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## Full Length Research Paper

# Phytochemical studies and thin layer chromatography of leaves and flower extracts of *Senna siamea lam* for possible biomedical applications

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*Senna siamea* is a medium-size, evergreen plant which has been utilized as a source of food, medicine and other agricultural purposes in different communities. However, there is dearth of information in regard to its possible biomedical uses, especially in Nigeria. Thus the preliminary phytochemical analysis and thin layer chromatography (TLC) separation was done using methanol, n-hexane and ethyl acetate (1:3:1) as solvent system while iodine vapour as spotting agent. The phytochemical screening of methanol extracts of leaves revealed the presence of cardiac glycoside, flavonoid, saponin, alkaloid and tannins while chloroform extracts of leaves revealed saponin only. Ethyl acetate and petroleum ether extracts revealed absence of all these phytochemicals. The chloroform, ethyl acetate and petroleum ether extracts of flower revealed absence of saponin, flavonoids, tannins and alkaloids but with traces of saponin and anthraquinones. TLC separation showed nine (9) spots each of chloroform and ethyl acetates, six (6) spots of methanol, three (3) spots of petroleum ether from leaves extracts. While, three (3) spots each of ethyl acetate and methanol, six (6) spots of chloroform were identified for flower extracts. No water spot separated from both leaves and flower extracts. From our findings, it can be concluded that *S. siamea lam* contains some significant phytochemicals that can exhibit desired therapeutic activities such as hypoglycemia, anti-arrhythmia and antimicrobial. However, there is the need to conduct further pharmaceutical analyses on test extracts in order to establish these biomedical applications.

**Key words:** *Senna siamea*, thin layer chromatography, antimicrobial, phytochemical.

## INTRODUCTION

Plants have been found to be the source of energy for the animal kingdom. In addition, plant can synthesize a large variety of chemical substances that are of physiological significance (Kretovich, 2005). The active phytochemical principles produced by plants include, alkaloids, phenolic,

anthraquinones, flavonoids, phenols, saponins, steroid, tannins, terpenes etc (Namukobe et al., 2011).

Medicinal plants are those that contains one or more of its phytochemicals that can be used for the synthesis of useful therapeutic agents (Sofowora, 2000). The wide

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range of medicinal plant parts like flowers, leaves, barks, stems, fruits, roots extracts are used as powerful raw drug possessing a variety of pharmacological activities (Momin et al., 2012). In the last two centuries, there have been serious investigations into the chemical and biological activities of plants and these have yielded compounds for the development of synthetic organic chemistry and the emergence of medicinal chemistry as a route for the discovery of more effective therapeutic agents (Roja and Rao, 2000).

*Senna siamea* is native to Southeast Asia from India, Sri Lanka, and Thailand to Indonesia, Burma, and Malaysia and forms part of the warm and wet tropical forests. The species has been introduced in Africa and America. *S. siamea* is effective in managing constipation association with a number of causes including surgery, childbirth and the use of narcotic pain relievers (Hill, 1992). It is used locally as antimalarial drugs especially when decocted (the leaves and bark) (Lose et al., 2000). In traditional medicine, the fruit is used to charm away intestinal worms and to prevent convulsion in children. The young fruits and leaves are also eaten as vegetables in Thailand. The flowers and young fruits are used as curries (Kiepe, 2001) and as an antimalarial (Otimenyin et al., 2010). The stem bark extract was reported to have analgesic and anti-inflammatory effects (Ntandu et al., 2010). Isolated compounds, emodu and lupeol from the ethyl acetate fraction of the stem bark of *S. siamea* were reported to be the active principles responsible for the antiplasmodial property with  $IC_{50}$  values of 5  $\mu\text{g/ml}$ , respectively (Ajaiyeoba et al., 2008). Sub-chronic studies of the aqueous stem bark extract of the plant in rats did not show significant toxic effect after seven weeks of administration (Mohammed et al., 2012).

This study was designed to determine the phytochemical compositions as well as to perform thin layer chromatography separation of the leaves and flowers extracts of *S. siamea* in order to create awareness of its possible medicinal and nutritional values.

## MATERIALS AND METHODS

These include the test plant (the fresh leaf and flower of *S. siamea*), beaker, conical flask, measuring cylinder (large and small), glass funnel, glass stirrer, cotton wool, spatula, bunsen burner, top mettler weighing balance, test tubes, stainless steel tray, thermostat water bath, oven, syringe and needle, aluminum foil paper, hand gloves, mortar and pestle, analytical weighing balance, test-tube holder, refrigerator, meter rule, sieves (No. 5), bottles, UV fluorescence analysis cabinet tripod stand, wire gauze, capillary tubes, retort stand, thin layer chromatography (TLC) paper, TLC tank, test tube rack, tiles and filter paper.

