and neutraceutical purposes due to their intense colouring abilities, role as precursors of vitamin A, and antioxidant activity in animals [1]. They act as photoprotective agents in a dose-dependent manner, and may reduce the risk of sunburns, photoallergy and even some types of skin cancer [1,2]. Epidemiologic studies indicate that an increased intake of carotenoids is associated with a decreased risk of many types of cancer including lung, breast and those affecting the gastrointestinal tract [3,4] a decreased risk of cardiovascular disease [4,5] less incidences of age-related macular degeneration and reduction in xeropthalmia in areas with low preformed vitamin A intake [4,5].

Natural β-carotene is the precursor of vitamin A and has preventive action against eye diseases and cancer. Carotenes enhance immune response, protect skin cells against UV radiations, lower the risk of cardiovascular diseases, age related vision disorders, asthma and reduce inflammation [4,6,7].

Lycopene, a non-provitamin A carotenoid and potent antioxidant, gives tomatoes their red colour and is effective at quenching the destructive singlet oxygen. Along with carotene and lutein, it protects against lung, breast, uterus and prostate cancers [4]. It produces significant reduction in blood pressure, serum lipids, and oxidative stress markers [8-10]. Xanthophyll's, like lutein and zeaxanthin function as protective antioxidants for the retinal part of human eye [4].

Phenolics are large family of plant secondary metabolites comprising many biologically active compounds. Depending on the number of phenol subunits, phenolics can be places into two basic groups – simple phenols and polyphenols [11]. The group of simple phenols includes the so-called "phenolic acids", including hydroxybenzoic and hydroxycinnamic acid derivatives. Phenolics protect plants against environmental and biological stress like higher energy radiation exposure, bacterial infection or fungal attacks [12], cold stress, hyperthermia [13] and oxidative stress [2]. They are also important for cell structure, signaling and pigmentation [13]. Some phenolic acids and flavonoids are allelochemicals [14].

The most representative cinnamic acid is acid, which occurs in fruit, vegetable and coffee, mainly as an ester with quinic and (chlorogenic- or 5-caffeoylquinic acid) [15,16]. Others include p-coumaric (4-hydroxycinnamic) and ferulic (4-hydroxy-3-methoxycinnamic) acids [17]. Hydroxycinnamic acid derivatives have a wide array of biological and pharmacological activities including antioxidative, antiviral and ant-listerial activities [18,19]. Their antioxidant properties can be expressed in several ways. For instance, 1, 5-dicaffeolquinic acid is an hepatoprotector when challenged by carbon tetrachloride, a mechanism that involves, among others, radical scavenging [16]. Chlorogenic acid is a powerful antioxidant, cholegogue and hypoglycemic agent [18,19].

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. The designation of tannin includes compounds of two distinct chemical groups: hydrolysable tannins (polymers of ellagic acid, or of gallic and ellagic acids, with glucose) [16] and condensed tannins, which result from the condensation of monomers of flavan-3-o1 units [16,20]. Given their relationship to phenolic acids and flavonoids, their antioxidant properties are not a surprise: they exert their antioxidant activity by scavenging free radicals, chelating trace metals and by binding proteins with suppression of their enzymatic activity [16]. Recent works [16,21,22] have described the capacity of tannins to enhance glucose uptake and inhibit adipogenesis, thus being potential drugs for the treatment of non-insulin dependent diabetes mellitus. Flavan-3-ols are thought to interfere in the pathogenesis of cardiovascular disease via several mechanisms: antioxidative, anti-thrombogenic, and

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anti-inflammatory. In particular, proanthocyanidins and flavan-3-o1 monomers lower plasma cholesterol levels, inhibit LDL oxidation, and activate endothelial nitric oxide synthase to prevent platelet adhesion and aggregation that contribute to blood clot formation [16,23,24]. Tannins also exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level and modulate immunoresponses [25]. They are constituents of several drugs because of their astringent property. They may be employed medicinally in anti-diarrhoeal, haemostatic, and anti-haemorrhoidal compounds. Their anti-inflammatory effect helps control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders [2,26].

