Alkaloids from aerial parts of *Annona senegalensis* against *Streptococcus mutans*

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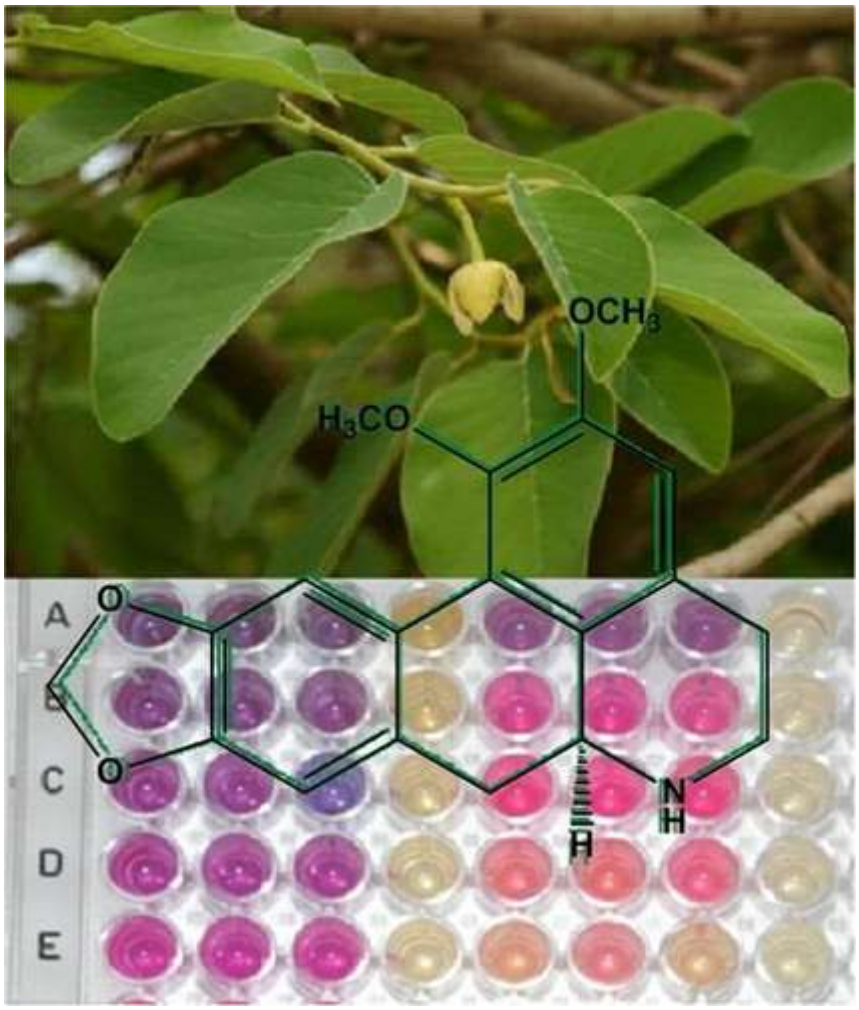
ABSTRACT

Antimicrobial potential of medicinal plants have been explored extensively these days. This study was carried out to evaluate the antibacterial potential from aerial parts of plant, called '*Annona senegalensis*' and its constituents. Bioassay guided fractionation led to the isolation of four metabolites, (+)-catechin (**1**), (-)-anonaine (**2**), (-)-asimilobine (**3**) and (+)-nornantenine (**4**). This is the first report on the isolation of compounds **1**, **3** and **4** from this plant. Compounds 2 and 4 showed good activity, whereas 1 and 3 displayed weak inhibition against *Streptococcus mutans* (ATCC 25175). The results showed that compound 2 and 3 showed significant activity with a minimum inhibition concentration (MIC) of 0.12 and 0.25 mg/mL respectively. The present study reports for the first time the antibacterial activity of the extract of *A. senegalensis* and its constituents. As *S. mutans* is a rather resistant bacteria, the MIC obtained during the present study is significant.

Antibacterial potential of alkaloids isolated from *Annona senegalensis*.