### Reagent used

Dragendoff's reagent, methanol, chloroform, 1% aqueous hydrochloric acid, Mayer's reagent, sodium chloride solution, glacial acetic acid, concentrated sulphuric acid, 10% Ferric chloride

solution, Molisch's reagent, Fehling's solution A and B, lead subacetate solution, 10% sodium hydroxide, 10% ferric chloride in 95% alcohol, Barfoed's reagent, 3,5 dinitro benzoic acid I, iodine solution, dilute hydrochloric acid, Wagner's reagent, concentrated hydrochloric acid, 3.2% ferric chloride in glacial acetic acid, 10% lead acetate, 10% tannic acid, 1% w/v picric acid, 5% sodium hydroxide, bromine water, potassium iodide solution, 3% hydrogen peroxide, 1 M sodium hydroxide, acetic anhydride.

### Sterilization

All work surfaces were comprehensively disinfected with cotton wool soaked in antiseptic fluid to minimize contamination during work process.

### Dry heat sterilization

An hot air oven was used to sterilize the conical flasks, forceps, office punch, wire loop and filter paper discs (wrapped in foil paper) and beaker at 160°C for 45 min.

### Moist heat sterilization

All materials used in the course of this research project that are not sensitive to moist heat sterilization were adequately sterilized using autoclave and detergents. Materials such as glass wares, beakers and conical flasks etc. were properly washed with detergent and water so as to remove dirt and contaminants and were allowed to dry prior to usage. These materials were then sterilized in a portable laboratory autoclave at 121°C for 15 min.

### Collection, authentication and processing of plant materials

The fresh leaves and flowers of *S. siamea* were collected from the botanical garden of University of Maiduguri. Plant materials were identified and authenticated by a taxonomist, Professor S. S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Nigeria in respect with the description in published literatures (Dalziel, 1958; Keay et al., 1989). The plant materials were dried under shade at our Pharmaceutical Chemistry Laboratory for about four weeks and then made into powdered form, using mortar and pestle and then sieved.

### Extraction

The method of extraction in this experiment was by maceration. The general process on a small scale, consist of placing the powdered plant material (250 g) of leave was soaked in 500 ml methanol while that of flower was soaked with different solvents that is water, petroleum ether, methanol, chloroform and ethyl acetate (in order of decreasing polarity) in 1 L capacity conical flasks stopper and kept for 48 h with intermittent shaking. The cold extracts thus obtained were filtered with Whatman No. 1 filter paper into different conical flask and allowed to dry at room temperature under normal atmospheric pressure. 50 g of the powdered leaves were soaked in 100 ml distilled water and the extract was obtained using the aforementioned method.

### Phytochemical analysis

Phytochemical analysis for the qualitative detection of alkaloids, anthraquinone, carbohydrates, flavonoids, tannins and saponins



**Table 1.** Leaves extraction results.

Parameter	Methanol	Chloroform	Ethyl acetate
Volume of solvent used (ml)	500	500	500
Weight of dried powdered (g)	200	200	200
Weight of solvent extract (g)	53.5	4.3	5
Extractive value (%)	26.8	2.15	2

**Table 2.** Leaves methanol extract partitioned

Parameter	Methanol/Water	Chloroform	Ethyl acetate	Petroleum ether
Volume of solvent used for partitioning (ml)	150	150	150	150
Weight of partitioned solvent extract (g)	47.6	18.8	9.9	20.8

was carried out on the extracts as described by Trease and Evans (2010), Sofowora (1993) and Harbone (1973).

#### Thin layer chromatography (TLC)

Commercially available standard TLC plate was used with standard particle size range to improve reproducibility. The absorbent silica gel coated on an aluminum foil of 22 cm length, 11.5 cm breadth and 0.3 cm thick plate for leaves while 22 cm length, 11.9 cm breadth and 0.3 cm thick plate for flower. Small spot of the solution containing the sample was applied on the plate 1.5 cm from the bottom marked by a line ruled using a pin. For a multiple spotted plate, the spots are applied 1 cm apart to avoid cross contamination and interference as they move up the plate.

#### Spotting and development

The sample spotted on the plate was allowed to dry before the plate was placed into the chromatographic tank which was covered immediately after which its atmosphere is completely saturated with solvent (mobile phase). The reaction was then monitored as the solvent moved up the plate (elutes the sample) using mobile phase solvent ratio 1:3:1 of methanol, n-hexane and ethyl acetate, respectively. When the solvent reaches the top of the plate, it is removed, marked and dried.

#### Visualization

Following separation of the solvent, the plate was removed and dried; the spots detected using various techniques and reagents. This includes visualization in daylight; viewing under UV at 254 and 366 nm i.e. short and long wavelengths and spraying with spotting reagent, using iodine vapour tank.

#### Findings

The phytochemical screening of methanol extracts of leaves revealed the presence of cardiac glycoside, flavonoid, saponin, alkaloid and tannins while chloroform extracts of leaves revealed saponin only. Ethyl acetate and petroleum ether extracts revealed absence of all these phytochemicals. The chloroform, ethyl acetate and petroleum ether extracts of flower revealed absence of

saponin, flavonoids, tannins and alkaloids but with traces of saponin. Anthraquinones glycosides was absent in all the extracts.