The anti-carcinogenic and anti-mutagenic potentials of tannins may be due to their antioxidative property, which is important in preventing cellular oxidative damage, including lipid peroxidation [2,25]. Tannins have antimicrobial activities. They inhibit the growth of many fungi, yeast, bacteria and viruses [2]. Tannic acid and propyl gallate, but no gallic acid, inhibits food borne and aquatic bacteria, and off-flavour-producing microorganisms. This antimicrobial property is due to the hydrolysis of ester linkage between gallic acid and polyols, cleaved after ripening of many edible fruits. Tannins in these fruits thus serve as a natural defense mechanism against microbial infections. The antimicrobial property of tannic acid can also be used in food processing to increase the shelf-life of foods [2,25,26].

Salacia senegalensis leaves have been reported to possess antimalarials activities [27,28] but its carotenoids, phenolic, hydroxycinnamic acids and tannin composition is yet to be reported. Therefore, the aim of this work is to assess the carotenoids, phenolic, hydroxycinnamic acids and tannin contents of the leaves of *Salacia senegalensis* (dry weight) using gas chromatography.

Materials and Methods

Determination of carotenoids composition

They were extracted by the method of Rodriguez-Amaya and Kimura [29] and gas chromatographically analysed.

Phenolic acids determination

The phenolic extract was obtained by the method of Li et al. [30]. The extract methylated with BF_3: MeOH (10). GC-FID analysis was performed to determine the phenolic acid contents.

Determination of the concentration of hydroxycinnamic acids

The extraction was carried according to method of Ortan et al. [31] and analyzed using gas chromatography.

Determination of the concentration of tannins

Extraction was carried out according to the method of Luthar [32] and the dry sample was subjected to gas chromatographic analysis.

Results

Ten known carotenoids were detected as shown in Table 1, mainly lutein (33.48%), carotene (21.31%), viola-xanthin (17.61%), zeaxanthin (15.0%), asta-xanthin (7.19%), beta-cryptoxanthin (1.84%), malvidin (1.64%), anthera-xanthin (0.03%) and lycopene (0.01%). The phenolic acid constituents of *Salacia senegalensis* leaves as shown Table 2 included ferulic acid (52.39%), vanillic acid (44.66%), ellagic acid (1.89%), piperic acid (0.61%), syringic acid (0.23%) and rosmarinio acid (0.23%).

Eight hydroxycinnamic acid were detected which totaling to 5.58 mg/100 g as shown in Table 3. They included mainly caffeic acid (60.08%), p-coumaric acid (39.76%), O-coumaric acid (0.08%), coumarin (0.05%), cinnamic acid (0.02%), and sinapinic acid (0.01%). A total of 162.86 mg/100 g tannin as tannic acid was found in the *Salacia senegalensis* leaf sample (Table 4).

Discussion

Relative high total carotenoids content (19.22 mg/100 g) was noted. Carotenoids are antioxidants, protect against cancer, cataract, and radiation damage, boost the immune system [33,34] and are precursors of vitamin A [35]. β-Carotene is used as food colourant.

The leaves of *Salacia senegalensis* had a total of 19.23 mg/100 g carotenoids as shown in Table 1. Lutein (33.48%) has antioxidant [1], photo-protective [36] and anti-cancer [1,2,37] activities. Carotenes have pro-vitamin A, antioxidant [1] and anti-cancer [2] activities. Lutein and zeaxanthin have been reported to have protective antioxidant effect in the macular region of the human retina [38-40]. Lycopene is an antioxidant-scavenger and destroyers of singlet oxygen [41]. Lycopene also prevents oxidation of low density lipoprotein (LDL) cholesterol and reduces the risk of developing atherosclerosis and coronary heart diseases [42], while asta-xanthin is also an antioxidant [41]. Beta-cryptoxanthin has been shown to have a unique anabolic effect on bone calcification-thus prevent osteoporosis [43-45].

