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In vitro Investigation of Antioxidant Phenolic Compounds in Extracts of *Senna alata*

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Abstract: Extracts of *Senna alata* were investigated for antioxidant phenolic compounds using High Performance Liquid Chromatography (HPLC). The dried aerial plant parts were macerated into powder and extracted in different organic solvent systems consisting of methanol, hexane, chloroform, ethyl acetate, butanol and water. Each extract was dried under reduced pressure using a rotary evaporator, freeze-dried and stored at a temperature of 4°C. The extracts were then subjected to high performance liquid chromatography studies. Two major phenolic compounds Naringin and Apigenin, were identified in some of the fractions of *Senna alata*. The presence of these flavoniods in *Senna alata* may explain its wide use in ethnomedicine practice for the treatment of hypertension, sickle cell anemia and diabetes in Southwestern Nigeria.

Key words: *Senna alata*, phenolic compounds, high performance liquid chromatography, naringin, apigenin

INTRODUCTION

Phenols are a class of low molecular weight secondary metabolites found in most land plants. These compounds are of great importance in foods and drinks because they are responsible for their organoleptic properties. Polyphenols such as anthocyanins, add colour to food which can be purple, black or red (Alonso *et al*., 2003) and this is desirable in red wines. Phenolic compounds are the largest group of phytochemicals and accounts for most of the antioxidant activity in plants or plant products (Aliyu *et al*., 2009). Phenolic substances such a flavonols, naringin, apigenin, myricetin, coumarins and caffeic acids are known to posses antioxidant properties which play important roles in protecting foods, cells and organs from oxidative degeneration (Osawa, 1999). In model systems, antioxidants are able to scavenge free radicals and thereby prevent free radicals from causing damage. Reports indicate that diets rich in phenolic compounds play a role in the prevention of various diseases associated with oxidative stress such as cancer, cardiovascular and neurodegenerative diseases (Manach *et al*., 2004; Hang *et al*., 2004). In addition, phenols constitute the active substances found in many medicinal plants with important pharmacological activities and modulate the activities of a wide range of enzymes and cell receptors (Middleton *et al*., 2000). Therefore, the isolation and identification of these compounds are of great interest and importance because of their role in drug development and in management of many chronic diseases.

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Senna alata Linn. Roxb (Leguminosae) synonym *Cassia alata* Linn. commonly referred to as Asunwon oyinbo by the Yoruba ethnic stock in Southwestern Nigerian, is indigenous to Africa. This plant grows in semidesert and sudano-sahelian zones of Africa (Iwu, 1993; Palamichamy *et al*., 1990; Awal *et al*., 2004). In Cameroon, the leaves and stem bark of *S. alata* are used to treat hepatitis, skin diseases, jaundice, gastroenteritis, eczema and ringworm. The young leaves are used in rural areas of Nigeria to treat constipation and food poisoning (Adedayo *et al*., 2001). In Northern Nigeria, the root, stem and leaves are used to treat burns, wounds, skin infection, diarrhea and upper respiratory tract infection (El-Mahmood *et al*., 2008). The bioactivity of the plant include antibacterial, antifungal, antimicrobial, diuretic, analgesic and choleretic (Reezal *et al*., 2002). There are reports also, on the antioxidant activity of the leaves of this plant. Wegwu *et al*. (2005), reported that *Senna alata* was able to induce antioxidant effects in the serum of rats exposed to carbon tetrachloride (CCl_4) with a concentration-dependent decrease in alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

In this study, we employed High Performance Liquid Chromatographic (HPLC) analysis coupled to an UV (Ultra violet) detector to identify and determine the phenolic contents of fractions of *Senna alata*.

MATERIALS AND METHODS

The study was conducted from Sept. 2008 to Feb. 2009. Plant experiments and analysis were done in the Department of Cell Biology and Genetics Laboratories, University of Lagos, Akoka, Lagos and HPLC studies were carried out at the Central Research Laboratory of the College of Medicine, University of Lagos.

Plant Material

The leaves of *Senna alata* were collected from Olokemeji reserves in Ogun State, Nigeria in August, 2008 and air dried in our laboratory. They were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Voucher specimen was deposited at the institute's herbaria.