#### Extraction process for leaves and flowers

This is seen in Tables 1 and 2.

Extractive value = weight of plant (part) extract/weight of dry powdered sample × 100

Volume of methanol used = 1 L.  
 Weight of dried powdered = 300 g.  
 Weight of methanol extract = 101 g.  
 Extractive value =  $101/300 \times 100 = 33.7\%$

#### Phytochemical screening results

This is seen in Tables 3 to 9.

#### Thin layer chromatography (TLC)

Extracts of leaves and flowers were individually applied on the origin, they dissolved and moved with the solvent, each extract separated into bio constituents and moved to different locations. After all the spots became clear. UV fluorescence lamp at 254 nm was used to visualize and identify all the various spots. However, at 366 nm and daylight, spots were not clearly visualized. On exposure to iodine vapour, spots of various extracts became darker. TLC separation showed nine (9) spots each of chloroform and ethyl acetates, six (6) spots of methanol, three (3) spots of petroleum ether from leaves extracts. While, three (3) spots each of ethyl acetate and methanol, six (6) spots of chloroform were identified for flower extracts. No water spot separated from both leaves and flower extracts.

#### Summary of TLC results

##### Leaves TLC

Length of the plate = 22 cm.  
 Breadth of the plate = 11.5 cm.  
 Thickness of the plate = 0.1 cm.



**Table 3.** Test for carbohydrate.

Tests	Extracts	Solvents used				
		Water	Methanol	Chloroform	Ethyl acetate	Petroleum ether
Molisch's test (for carbohydrate)	leaves	++	++	-	++	+
	flowers	++	++	+	+	
Iodine test (for starch)	leaves	-	+	++	+	-
	flowers	-	+	++	+	
Fehling's test (for reducing sugar)	leaves	-	++	+	+	+
	flower	-	++	-	++	
Combined reducing sugar test	leaves	-	+	++	++	+
	flowers	+	++	-	+	
Barfoed test (for monosaccharides)	leaves	-	-	-	-	-
	flowers	-	-	-	-	-

++ More abundance, + Abundance, - Absence

**Table 4.** Tests for tannins (hydrolysable and condensed).

Tests	Extracts	Solvents used				
		Water	Methanol	Chloroform	Ethyl acetate	Petroleum ether
Lead sub- acetate test	Leaves	+	++	-	++	-
	Flowers	+	++	-	-	-
Ferric chloride test	Leaves	++	+	-	+	-
	Flowers	++	+	-	+	-
Bromine water test	Leaves	-	-	-	-	-
	Flower	-	-	-	-	-

++ More abundance, + Abundance, - Absence

Solvent front of the plate = 18.3 cm.

$R_f$  value = distance move by the solute ÷ distance move by the solvent.

Tables 10 to 13 shows the TLC results of leaves.

#### Flowers TLC

Length of the plate = 22 cm.

Breadth of the plate = 11.9 cm.

Thickness of the plate = 0.1 cm.

Solvent front of the plate = 17.9 cm.

$R_f$  value = distance move by the solute ÷ distance move by the solvent.

Tables 14 to 16 shows TLC results of flowers.

## DISCUSSION

*S. siamea* plants grow virtually everywhere in Nigeria and Maiduguri in particular. The plant has been used in this region for the treatment of typhoid fever and fever related conditions. Traditionally, it has also been used for treatment of jaundice, abdominal pain, menstrual pain, and hypoglycemic agent among diabetics. Ethno medicinally, *S. siamea* is used as laxative, blood cleaning agent, cure for digestive system and genitourinary

**Table 5.** Tests for anthraquinones glycosides.

Tests	Extracts	Solvents used				
		Water	Methanol	Chloroform	Ethyl acetate	Petroleum ether
Borntrager's Test	Leaves	-	-	-	-	-
	Flowers	-	-	-	-	-

++ More abundance, + Abundance, - Absence

**Table 6.** Tests for cardiac glycosides.

Tests	Extracts	Solvents used				
		Water	Methanol	Chloroform	Ethyl acetate	Petroleum ether
Burchard test (for glycosides steroids)	Leaves	-	-	-	+	++
	Flowers	-	-	-	+	-
Salkwoskii test (for steroidal nucleus)	Leaves	-	++	-	+	+
	Flowers	-	+	-	+	-
Keller killiani's test	leaves	++	+	-	-	++
	flower	++	-	+	+	-
Kedde test	leaves	-	-	-	-	-
	flowers	-	-	-	-	-

++ More abundance, + Abundance, - Absence

**Table 7.** Tests for Saponin.

Tests	Extracts	Solvents used				
		Water	Methanol	Chloroform	Ethyl acetate	Petroleum ether
Frothing test	Leaves	++	+	++	-	-
	Flowers	+	-	-	-	-
Haemolysis test	Leaves	+	+	+	-	-
	Flowers	+	+	-	-	-

++ More abundance, + Abundance, - Absence

disorders, herpes and rhinitis (Aliyu, 2006). When decocted, *S. siamea* leaves are locally used as anti-malaria drug (Lose et al., 2000). Previous studies on *S. siamea* extracts have confirmed some of the traditional uses: antiplasmodial activity (Gbeassor et al., 1990; Nsonde-Ntandou et al., 2005; Mbatchi et al., 2006). Antibacterial activities of the extract were tested against thirteen pathogenic bacteria and were compared with the standard antibiotic, kanamycin by measuring the zone of inhibition diameter and expressed in millimeter (mm) (Hailu et al., 2005; Dahiru et al., 2013).