KEYWORDS: *Annona senegalensis*; alkaloids; flavonoids; *Streptococcus mutans* (ATCC 25175); antibacterial activity

1. Introduction

Oral diseases remain to be universal health problems and the association between oral diseases and bacteria is quite common. The oral cavity holds several bacterium species are linked to the periodontal diseases. There are few specific species found in the human oral cavity including *Streptococcus mutans*, which cause dental caries and significantly contribute to the tooth decay. The dental caries occurs up to 90% in school aged children but adults are also affected (Makunga et. al. 2008). Consequently, the search for plant based natural medicine from the source of traditional knowledge is still considered as best replacements. Therefore, the global needs for alternative medicine from the traditional sources are rising up day by day and many researchers are exploring the medicinal potential of plants these days.

Annona senegalensis Pers. is a perennial shrub belonging to family Annonaceae, commonly grown in African countries including South Africa. It has been found to be used in traditional medicine for the treatment of various ailments. In Nigeria, the leaf decoction is used for fever while the root forms the major components of blends used for the cure of sexually transmitted ailments (Ogbadoyi et al. 2007; Suleiman et al. 2008). Several potential metabolites and essential oils have been previously reported from this plant (Fatope et al. 1996). The other species of plant viz. *Annona crassiflora, and Annona squamosal* have been characterized to show biological activity for various ailments like malaria, cancer respectively (Lage et al. 2014, Pimenta et al. 2014, Miao et al. 2015). *Annona diversifolia* reported to show anticonvulsant and antidepressant activity, however, alkaloid content in *Annona diversifolia* is reported to influence seedling during diurnal periods (Gonzalez-Trujano et al. 2006, Orozco-Castillo et al. 2016).

2. Results and discussion

2.1 *Identification of isolated compounds*

The identification of isolated alkaloids was done using previously reported spectroscopic data. Four compounds isolated from the methanol extract of aerial parts of *A. senegalensis* were identified as catechin (Dong et al. 2011), nornantenine (Vendramin et al. 2013), anonaine and asimilobine (Fofana et al. 2013) (Figure 1). Catechin is a known secondary metabolite which has been previously isolated from many plant species including green tea (Dong et al. 2011). However, this is the first report on the isolation of catechin from the plant, *A. senegalensis*. Whereas, anonaine has been previously isolated from the chloroform extract of leaves and bark of *A. senegalensis* (Fofana et al. 2013). Similarly, asimilobine has been previously isolated from the other plants but has not reported from any plant part of *A. senegalensis*. The compound nornantenine also has not been previously reported from this plant. To the best of our knowledge, the compounds, catechin, asimilobine and nornantenine have been isolated for the first time from the plant *A. senegalensis*.

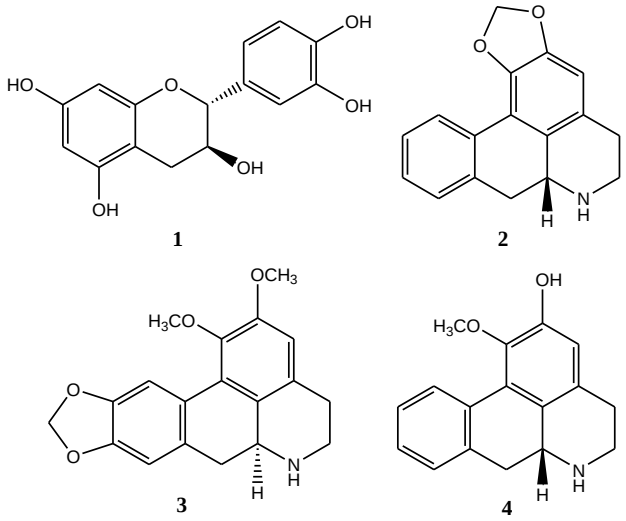


Figure 1. Structures of isolated compounds, 1-4 from *A. senegalensis.*

2.2 *Antimicrobial activity of crude extract and isolated compounds*

The methanol extract showed significant antibacterial activity against *S. mutans* with MIC value of 2 mg/ml. The ethanol extract from the bark of *A. senegalensis* exhibited an MIC of 12.5 mg/ml in our evaluation conducted earlier. Four compounds tested showed moderate antibacterial activity. Anonaine was the most significant alkaloid with

MIC value of 0.12 mg/ml followed by the compound, Nornantenine which showed an MIC of 0.25 mg/ml. Catechin and Asimilobine showed weak activity with MIC of 2.0 mg/ml. Chlorhexidine (CHX) used as a standard drug, displayed the MIC 0.004 mg/ml in present study. The inhibitory effect of different types of catechins on *S. mutans* have been previously reported by many scholars (MIC ranging from 50-1000 µg/ml) (Taylor et al. 2005), while in our study it displayed an MIC of 2.0 mg/ml against *S. mutans*. In addition, several research groups previously reported the antibacterial activity of these compounds against various pathogenic bacteria viz. *Staphylococcus aureus, S. epidermidis, Bacillus cereus, K. rhizophila, Microsporum gypseum and Tricophyton rubrum* (Paulo et al. 1992, Taylor et al. 2005, Costa et al. 2016).