Preparation of Crude Extract

The dried leaves of *Senna alata* were pulverized with a blender into a fluffy mass. One hundred gram of powdered leaves of *Senna alata* was extracted with 250 mL of Methanol (MeOH) using Soxhlet apparatus. The extraction process was repeated twice to obtain extractable components of the plant. Combined extract solution was evaporated to dryness under reduced pressure at below 40°C to yield 9.47 g crude extract. The ratio of crude drug to extract was 1:10

Fractionation of Plant Extract

This was done according to the method of Yesilada and Kupeli (2002). Seven gram of the methanol extract was reconstituted with 200 mL of MeOH:H_2O (9:1) mixture and shaken with n-hexane (3×100 mL). Combined hexane extract was evaporated under reduced pressure to yield Hexane fraction (2.17 g). MeOH was evaporated from the remaining extract and diluted with distilled water to 200 mL and further fractionated by successive solvent extraction with chloroform (4×100 mL^{-1}), ethyl acetate (2×100 mL^{-1}) and n-butanol saturated with H_2O (3×100 mL^{-1}). Each extract was evaporated to dryness under reduced pressure to yield chloroform fraction (1.38 g), ethyl acetate (0.84 g), butanol fraction (0.82 g) and the remaining extract is the water fraction (2.08 g).

HPLC (High Performance Liquid Chromatography) Analysis

HPLC analysis was carried out at the Central Research Laboratory of the College of Medicine, University of Lagos, on 1100 series Agilent Technologies equipment comprising of the Thermostatic column compartment G1316A (Germany), Variable wavelength detector (UV) G1314A (Japan), Quaternary pump G1311A (Germany) and Degasser G1719A (Japan) incorporated with Rheodyne auto injector USA (20 μL loop Volume).

Chemicals and Reagents Used for HPLC Analysis

The reagents used include HPLC grade methanol, 70% aqueous methanol, acetonitrile (ACN) ammonium acetate buffer and standard phenolic compounds (naringin, apigenin, rutin and myricetin).

Chromatographic Conditions

The automated HPLC system was driven by a CHEM STATION software and the chromatographic separations were performed using a Hypersil 100-5 (Macherey-Nagel) silica gel coated column (250×4.6 mm). Identification of phenolic compounds was based on co-chromatography using standards of the phenolic compounds to be analyzed (apigenin, naringin, rutin and myricetin), chemical tests on pure compounds and characteristics of UV-VIS spectra recorded by PDA detector and Perkin-Elmer spectrophotometer for pure compounds. The mobile phase selected for the method validation and for the determination of the phenolic compounds were Methanol (MeOH), Acetinitrile (ACN) and Ammonium acetate buffer (25:55:20%). Before use, the mobile phase was degassed for 15 min in an ultrasonic bath. The samples were monitored with UV detection at 260 nm at the flow rate of 1 mL min^{-1}. Ambient temperature was maintained.

Preparation of Standard Stock and Test Solutions

This was carried out according to the methods of Goren *et al.* (2004). The standard phenolic compounds were prepared at varying concentrations of 5, 10, 25, 50, 100, 175, 250 and 500 μg mL^{-1} for naringin and apigenin, rutin and myricetin. The 10 mg mL^{-1} solutions of test samples (CH_3OH, hexane, chloroform, ethyl acetate, butanol and water) fractions of *S. alata* were also prepared. Methanol 10% v/v aqueous (1 mL each) served as the suitable solvent medium for both the standard and test samples.

The measurement was done 3 times at the various concentrations for precision of the method.

RESULTS AND DISCUSSION

The compounds were identified by peak integrations of the elution time (mins) and the peak area (mAU). The concentrations of phenolic compounds (Table 1) in the various

Table 1: Types of phenolic compounds and their concentration in different fractions of *Senna alata*

Phenol compounds	Crude	Hexane	Ethylacetate	Chloroform	Butanol	Water
Naringin	+	+	+	+	+	+
Apigenin	+	+	+	-	-	-
Myricetin	-	-	-	-	-	-
Rutin	-	-	-	-	-	-
Conc. of naringin (mg mL^{-1})	NQ	0.007	NQ	0.010	NQ	0.025
Conc. of apigenin (mg mL^{-1})	0.018	0.008	0.01	-	-	-
mAU of naringin	4715	374	4338	852	5000	2052
mAU of apigenin	4619	281	2350	-	-	-

+: Present, -: Absent, NQ: Not Quantified, mAU: Mean area unit, Conc.: Concentration