Phytochemical screening reveals that methanolic extract contains carbohydrate, cardiac glycosides,

saponins, flavonoids, tannins and alkaloids. The extracting solvent used are decreasing order of polarity in which each of them extract a number of solvent to their own polarity depending on the active metabolites the plant contained. Based on this experiment the alkaloid, tannins and saponins content of this can be responsible for its antibacterial activity (Dahiru et al., 2013)

Preliminary phytochemical analysis showed that leaf extracts of *S. siamae* possesses alkaloids, saponins, tannins and glycosides which is in support with studies done by Momin et al. (2012), Edeoga et al. (2005) and Bukar et al. (2009). Phytoconstituents such as saponins, phenolic compounds and glycosides when present in *S.*

**Table 8.** Tests for Flavonoids.

Tests	Extracts	Solvents used				
		Water	Methanol	Chloroform	Ethyl acetate	Petroleum ether
Shinoda's test	Leaves	-	+	-	-	-
	Flowers	-	+	-	-	-
Ferric chloride test	Leaves	++	+	+	+	-
	Flowers	+	-	-	-	-
Lead acetate test	Leaves	+	++	-	-	-
	Flower	+	++	-	-	-
Sodium hydroxide test	Leaves	+	-	-	-	-
	Flowers	+	-	-	-	-

++ More abundance, + Abundance, - Absence

**Table 9.** Tests for alkaloids.

Tests	Extracts	Solvents used				
		Water	Methanol	Chloroform	Ethyl acetate	Petroleum ether
Mayers reagent	Leaves	-	-	-	-	-
	Flowers	-	-	-	-	-
Dragendorff A × B	Leaves	-	-	-	-	-
	Flowers	-	-	-	-	-
Wagners reagent	Leaves	+	+	-	-	-
	Flower	+	+	-	-	-
10% w/v tannic acid	Leaves	-	+	-	-	-
	Flowers	-	-	-	-	-
1% w/v picric acid	Leaves	-	+	+	-	+
	Flowers	-	+	-	-	-

++ More abundance, + Abundance, - Absence

**Table 10.** Leaves methanol extract TLC results.

Spots positions (cm)	R <sub>f</sub> values (cm)	Day light	UV-254 nm	UV-366 nm	Iodine vapour
16.3	0.89	Green	Green	Blue black	Light Green
15.2	0.83	Green	Green	Blue black	Light Green
14.1	0.77	Light green	Light green	Blue black	Yellow
11.5	0.62	Light yellow	Light yellow	-	Yellow
10.8	0.59	Yellow	Light brown	-	White
9.3	0.50	White	White	-	Brown

- No colour

*siamea* have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal

infections (Gonzalez and Mather, 1982; Okwute, 1992). Cardiac glycosides have also been found useful in

**Table 11.** Leaves Chloroform extract TLC results.

Spots positions (cm)	R <sub>f</sub> values (cm)	Day light	UV-254 nm	UV-366 nm	Iodine vapour
16.8	0.91	White	White	White	Yellow
16.1	0.87	Green	Violet	Blur black	Green
14.6	0.79	Green	Green	Blue black	Green
14.4	0.78	Light green	Green	-	Yellow
13.7	0.74	Light green	Light green	-	Yellow
13.5	0.73	Light brown	White	-	Yellow
11.9	0.65	White	Violet	-	Brown
10.8	0.59	Light brown	Light brown	-	Brown
9.8	0.53	Light yellow	Violet	-	Brown

- No colour

**Table 12.** Leaves ethyl acetate extract TLC results.

Spots positions (cm)	R <sub>f</sub> values (cm)	Day light	UV-254 nm	UV-366 nm	Iodine vapour
15.9	0.86	Green	Light green	Blue black	Yellow
14.8	0.80	Green	Green	Blur black	Green
13.9	0.75	Light green	Green	Blue black	Green
13.1	0.71	Light green	Light brown	-	Yellow
11.3	0.61	Light green	Light brown	-	Yellow
10.5	0.57	Yellow	Light brown	-	Yellow
9.4	0.51	Brown	Brown	-	Yellow
8.7	0.47	Green	Brown	-	Brown
7.6	0.41	Light green	Violet	-	Brown

- No colour

**Table 13.** Leaves petroleum ether extract TLC results.

Spots positions (cm)	R <sub>f</sub> values (cm)	Day light	UV-254 nm	UV-366 nm	Iodine vapour
16.3	0.89	Green	Green	Blue black	Green
15.0	0.81	Green	Green	-	Light green
14.6	0.79	Yellow	Violet	-	Yellow