The leaves of *Salacia senegalensis* had relative high content of caffeic acid (60.08%) as presented in Table 2. Caffeic acid has been shown to have neuro-protective and antioxidant properties [46,47]. P-commaric acid found is an antioxidant and reduces stomach cancer by reducing the formation of carcinogenic nitrosamines [48,49]. Coumarin was also found and it is used in the treatment of asthma [50] and lymphedema [51].

Generally, phenolics are important for cell structure, signaling and pigmentation [13]. Phenolic acids (Table 3) are known to act as allelochemicals [14], protect plants against environmental and biological stress such as high energy radiation exposure, bacterial infection or fungal attacks [12], cold stress, hyperthermia [13] and oxidative stress [2]. Therefore, the present results suggest a likely allelopathic potential of *Salacia senegalensis* leaves.

Ferulic acid found has many physiological roles, such as hypo cholesterolemic activity, prevention of coronary diseases, and increasing sperm viability [52].

Vanillic acid (4-hyroxy-3- methoxybenzoic acid) is used as a flavouring agent. It has anti-sickling, anthelmintic, hepatoprotective

Name	Amount (mg/100 g)	**Amount (%)
Malvidin	0.3150850	1.64
Carotene	4.0964900	21.31
Lycopene	0.0010883	0.01
Beta-cryptoxanthin	0.3527960	1.84
Lutein	6.4370100	33.48
Zeaxanthin	2.8979100	15.07
Anthera-xanthin	0.0063927	0.03
Asta-xanthin	1.3826700	7.19
Viola-xanthin	3.3858800	17.61
Neo –xanthin	0.3501600	1.82
Total	**19.2254800 mg/100 g**	

***Percentage is based on the weight of the compounds per the total extract of its family.*

Table 1: Carotenoid composition of *Salacia senegalensis* leaves.

Name	Amount (mg/100 g)	**Amount (%)
Vanillic acid	0.1168070	44.66
Ferulic acid	0.1370260	52.39
Syringic acid	0.0005970	0.23
Piperic acid	0.0015819	0.61
Ellagic acid	0.0049325	1.89
Rosmarinio acid	0.0005967	0.23
Total	**0.2615410 mg/100 g**	

***Percentage is based on the weight of the compounds per the total extract of its family.*

Table 2: Phenolic composition of *Salacia senegalensis* leaves.

Name	Amount (mg/100 g)	**Amount (%)
Cinnamic acid	0.0012745	0.02
Coumarin	0.0027936	0.05
P-coumaric acid	2.2189400	39.76
O-coumaric acid	0.0042574	0.08
Caffeic acid	3.3525900	60.08
Sinapinic acid	0.0003049	0.01
Total	**5.5803800 mg/100 g**	

***Percentage is based on the weight of the compounds per the total extract of its family.*

Table 3: Hydroxycinnamic acid composition of *Salacia senegalensis* leaves.

Name	Amount (mg/100 g)	**Amount (%)
Tannic acid	162.86	100
Total	**162.86 mg/100 g**	

***Percentage is based on the weight of the compounds per the total extract of its family.*

Table 4: Tannin composition of *Salacia senegalensis* leaves.

[53-56], immune-modulating and anti-inflammatory [57] activities. It also, inhibits snake venom 5^{1}-nucleotidase [55,56,58].

The total tannin was relatively high (162.86 mg/100 g). Tannin reduces blood cholesterol [34]. Tannic acid was the only tannin found in the leaves of *Salacia senegalensis* as shown in Table 4. Tannic acid is an antioxidant, hepatoprotective, hypocholesterolemic and hypoglycemic agent [34,50,59,60].

Conclusion

The leaves of *Salacia senegalensis* are rich in these bioactive compounds.

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