This the first report on the antibacterial activity of *A. senegalensis* from the aerial parts of the plant and its isolated compounds anonaine, asimilobine and nornantenine against *S. mutans*. To the best of our knowledge, all the three alkaloids isolated in present study have been tested for the first time against *S. mutans* (ATCC 25175) strain.

3. Conclusion

The results of the present study reports on the *in vitro* antibacterial activity of isolated alkaloids and flavonoid against bacterial strain, *Streptococcus mutans* (ATCC 25175). The compound, anonaine (0.12 mg/ml) displayed the best activity followed by the asimilobine (0.25 mg/ml) in micro dilution assay. These results indicated the antibacterial potential of traditionally used medicine for the prevention and treatment of oral problems and other ailments.

Acknowledgements

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Disclosure statement

No potential conflict of interest was reported by authors.

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SUPPLEMENTARY MATERIAL

Experimental

1 Microbial strain and cell culture

The microorganism used in this study was *Streptococcus mutans* (ATCC 25175, Anatech Company, Johannesburg). Bacteria were grown in Casein-peptone Soymeal-peptone (CASO) Broth (Merck Chemicals (Pty) Ltd Wadeville, South Africa) enriched with 1% sucrose (Merck Chemicals (Pty) Ltd Wadeville, South Africa) under anaerobic conditions in an anaerobic jar with Anaerocult® A (Merck Chemicals (Pty) Ltd Wadeville, South Africa), at 37 °C for 24 hours. Sub culturing was done once weekly on (CASO) Agar.

2 Plant material and chemicals

Aerial parts of the plant *A. senegalensis* were collected from the campus of University of Pretoria. A voucher specimen (PRU 074974) was deposited for future reference at H.G.W.J Schwelckerdt Herbarium, Department of Plant Science, University of Pretoria, Pretoria. The collected plant material was washed with distilled water and shade dried. All the chemicals and solvents were purchased from Sigma-Aldrich and Merck SA Pty Ltd. Silica gel 60 (70-230 mesh) and Sephadex LH-20 purchased from Sigma-Aldrich for column chromatography.

3 Extraction and isolation of compounds

The dried and powdered plant material (650 g) was extracted with 2.5 liters of methanol for 36 h on a shaker. The filtrate was collected, then evaporated under reduced pressure at 40 °C using a rotary evaporator. The crude methanol extract was (27.40 g) partitioned using ethyl acetate and water. The water layer was again partitioned using n-butanol and water soluble fraction. Three major fractions were obtained; ethyl acetate (AS-e) 6.99 g, n-butanol (AS-b) 17.60 g and H_2O fractions (AS-w) 5.33 g, were obtained. The EtOAc fraction (6.99 g) was subjected to silica gel column using n-hexane- EtOAc-MeOH as eluents in increasing order of polarity. Similar fractions were pooled based on the TLC profile, which resulted in a total of six fractions (AS-e-1 to AS-e-6). During investigating the antibacterial activity of these major fractions, fraction AS-e-3 and AS-e-6 were proven to be better than the other fractions (Table S1). Fraction AS-e-3 (1.40

g) was subjected to silica gel column chromatography using hexane-EtOAc-MeOH as eluents of increasing polarity. Sub-fraction 2 afforded compound **1** (0.12 g, 0.01% of dry plant material) using another column when $CHCl_3$-MeOH was used as eluents. Fraction AS-e-6 (0.63 g) was subjected to repeated column on silica gel using $CHCl_3$-MeOH as eluents which afforded compound **2** (0.05 g, 0.008% of dry plant material). The n-butanol fraction (17.60 g) was subjected to silica gel column which resulted in four main fractions (AS-b-1 to AS-b-4). The most active fraction (Table S1) AS-b-3 (0.69 g) was subjected to repeated columns on silica gel using $CHCl_3$-MeOH as eluents, which afforded compound **3** (0.02 g, 0.003% of dry plant material) and compound **4** (18 mg, 0.002% of dry plant material).