- No colour

**Table 14.** Flowers methanol extract TLC results.

Spots positions (cm)	R <sub>f</sub> values (cm)	Day light	UV-254 nm	UV-366 nm	Iodine vapour
11.9	0.66	Yellow	Violet	Blue black	Brown
10.8	0.60	Light brown	Brown	-	Brown
9.7	0.54	Yellow	Violet	-	brown

- No colour

treatment of heart failure and supraventricular arrhythmias (Zamotaev et al., 2005). The traditional uses indicate that both the leaves and flowers have been used together for therapeutic purposes. From this study, findings showed that the leaves have more phyto-

chemicals than the flowers and since all active metabolites in the flowers are also present in the leaves, using leaves alone might suffice for treatments.

Presence of alkaloids, tannins, saponins, glycosides, steroids, phenolic compounds and flavonoids in all the

**Table 15.** Flowers chloroform extract TLC results.

Spots positions (cm)	R <sub>f</sub> values (cm)	Day light	UV-254 nm	UV-366 nm	Iodine vapour
15.9	0.88	Green	Light green	White	Brown
15.5	0.86	Light green	Yellow	White	Brown
14.5	0.81	White	White	White	Brown
12.9	0.72	Yellow	Violet	White	Brown
11.9	0.66	Yellow	Violet	White	Brown
8.9	0.49	Light brown	Brown	White	brown

- No colour

**Table 16.** Flowers ethyl acetate extract TLC results.

Spots positions (cm)	R <sub>f</sub> values (cm)	Day light	UV-254 nm	UV-366 nm	Iodine vapour
15.9	0.88	Light green	Green	-	Brown
15.5	0.86	Yellow	Yellow	-	Brown
14.6	0.81	Light yellow	Violet	-	brown

- No colour, Solvent system- methanol: n-hexane: ethyl acetate (20: 60: 20), Running time- 53 min, Adsorbent used- silica aluminum sheet, Locating reagent- Day light, UV 254 nm, UV 366 nm and iodine vapour

extracts confirmed the presence of rich bioactive principles in the leaf. Tannins, steroids and glycosides had been reported in ethanol extract of the leaf of *S. siamea* (Bukar et al., 2009; Muhammad et al., 2012) while alkaloids, saponins, phenolics and flavonoids by Momin et al. (2012). Secondary metabolites are mostly produced by plant during adverse condition for protection against herbivores (Chitra et al., 1999). Alkaloids, flavonoids, tannins and saponins were known to show medicinal activity as well as exhibiting physiological activity (Edeoga et al., 2005). The presence of phenolic group in plants is to protect them from microbial, insect and herbivores damage (Conco, 2000). Many of these active compounds also possess other functional attributes like anti-inflammatory, antimutagenic, hypocholesteremic and antiplatelet aggregation properties (Praveena et al., 2012). These phytochemical compounds carry out their activity by combining with protein, lipids or other components of the bacterial cell membrane that are relevant to one or more vital physiological roles thereby disrupting the integrity and functional behaviour of the membrane (de Kruijff et al., 2000).

## Conclusion

From our findings, it can be concluded that *S. siamea lam* contains some significant phytochemicals that can exhibit

desired therapeutic activities such as hypoglycemia, anti-arrhythmia and antimicrobial. However there is the need to conduct further pharmaceutical analyses on test extracts in order to establish these biomedical applications.

## Conflict of interest

There are none to declare.

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Full Length Research Paper

## Separate and co-administration of *Amaranthus spinosus* and vitamin C modulates cardiovascular disease risk in high fat diet-fed experimental rats

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Report indicates that global death from cardiovascular diseases is more than any other diseases hence research is being intensified to provide scientific data in support of the use of traditional plants for its management. In this study, the efficacy of *Amaranthus spinosus* on lipid disorder occasioned by dietary regimen supplemented with lard and its role in oxidative stress was compared with vitamin C. Forty adult male rats randomized into 5 groups of 8 each were used. Group 1 was the control, while groups 2, 3, 4 and 5 were placed on lard supplemented diet. Leaf extract of *A. spinosus* was administered to rats in groups 3 while group 4 was co-administered with *A. spinosus* and vitamin C. Group 5 was administered with vitamin C alone. The extract was administered at a dose of 250 mg/kg while vitamin C was administered at a dose of 10 UI/kg. All administrations were performed orally as a single dose continuously for 28 days. High fat diet increased malondialdehyde concentration but reduced the concentrations of glutathione (GSH) and the activities of catalase and superoxide dismutase in the heart. It also increased plasma cholesterol, triglyceride, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol but lowered high density lipoprotein (HDL) cholesterol concentration. Although, no significant alterations were observed in the cholesterol and triglyceride levels of the heart, there was a significant increase in the atherogenic indices of plasma. Separate and combined administration of *A. spinosus* and vitamin C reverses these unfavorable alterations. The effect of separate administration of *A. spinosus* was also observed in the study to compare effectively with its combined administration with vitamin C. Based on this study, *A. spinosus* may be useful as a base medicine for the management of cardiovascular diseases (CVD) related disorder.