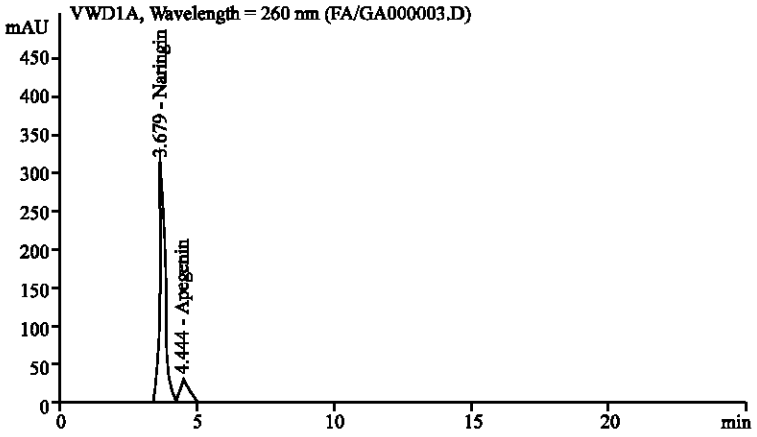

Fig. 1: Elution profile of naringin standard

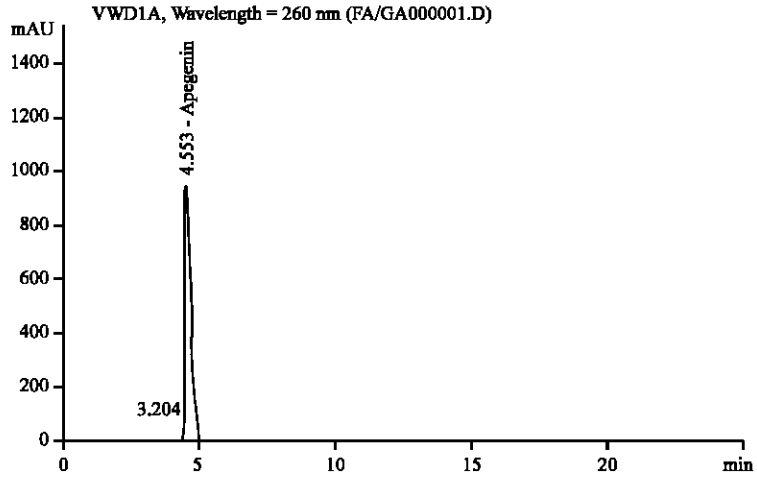

Fig. 2: Elution profile of apeginin standard

fractions were extrapolated from a standard curve obtained by plotting the peak area unit for the different elution times against the various concentrations of the standards compounds-naringin, apigenin, rutin and myricetin.

Naringin and Apeginin were detected in all or some of the different fractions respectively, while rutin and myricetin were not detected in any of the fractions (Table 1).

Figure 1 and 2 show the different elution times and the peak area units obtained for standard phenolic compounds naringin and apigenin. Naringin and apigenin were detected in the crude, hexane and ethyl acetate fractions (Fig. 3-5) while only naringin was present in the chloroform, butanol (though too small to be quantified) and water fractions of *Senna alata* (Fig. 6-8).

Naringin (5, 4, 7-Trihydroxyflavonone 7-rhamnoglucoside) a prenylated flavonone, is a known antioxidant compound found in grapefruit and is responsible for its bitter taste.