4 Identification of isolated compounds

The bioassay guided fractionation of the methanol extract of *A. senegalensis* (Table S1), resulted in the isolation of four compounds, one flavonoid and three alkaloids. ^{1}H and ^{13}C NMRs of all the samples were recorded on (600 MHz) and (150 MHz), respectively. Structural assessment of these compounds was done by HR-MS, ^{1}H and ^{13}C NMR spectroscopic data (Fig S1 and S2). The compounds obtained in this study which were Catechin (**1**) Anonaine (**2**), Nornantenine (**3**) and Asimilobine (**4**) were identified based on the spectral analysis as well as comparison with respective literature reports (Fig. 1), To the best of our knowledge, compounds **1**, **3** and **4** have been isolated from aerial parts of *A. senegalensis* for the first time.

5 Antibacterial activities of extract and isolated compounds

The microdilution technique using 96 well micro-plates, as described by Eloff (1998) was used with some modification for antibacterial bioassay to obtain the MIC values of the crude extracts, major fractions and pure compounds against the microorganism in the present study. All the major fractions of *A. senegalensis* and compounds were dissolved in 10% DMSO to make up a stock solution of 25 mg/ml. One hundred microlitres (100µl) of the stock solution was serially diluted in the 96-well plate to give final concentrations ranging from 12.5 -0.1 mg/ml for the extracts and 2 – 0.015 mg/ml for the pure compounds. After which 100µl of 24 hours old bacteria (3×10^{8} cfu/ml) grown at 37°C were added to the plates and incubated for a further 24hours at 37°C . The positive drug control 1.25% v/v chlorhexidine gluconate (CHX) (Dental Warehouse, Sandton, South Africa), ranged from 0.62 - 4.8 x 10^{-4}% v/v. Microbial

growth was indicated by adding 40µl PrestoBlue to micro-plate wells. MIC was defined as the lowest concentration that inhibited PrestoBlue from changing to pink from blue, an indication of bacterial growth (Lall et al. 2013).