**Key words:** Atherogenic index, *Amaranthus spinosus*, cardiovascular risk, herbal medicine, high-fat diet, lipoprotein.



## INTRODUCTION

Cardiovascular diseases (CVDs) are a class of pathologies involving the heart or blood vessels (arteries, capillaries, and veins). They refer to any disease that affects the cardiovascular system, mainly cardiac diseases, vascular diseases of the brain and kidney, and peripheral arterial disease (Ursula et al., 2014). Reported data (Sydney et al., 2012) indicated that an estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke and the number is expected to grow to 23.6 million by 2030 (World Health Organization (WHO), 2015). This report is supported by data from previous studies (Ignarro et al., 2007; Burta et al., 2008; Norozi et al., 2011).

Obesity, high blood pressure, insulin resistance, and aging are associated with the development of CVDs (De Marchi et al., 2013). Other important factors are diet, lifestyle, environmental, genetic and epigenetic interactions (Haslam and James, 2002). These factors reflect complex pathological processes in which oxidative stress caused by reactive oxygen species (ROS) plays a pivotal role. Oxidative stress represents an imbalance between reactive oxygen species (ROS) production and the cellular antioxidant defense system. In stress conditions, ROS levels increase, and because of their high reactivity, participate in a variety of chemical reactions. They are involved in cell damage, necrosis and apoptosis via oxidation of lipids, proteins and DNA (Elahi et al., 2009) and provoke also endothelial dysfunction, infiltration and activation of inflammatory cells (Hulsmans et al., 2012). Oxidative stress has been noted to play a central role in the pathogenesis of atherosclerosis and thus a critical feature in atherogenesis (Lee et al., 2012). An increased generation of ROS in the vascular wall and a reduction of nitric oxide (NO) bioavailability lead to endothelial dysfunction in atherogenesis (Lee et al., 2012; Channon and Guzik, 2012). ROS cause damage to cellular structures within the vascular wall, and trigger several redox-sensitive transcriptional pathways, shifting the cell towards a proatherogenic transcriptomic profile.

Increasing evidence indicates that certain natural products can influence the aetiology, progression and treatment of CVD (Osadolor et al., 2005; Amadou et al., 2009; Zuchi et al., 2010; Sahebkar, 2013) and this may occur by modifying risk factors such as obesity, dyslipidaemia as well as factors involved in systemic inflammation, oxidative stress and thrombosis (Parikh et al., 2005; Napoli et al., 2007). The role of dietary fat in

health has been under intensive research during the past decades (Ursula et al., 2014). Many observational studies reported that the total amount of dietary fat has only a minor or no effect on the risk of lifestyle diseases such as CVD, type 2 diabetes mellitus (T2DM), and cancer or the level of the risk factors of these diseases, or markers (abdominal adiposity, blood pressure (BP), serum lipid profile, and insulin sensitivity) (Food and Agriculture Organization (FAO), 2010). However, the quality of fat has been shown to have a significant effect on serum lipid profile and blood pressure (BP) (Uusitupa et al., 2013) as well as endothelial function and low-grade inflammation, and these has been shown to affect the risk of CVD (Graham et al., 2007; FAO, 2010; Astrup et al., 2011; Lopez-Garcia et al., 2004; Uusitupa et al., 2013). Current studies are focused at investigating certain nutraceuticals with potential to reduce cardiovascular disease risk by improving plasma lipid profile (Cinzia et al., 2010; Norata et al., 2013; Sahebkar et al., 2014).

*Amaranthus spinosus* L. (prickly amaranthus or water leaf) is known among the Yoruba tribe of Nigeria as “efo tete” or “tete eleegun”. It is a medicinal plant under the family of Amaranthaceae and an annual or perennial herb native to Tropical America (Bagepalli et al., 2010). The plant grows in cultivated areas as well as in waste places. The leaves are stacked and alternate (Debiprasad et al., 2013). Medicinal uses of *A. spinosus* as reported in the literatures (Bagepalli et al., 2010; Debiprasad et al., 2013) include: diuretic, stomach disorder, peptic ulcer and anemia (leaf infusion); gonorrhoea, eczema and menorrhoea (root paste). In some traditional homes in Nigeria, the plant is being used as analgesic, antipyretic, laxative, diuretic, anti-snake venom and antidiabetic.

Although the plant is very popular in the traditional medicine in Nigeria as anti-diabetic and anti-lipidemic agent suggesting that it may be a good agent in the management of cardiovascular diseases, but scientific evidence is lacking in support of this use. The aim of this study therefore is to provide scientific data to validate the use of the plant in the management of cardiovascular disorders and oxidative stress and compare its efficacy with that of vitamin C.