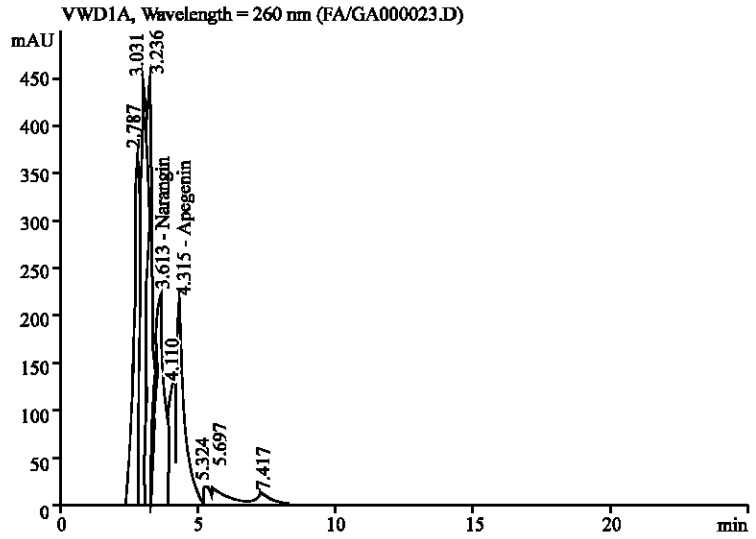

Fig. 3: Elution profile of crude fraction of *Senna alata*

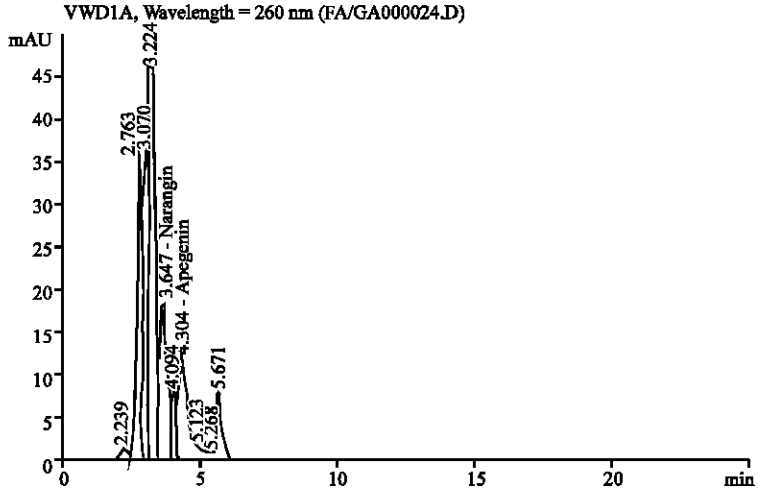

Fig. 4: Elution profile of hexane fraction of *Senna alata*

Mamdouh *et al.* (2004), reported the hypoglycemic and antioxidant activity of naringin in streptozocin-induced diabetic rats. Their study showed that gradual exogenous administration of doses of naringin to hyperglycaemic rats caused a dose-dependent decrease of the glucose level, a decrease of the H_2O_2 and TBARS levels, as well as the increase of the total antioxidant status while antioxidant enzyme activities such as catalase, superoxide dismutase and glutathione peroxidase also increased. The compound naringin was also identified in a fraction of *Globumentula branuii* (Okpuzor *et al.*, 2009). Pereira *et al.* (2007) also reported its anti-inflammatory and anti-carcinogenic activities. However, apigenin was absent in some of the fractions and this could be as a result of their presence in undetectable amounts or they were probably not extractable in that particular