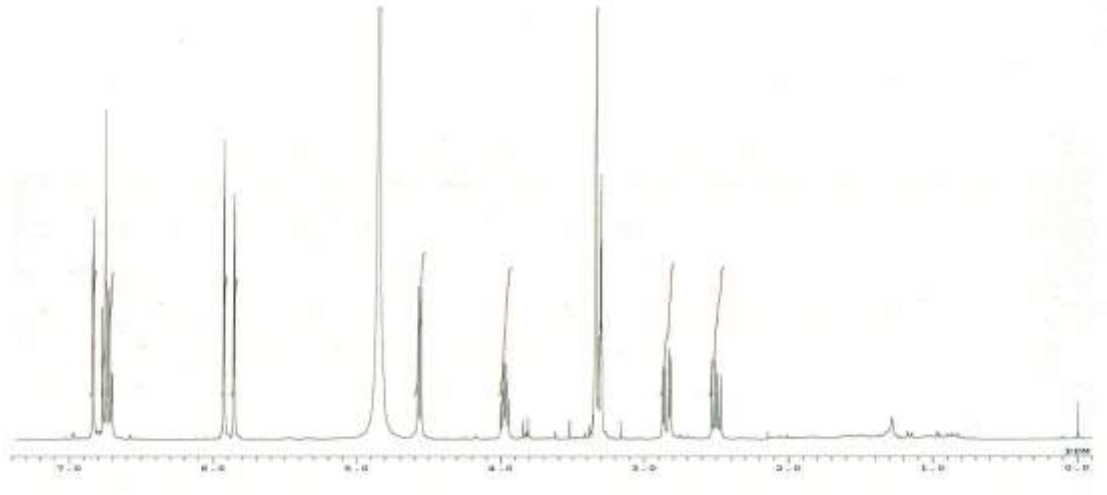

Figure S1. ^{1}H NMR of Catechin

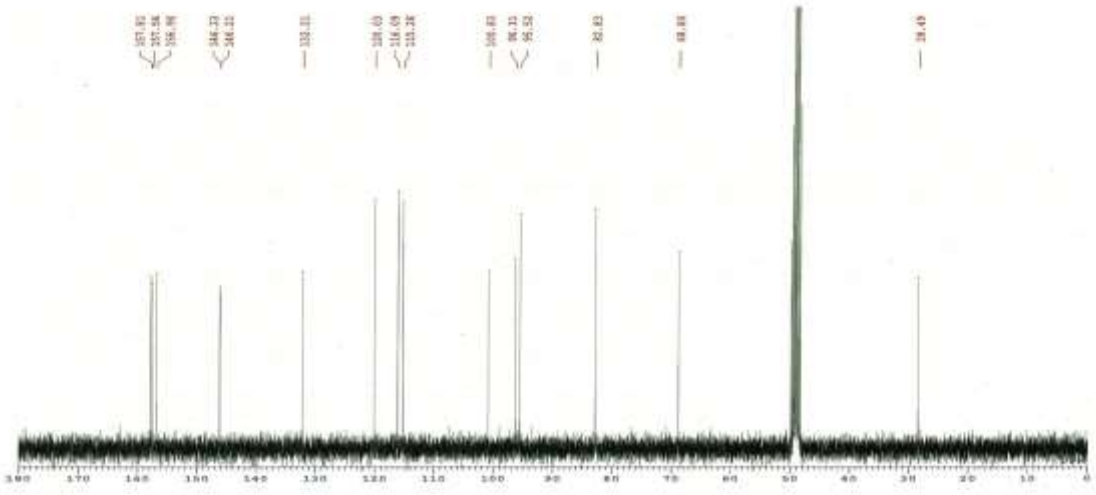

Figure S2. ^{13}C NMR of Catechin

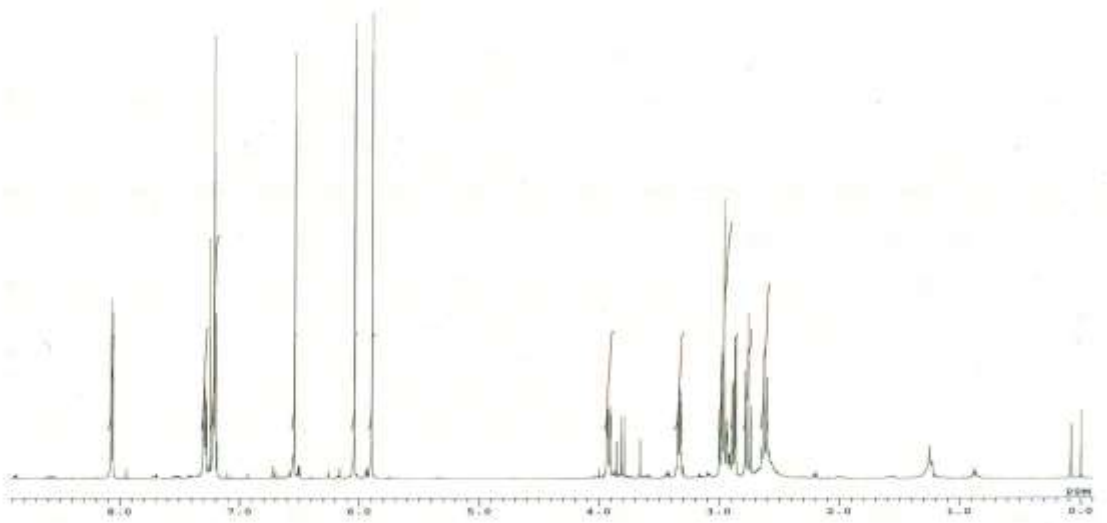

Figure S3. ^{1}H NMR of Anonaine

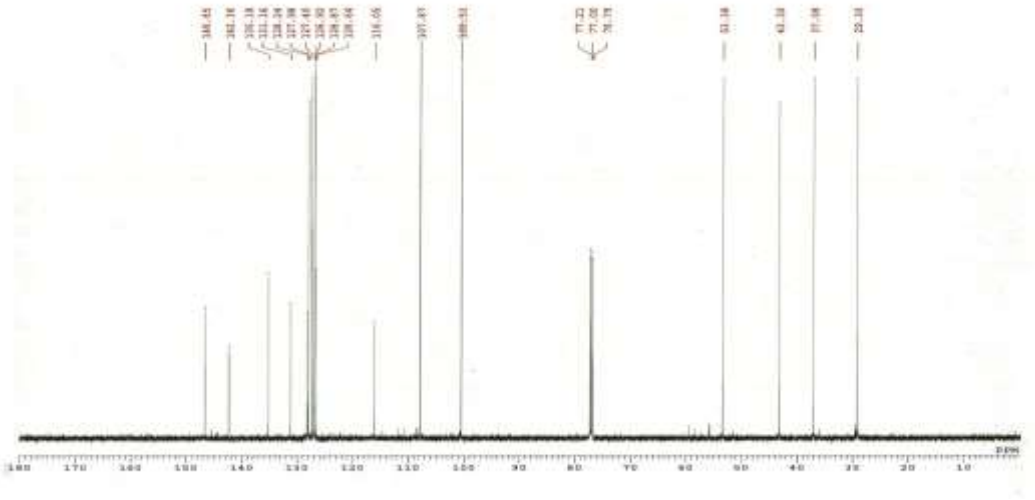

Figure S4. ^{13}C NMR of Anonaine

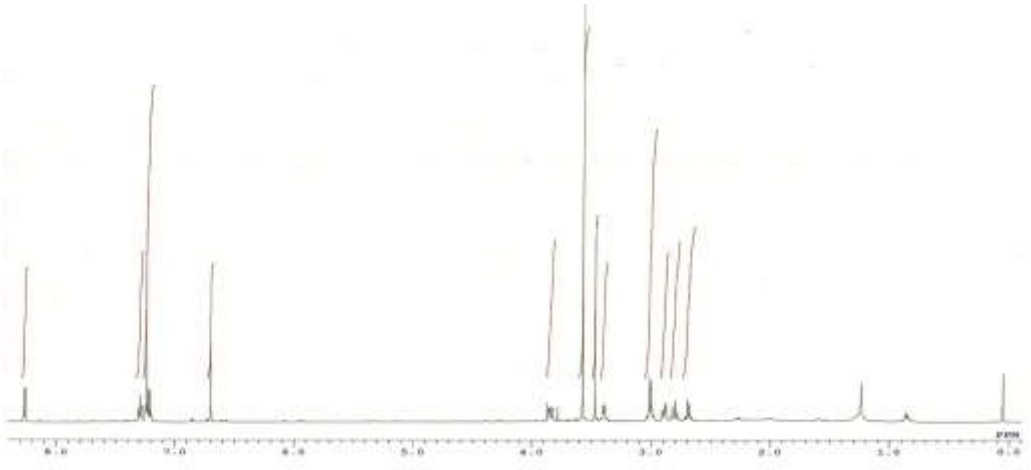

Figure S5. ^{1}H NMR of Asimilobine