## MATERIALS AND METHODS

### Preparation of plant extract

Fresh leaves of *A. spinosus* were collected from a local garden in Ikenne, Nigeria in July, 2013. The plant materials were

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authenticated at the Department of Plant Science, Faculty of Science, Olabisi Onabanjo University, Nigeria. A voucher number Ars 013 NF was assigned and voucher specimen was thereafter deposited at the Herbarium. Twenty (20) grams of the powdered sample was macerated with 100 ml 70% methanol, filtered, concentrated using rotary evaporator (Yamato Scientific RE301A- W, Tokyo) and lyophilized with Hull brand (SP Scientific Series, USA) freeze-drier. Stock solution was prepared by dissolving the dried extracts in distilled water and was stored at -20°C until used.

### Animal handling

Forty male Wistar strain rats, weighing between 150 and 220 g self-reared at the Animal holding, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ikenne, Nigeria were used in the study. The animals were kept under ambient condition and were allowed to acclimatize for a week while being fed with standard rat chow (obtained from Animal Care Nig. Ltd) and water *ad libitum*. Experimental animals were handled appropriately as outlined by the guidelines of Experimental Animals Ethics Code of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Nigeria. The protocol conforms to the guidelines of the National Institute of Health for laboratory animal care and use (NIH, 2011), and in accordance with the principles of good laboratory procedure (WHO, 1998). The rats were randomly assigned into 5 groups of 8 rats each. The rats were then treated as follows:

- Group 1: Normal control (standard rat chow),
- Group 2: Test control (high dietary fat),
- Group 3: Test I (high dietary fat and administered with *A. spinosus*, extract),
- Group 4: Test II (high-fat fat and administered with *A. spinosus* extract + vitamin c),
- Group 5: Test III (high dietary fat and administered with vitamin c).

High fat diet was prepared by supplementing the normal rat chow with 15% lard (Chi et al., 1982). All the rats were fed with their respective diet *ad libitum*. The extract and vitamin C were administered orally using oral intubator at a dose of 250 mg kg<sup>-1</sup> and 10 UI kg<sup>-1</sup>, respectively. All administrations were carried out once daily for 28 consecutive days.

### Preparation of organ homogenate and blood sample

After the administration of last dose, the rats were fasted for 12 h and then anesthetized in a closed jar of cotton-soaked diethyl ether. Blood was withdrawn from the rats by cardiac puncture after which they were sacrificed by cervical dislocation. The blood samples were collected in heparinized bottles and the heart excised, weighed and stored in buffered petri-dishes before being homogenized in phosphate buffer (pH 7.3) (Paul et al., 2013). Afterwards, blood samples and heart homogenates were centrifuged and supernatant collected for biochemical analyses.

### Biochemical assay

Assay for catalase and superoxide dismutase activities was according to the previously described methods of Sinha (1972) and Marklund and Marklund (1974), respectively. Lipid peroxidation was assessed by determining the malondialdehyde as described by

Varshney and Kale (1990). Reduced glutathione was measured by the method of Beutler et al. (1963). Heart lipid was extracted using the method of Folch et al. (1957). Randox<sup>TM</sup> diagnostic kits (Randox Laboratories, U.K.) was then used for estimation of the lipid content. Cholesterol and triglyceride were determined enzymatically (Belcher et al., 1991). HDL-cholesterol (HDL-C) was estimated by the HDL precipitant method (Bachorik and Albers, 1996). VLDL-cholesterol (VLDL-C) and LDL-cholesterol (LDL-C) were calculated using Friedewald et al. (1972) formula. Atherogenic index of plasma (AIP) was calculated using the formula of Abot et al. (1998) and coronary risk ratio (CRR) was obtained by the method of Allard et al. (1994).

VLDL-C = Serum triglyceride/2.2,

LDL-C = Total serum cholesterol-Total serum triglycerides/5-Total serum HDL-C,

AIP = Total serum triglycerides/Total serum HDL-C,

CRR = Total cholesterol/HDL-C.

### Statistical analysis

Statistical package for social sciences (SPSS) v20.0 software package was used for data processing. Data analyses were done with one-way analysis of variance (ANOVA) and level of significance tested at  $p \leq 0.05$  with Duncan multiple range test (DMRT).

## RESULTS

When compared with the normal control, the result of the lipid peroxidation and antioxidant status (Table 1) showed that increased dietary fat significantly ( $p \leq 0.05$ ) raised the level of malondialdehyde (MDA) in the rat and lowered significantly the level of reduced GSH and catalase activity. Separate administration of *A. spinosus* and vitamin C and when co-administered into rats placed on high dietary fat prevented increase in the level of heart MDA and significantly raised the level of reduced GSH and catalase activity. Although the catalase activity of rats placed on vitamin C ( $61.59 \pm 2.09$ ) was not different from that of those treated with *A. spinosus* ( $61.09 \pm 1.47$ ) and that which was co-administered with vitamin C and *A. spinosus* ( $65.94 \pm 2.17$ ), the level of reduced GSH of rats placed on vitamin C alone ( $25.97 \pm 1.18$ ) was observed to be lower than that of rats placed on *A. spinosus* ( $29.02 \pm 1.58$ ) and that of rats co-administered with vitamin C and *A. spinosus* ( $28.71 \pm 1.18$ ). No significant ( $p \geq 0.05$ ) difference was observed in the superoxide dismutase (SOD) activity of the treated groups when compared among each other and the activities were also not significantly different from that of the control groups. No significant alteration was observed in the total cholesterol and triglyceride level of the heart both with high fat diet and when vitamin C and *A. spinosus* were either separately administered or co-administered to rats placed on high fat diet (Table 2).

