Epicatechin and Procyanidin B2 in the Stem and Root bark of Sterculia tragacantha Lindl (Sterculiaceae)

Orisakeye OT* and Olugbade TA

Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract

This study investigated the anti-oxidant activities of both the stem and root bark of Sterculia tragacantha, isolated its active compounds and characterised the isolated compounds.

The plant parts and the isolated compounds were screened for anti-oxidant activity by Thin Layer Chromatography technique using 2, 2-diphenyl-1-dipicyrylhydrazyl (DPPH). Extracts were fractionated using Medium Pressure Liquid Chromatography guided by bioautography to isolate active substances. The isolated compounds were characterised using Nuclear Magnetic Resonance spectroscopy and by comparison of spectrosopic data with literature values.

The results revealed that the crude extracts and two compounds possess strong anti-oxidant activity and antibacterial activity. Epicatechin was isolated from both the stem and the root bark while Procyanidin B2, C4-C8 dimer of epicatechin, was isolated from the stem bark.

In conclusion, this study suggests that the crude extracts, epicatechin and procyanidin B2 which demonstrated strong anti-oxidant activities can provide efficient antioxidant protection by complementary mechanism, such as free radical scavenging and metal ions reduction.

Keywords: Sterculia tragacantha Lindl; Epicatechin; Procyanidin; B2; DPPH; Antioxidant activity

Introduction

The family sterculiaceae is mostly found in the tropical and warm areas but a few are found in the temperate areas [1]. Sterculia tragacantha (sterculiaceae) is one of the 150 species of the 60 genera of the genus Sterculia [1,2]. Sterculia species have been extensively used in traditional medicine in the various countries where they are found.

In the Solomon Islands, Sterculia lepidoto-stellata is used to treat common cold. Sterculia lychnophora is used as tea in Cambodia. Sterculia macrophylla is used as an aphrodiasiac in Java. In Australia, Sterculia quadrifolia is used as peanut tree. Sterculia rubiginosa is used as fruit diet in Guiana. Sterculia scaphigera, in Thailand, is used to treat fever, and action is tonic [3].

Sterculia tragacantha has been employed in the treatment of boils, diarrhoea, dyspepsia, fever, gonorrhea, snake bite, syphilis, tapeworm in Burkina Faso, Cameroon, Ghana, Guinea, Ivory coast, Nigeria and Sierra Leone, action is anodyne and vermifuge [4,5]. Also infusions of the leaves of Sterculia tragacantha are used to treat malaria [6]. In India, Sterculia urens is used to treat fistula, rhagades, with the indigenous use which is known as santal [5]. Sterculia villosa is used as an antidote (Lizard) to treat fistula, night fever. Both C. acuminata and C. nitida are used as nerve stimulants, diuretics and diolotomics [7]. Cola nitida is specially used for pains [8].

The only literature report [9] on the Chemistry of Sterculia tragacantha is the fatty acid composition of the seeds. Phytochemical groups have also been reported to contain phenolics, peptides and cyclo-peptides [10]. Sterculia tragacantha leaves have been reported to show anti-ulcer, anticholinergic, antispasmodic and smooth muscle relaxant effects [9].

Epicatechin the major component isolated from both the root and stem barks, in this research work, is known in literature to possess antioxidant, anti-ulcer and anti-inflammatory [8]. The biological properties of procyanidins have also been extensively reviewed to possess anti-oxidant properties, antitumor activities and antimicrobial activity [11-13]. So also plant parts of Sterculia tragacantha have been reported to possess strong antibacterial properties [10].

The present study obtained the crude extracts of the stem and root barks of Sterculia tragacantha, determines their antioxidant properties, anti-bacterial properties isolate and characterise isolated compounds.

Experimental and Results

Plant materials and general experimental details

Fresh plant parts (stem and root bark) of Sterculia tragacantha were collected along Road 1, OAU campus. The plants were authenticated by comparison with herbarium sample at the herbarium of Dept of Botany, OAU, Ile-Ife. All organic solvents were redistilled before use. The observed rotation was obtained using Digital Polarimeter Polax-2L, ATAGO CO., LTD. USA. Isolation and purification process were monitored by a DPPH guided bioautography method.

Extraction and Isolation

Compound 1 from the stem bark: Fresh stem bark was collected, air-dried (930 g), milled and extracted with methanol at room temperature for 48 h and filtered. The filtrate was concentrated to dryness in vacuo to yield crude extract Sterculia tragacantha (20 g).

The crude extract (20 g) was dissolved in 5% methanol and ethylacetate. This was passed through silica gel by vacuum chromatography. Further elution with the methanol and ethylacetate yielded 4 UV active spots. These fractions were pooled and subjected to Thin Layer Chromatography monitored by a DPPH guided bioautography method.

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Therefore, compound 3 was identified as epicatechin (Figure 1).