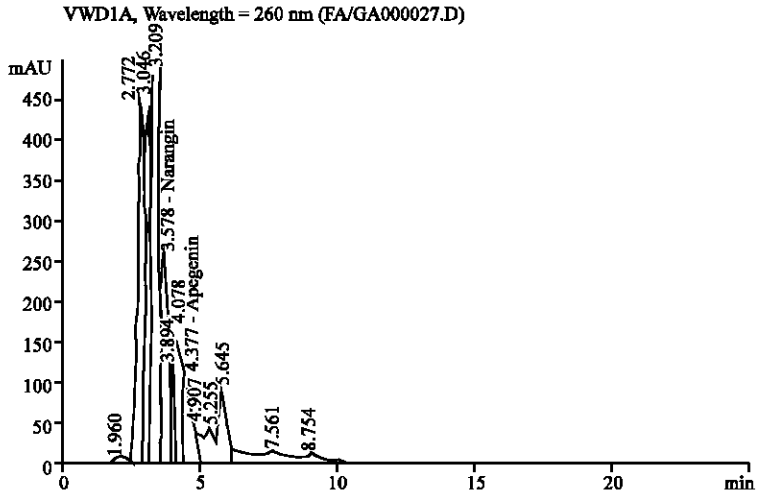

Fig. 5: Elution profile of ethyl acetate fraction of *Senna alata*

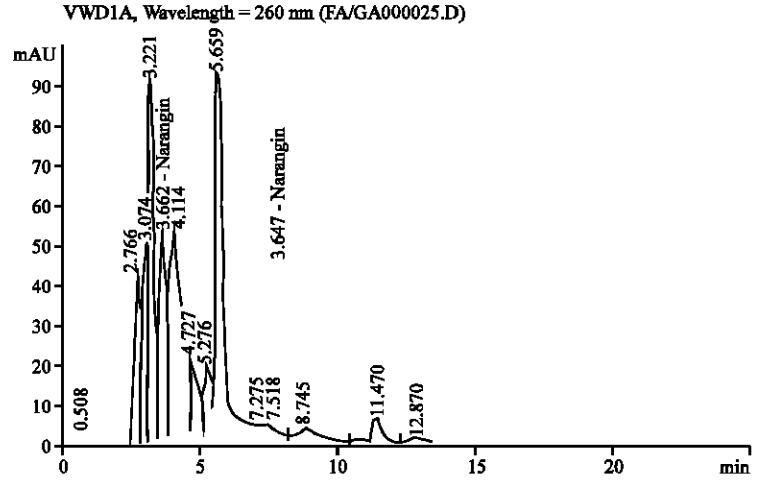

Fig. 6: Elution profile of chloroform fraction of *Senna alata*

solvent system. Panichayupakaranant and Kaewsuwan (2004) using bioassay guided studies identified Kaempferol as the major bioactive component of *Cassia alata* and reported its antioxidative properties. We did not identify kaempferol in this study. It is likely that one of the unidentified peaks observed from this study may have been kaempferol because we did not include kaempferol among the standard compounds used. It is also possible that the local specie of *Senna alata* used does not contain kaempferol as a major phenolic compound. This is supported by the fact that rutin which is one of the most easily detectible compounds with antioxidative properties in most Nigerian medicinal plants was not detected in *Senna alata*. Further studies are suggested to identify most of the peaks observed in the chromatogram.

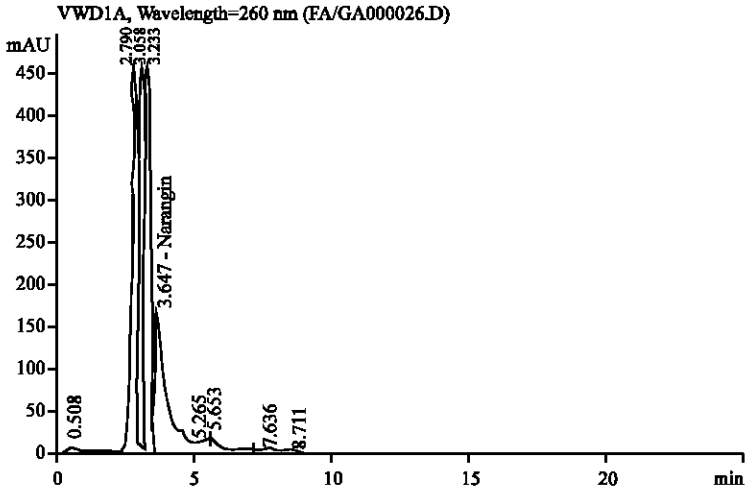

Fig. 7: Elution profile of butanol fraction of *Senna alata*

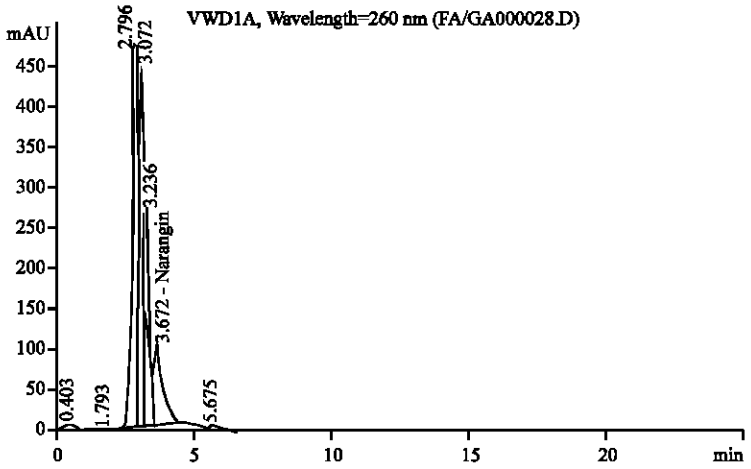

Fig. 8: Elution profile of water fraction of *Senna alata*

CONCLUSION

This study suggests that *Senna alata* possesses antioxidative properties as revealed from *in vitro* HPLC studies and that the two phenolic compounds identified in *Senna alata* fractions may possibly contribute to the antioxidative and other pharmacological activities associated with the plant. HPLC method is also reported to be effective for this type of studies because previous studies have utilized it to identify some bioactive compounds in plants. Goren *et al.* (2004) had reported that this method is a good tool for the screening of phenolic compounds in plants. In addition, Hanachi (2009) determined the composition and antioxidant activity of *Berberis vulgaris* using HPLC studies. Sun *et al.* (2009) also employed this method to identify polyphenol C-glycosides in *Swertia franchetiana*, a Chinese/Tibetan herb. The use of HPLC to identify the *in vitro* antioxidative compounds of *Senna alata* also

corroborates the findings of Okpuzor *et al*. (2009) in *in vivo* studies of the extract of *Globimetula braunii* in rats. Results from that study showed that the ethyl acetate fraction possesses antioxidant properties expressed by increase in antioxidant enzymes and the presence of naringin.

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