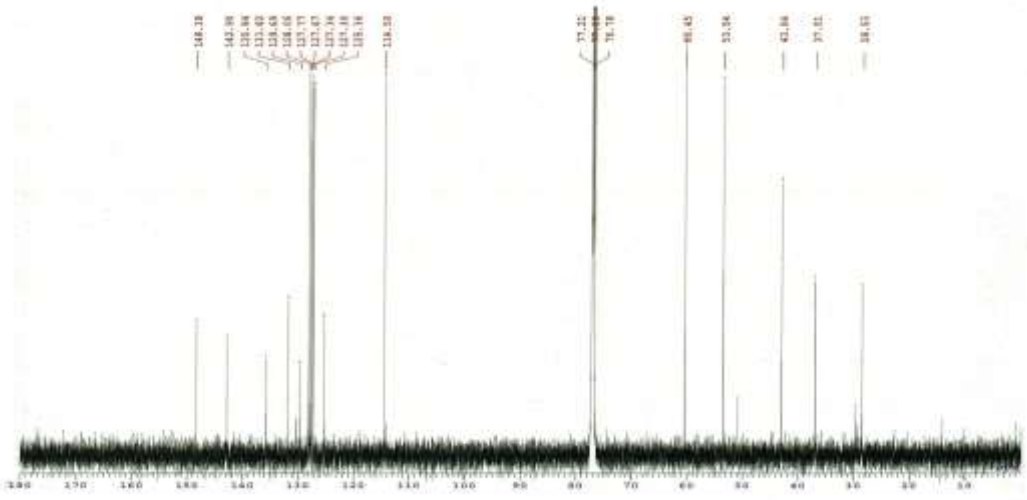

Figure S6. ^{13}C NMR of Asimilobine

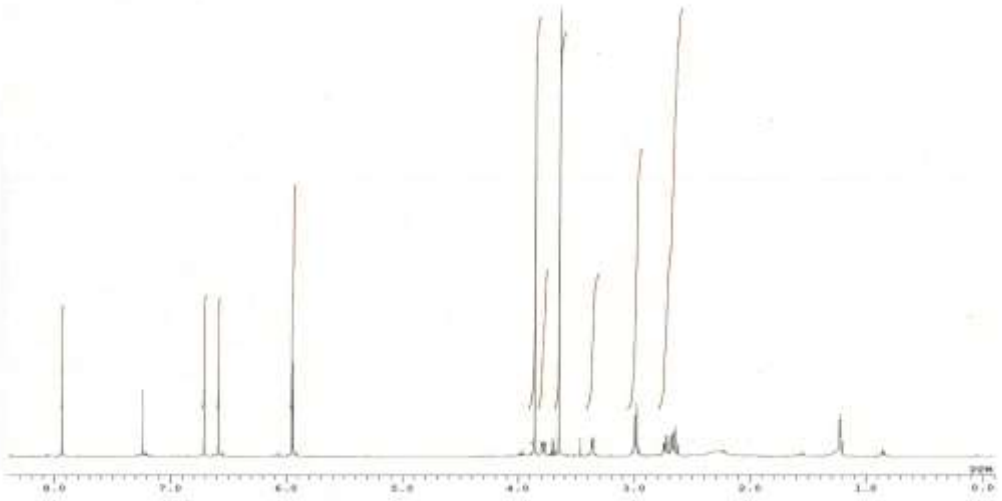

Figure S7. ^{1}H NMR of Nornantenine

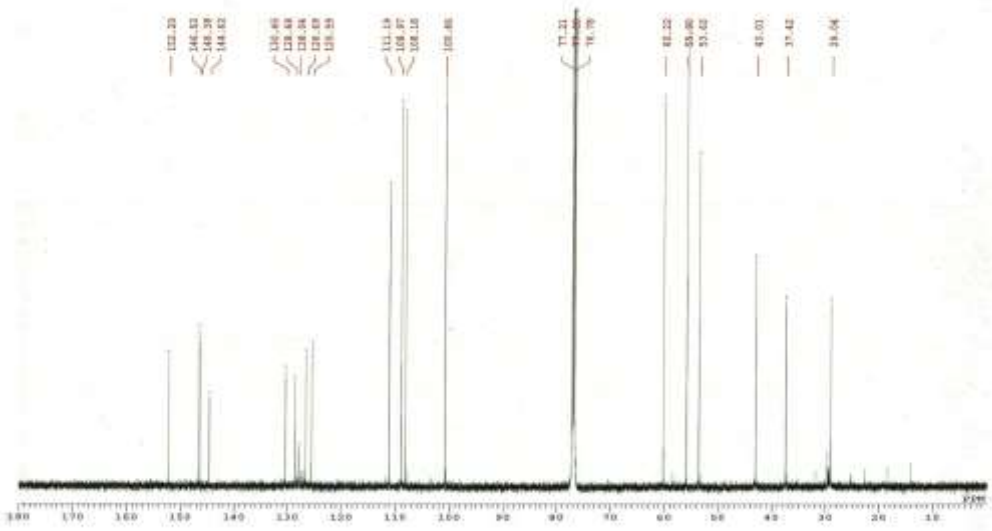

Figure S8. ^{13}H NMR of Nornantenine

Table S1. The minimum inhibitory concentration of major fractions of methanol extract of *A. senegalensis* and its isolated compounds against *Streptococcus mutans*

S.N.	Fractions/Samples	MIC[a] (mg/ml)
	Crude extract	12.5
	as-b-3	0.4
	as-e-5	12.5
	as-e	3.125
	as-w	12.5
	as-e-6	0.8
	as-e-3	3.125
	as-b	6.25
	as-e-1	12.5
	Catechin (**1**)	2.0
	Anonaine (**2**)	0.125
	Nornantenine (**3**)	2.0
	Asimilobine (**4**)	0.25
	Chlorhexidine (CHX)[b]	0.004

[a]MIC- Minimum Inhibitory Concentration

[b]Chlorhexidine (CHX)- Standard used

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