The active spots fractions yielded (Stem bark Sterculia tragacantha) Sst 001 (72 mg), known as compound 1, and Sst002 (74 mg). They were obtained after combining fractions with similar TLC characteristics.

Sst 002 (74 mg) was further purified by Preparative Thin Layer Chromatography using silica plate of 1 mm thickness and developed in dichloromethane:methanol (7:3) to give a major UV detectable band. This band was scrapped and eluted with dichloromethane: methanol (1:1) and the resulting eluate was concentrated in vacuo to yield 3sst 002 (57 mg) known as compound 2.

Compound 1 was obtained as a pink reacting substance on silica (vanillin-H$_2$SO$_4$ spray) with $R_f=0.5$. It was UV active and had immediate anti-oxidant property.

Compound 2 was also obtained as a deep red reacting substance on silica (vanillin-H$_2$SO$_4$ spray) with $R_f=0.22$.

Compound 3 from the root bark: Fresh root bark was collected, (wet weight=950 g) and extracted with methanol at room temperature for 48 hr and filtered. The filtrate was concentrated to dryness in vacuo to yield crude S. tragacantha (26 g). The crude extract (26 g) was subjected to AGC and the constituents eluted on silica gel using hexane-dichloromethane-methanol in gradient. The test tubes collected were analyzed by TLC using solvent systems 30% methanol (reverse phase).

Tube fractions showing similar tlc characteristics were bulked together and concentrated in vacuo to yield fractions STRB/RP/0.42 (Sterculia tragacantha root bark) at $R_f=0.42$ (eluted in tubes 28-30) (15mg) and STRB/RP/0.31 a $R_f=0.31$ (eluted in tubes 35-40) (10mg). STRB4/RP/0.42 could not be processed further because of the quantity.

Compound 3 was obtained as a pink colour after spraying with vanillin sulphuric spraying reagent which is characteristics of $R_f=0.5$. It was UV active compound with immediate antioxidant property and strongly phenolic. This pure product of compound 3 was obtained as a pink reacting substance. The NMR data of compound 3 isolated from the root bark is identical with compound 1 isolated from the stem bark. Therefore, compound 3 was identified as epicatechin (Figure 1).

Antioxidant test

A solution of the test material in method was spotted on the tlc plate and developed using a suitable solvent system. This was sprayed with 2,2-diphenyl-1-dipicyrylhydrazyl (DPPH) reagent. The chromatogram was exposed to daylight until the purple to yellow within the first fifteen minutes after spraying were recorded as positive results (i.e possess antioxidant activity). The result of the screening of the different plant materials are summarized in plate 1.

Preparation of 2,2-diphenyl-1-dipicyrylhydrazyl (DPPH) solution: (0.3 mM) DPPH (12 mg) was dissolved in MeOH in volumetric flask (100 ml) and kept in the dark until use.

Antimicrobial screening

The agar diffusion (cup plate) method was used for this examination. Molten and cooled agar 60 ml (45°C) were separately inoculated with the nutrient broth culture of the test organisms (0.6 ml) and mixed thoroughly. The inoculated medium was then carefully poured into sterile Petri dishes (24 cm Petri dish) and allowed to set. Thereafter, cups (9 mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer. The test solutions 100 µl each were then introduced into each of the cups ensuring that no spillage occurred. Also the same volume of the standard antimicrobial agent and the solvent were introduced into some of the cups to act as positive and negative controls respectively. The plates were left at room temperature for 2 hours to allow for diffusion into the medium and thereafter incubated face upwards at 37°C for 24 hours. Sample was tested in duplicate and the diameters of zone of inhibition were measured to the nearest millimeter using a transparent ruler.

The result of the antimicrobial assays show that the crude extract of Sterculia tragacantha root bark and stem bark in 80 mg/ml in 50% methanol were active against Staphylococcus aureus showing the zone of inhibition to be 5 mm and Bacillus subtilis showing the zone of inhibition to be 6 mm and 7 mm respectively.

Antioxidant properties (A) of (sst001)(1), (3sst002)(2), and a mixture (m) compared with vanillin-H$_2$SO$_4$ sprayed plate (B).
Bioautography technique for anti-microbial screening

The method involved an overlay of inoculated agar medium on developed silica gel TLC glass plates followed by incubation at 37°C for 24 h. After incubation and revelation with tetrazolium salt, inhibition zones are visible as decolorized spots against purple background (Plate 2).

Discussion

Characterisation of sst001 as Epicatechin

The Sst 001 was a brown powder. It gave a deep pink colour with vanillin-sulphuric after heating with Rf = 0.63 in dichloromethane: methanol (30:10) silica. It showed antioxidant activity with DPPH. It showed strong reaction with ferric chloride indicating the presence of phenolic. Interestingly, both the crude extracts and the two compounds isolated showed anti-microbial activity.

Sst 001 was unequivocally identified as Epicatechin (2,3 cis configuration) rather than its C-3 epimer, Catechin (2,3 trans configuration), by virtue of the weak coupling between H-3 and H-2, (H-2, δ4.87 , s) indicating cis disposition as detailed in Table 1. C-2 of δ78.8 confirms epicatechin as the C-2 of Catechin resonates at 82.0 [2]. Interestingly the alcoholic OH at position 3 showed coupling with H-3 (δ5.6Hz) in the acetone spectra.

Characterisation of 3sst002 as Procyanidin B2

The 3sst002 gave a deep red colour after spraying with vanillin sulphuric spraying reagent which is characteristics of Procyanidin (a dimer of epicatechin) with Rf = 0.22 in dichloromethane: methanol (25:5) on silica (Figure 2).

The observed strong reactions with ferric chloride indicate the presence of phenolic function. It also showed strong reaction with DPPH indicating strong antioxidant activity.

The [H-NMR spectrum of 3sst002 showed two broad singlet signals at δ4.98(1H) and 5.11(1H), which are ascribable to H-2 of flavan-3-ol frameworks.

These observations were also consistent with the 13C-NMR data which clearly showed the presence of two flavan-3-ol C atoms (δ76.2 and 78.5). The absence of downfield signal at about δ82 indicates the absence of catechin and confirms the two flavan-3-ol residues as epicatechin. In order to clarify the points of the interflavanoid linkages, the appearance of an aliphatic CH (δ4.74) and one CH2 (δ2.73, 2.93) epicatechin. In order to clarify the points of the interflavanoid linkages, the appearance of an aliphatic CH (δ4.74) and one CH2 (δ2.73, 2.93) indicate the involvement of a C-4 position in the interflavanoid linkage. The position of linkage on the second flavanol ring is evidently on the aromatic ring A with only three H bearing Carbon signals for the C-6 and C-8 positions (δ79.5, 95.1, 96.4), the fourth signal being a quaternary C (δ106.3).

The interflavanol linkage is thus C4/C8 (Procyanidin B2) or C4/C6 (Procyanidin B5). However, Procyanidins B2 and B5 are practically indistinguishable by NMR chemical shift values (Table 2).

The difference in the optical rotation of B2 [α] =+29.2° [8] and B5 [α] =+102° [4] as reported in literature is significant. The observed rotation of 3sst002 in the present study was +29.4°. Compound 2 was therefore unequivocally established as Procyanidin B2.

Conclusion

The crude extracts of S.tragacantha showed anti-oxidant property in the DPPH model and anti-microbial activity.

Epicatechin was isolated for the first time from the root bark as well as the stem bark of S. tragacantha. Procyanidin B2 was isolated for the first time from the stem bark of the plant.

The presence of epicatechin and procyanidin B2 in the plant is significant in view of the known important anti-oxidant activities of the two compounds in the inhibition of peroxidation of lipid in human body and to extend the shelf life of food.

This study suggests that the crude extracts and the isolated
Compounds of both the stem and root bark of the plant are rich sources of antioxidant potential. *S. tragacantha* crude extract might also be useful in the treatment of any damage tissue cause by oxidative agents in human body and it can also serve as a natural antioxidant in food industry instead of synthetic preservative antioxidant.

The presence of anti-microbial activity of the two compounds explains its use in the treatment of boils and diarrhoea.

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Table 2: NMR Data of B2 and B5.

| Position | B2 | 3sat02 (acetone +D2O) | B5 | 3sat02 (acetone +D2O) |
|----------|----|-----------------------|----|-----------------------|
| 2        | (U)4.99 | (L)4.92 | (U)5.111 | (L)4.379 |
| 3        | (U)3.85 | (L)4.29 | (U)4.010 | (L)4.312 |
| 4        | (U)4.63 | (L)8.32.69 | (U)4.741 | (L)7.312.925 |
| 5,7,8a   | (U)5.9.92 | (L)6.02 | (U)6.056 | (L)6.04(U,L) |
| 6        | 5.91, 5.92(U,L) | 6.02 | 6.02 | (U)96.5 | (L)96.5 |
| 7        | 5.98 | 6.066,0.04(U,L) | (U)96.0 | (L)107.7 |
| 8        | 5.9 | 6.066,0.04(U,L) | (U)96.0 | (L)96.0 |
| 9        | 99.8 | 100.22 | 100.18 | 101.0,0.12(U,L) |
| 10       | 131.5 | 130.98 | 131.41 | 131.90 |
| 11       | (U)144.6 | (L)114.3 | (U)115.05 | (L)114.77 |
| 12       | 144.8,144.6,144.4 | 144.76,144.95, | 145.14 | 145.0,145.12, |
| 13       | 157.6,156.8,155.2 | (U)115.0 | (L)115.38 | (U)115.01, |
| 14       | (U)118.5 | (L)118.3 | 118.95 | 118.95, |
| 15       | (U)118.9 | (L)118.9 | 118.95 | 118.95, |

1H nmr 13C nmr