Result of plasma lipid profile (Table 3) indicates that

**Table 1.** Effects of treatment on heart oxidative status.

Group	Treatment	MDA (nmol MDA/g tissue)	Catalase ( $\mu\text{g}/\text{mg}$ protein) $\times 10^3$	SOD (ng/mg protein) $\times 10^3$	Reduced GSH ( $\mu\text{g}/\text{g}$ tissue) $\times 10^3$
1 (Normal control)	SRF	5.21 $\pm$ 0.19 <sup>a</sup>	54.61 $\pm$ 4.45 <sup>a</sup>	3.30 $\pm$ 0.01 <sup>a,b</sup>	28.13 $\pm$ 1.16 <sup>a</sup>
2 (Test Control)	HF	6.56 $\pm$ 0.08 <sup>b</sup>	25.15 $\pm$ 2.90 <sup>b</sup>	3.33 $\pm$ 0.00 <sup>b</sup>	22.20 $\pm$ 0.46 <sup>b</sup>
3 (Test 1)	HF + AS	5.21 $\pm$ 0.08 <sup>a</sup>	61.09 $\pm$ 1.47 <sup>c</sup>	3.25 $\pm$ 0.02 <sup>a</sup>	29.02 $\pm$ 1.58 <sup>a</sup>
4 (Test 2)	HF + As + VC	5.26 $\pm$ 0.12 <sup>a</sup>	65.94 $\pm$ 2.17 <sup>c</sup>	3.30 $\pm$ 0.01 <sup>a,b</sup>	28.71 $\pm$ 1.18 <sup>a</sup>
5 (Test 3)	HF + VC	4.56 $\pm$ 0.04 <sup>c</sup>	61.59 $\pm$ 2.09 <sup>c</sup>	3.32 $\pm$ 0.01 <sup>b</sup>	25.97 $\pm$ 0.85 <sup>c</sup>

Results presented are mean  $\pm$  SEM (n=8). Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan's multiple range test (DMRT). Value in the same column with similar superscript are not significantly different ( $p > 0.05$ ) from each other. SRF = Standard Rat Feed, HF = High fat diet, AS= *Amaranthus spinosus*, VC = Vitamin C, MDA = Malondialdehyde, SOD = Superoxidedismutase and GSH = Reduced glutathione.

**Table 2.** Effect of treatment on the heart cholesterol and protein level.

Group	Treatment	Cholesterol (mmol/L)	Triglyceride (mmol/L)	Total protein (mg/dl)
1 (Normal control)	SRF	89.70 $\pm$ 3.47 <sup>a</sup>	54.50 $\pm$ 0.93 <sup>a</sup>	5.88 $\pm$ 0.33 <sup>a</sup>
2 (Test control)	HF	88.70 $\pm$ 3.47 <sup>a</sup>	56.17 $\pm$ 2.55 <sup>a</sup>	5.46 $\pm$ 0.09 <sup>a</sup>
3 (Test 1)	HF + AS	86.90 $\pm$ 3.05 <sup>a</sup>	51.79 $\pm$ 3.58 <sup>a</sup>	5.20 $\pm$ 0.19 <sup>a</sup>
4 (Test 2)	HF + As + VC	82.00 $\pm$ 4.64 <sup>a</sup>	57.46 $\pm$ 3.27 <sup>a</sup>	5.17 $\pm$ 0.18 <sup>a</sup>
5 (Test 3)	HF + VC	87.60 $\pm$ 6.99 <sup>a</sup>	57.17 $\pm$ 1.08 <sup>a</sup>	5.22 $\pm$ 0.12 <sup>a</sup>

Results presented are mean  $\pm$  SEM (n=8). Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan's multiple range test (DMRT). Value in the same column with similar superscript are not significantly different ( $p > 0.05$ ) from each other. SRF = Standard Rat Feed, HF = High fat diet, AS= *Amaranthus spinosus*, VC = Vitamin C, MDA = Malondialdehyde, SOD = Superoxidedismutase, GSH = Reduced glutathione.

increased dietary fat significantly raised both the total cholesterol concentration (224.56  $\pm$  9.03 mmol/L) and plasma triglyceride concentration (101.30  $\pm$  6.68 mmol/L) significantly ( $p \leq 0.05$ ) above the normal control (133.94  $\pm$  0.95 mmol/L) concentration. Separate treatment with *A. spinosus* and vitamin C and their co-administration prevented this increase. The result also indicates that when separately administered, the concentration of plasma cholesterol of rats treated with vitamin C (158.18  $\pm$  4.08 mmol/L) was significantly higher than that of the rat treated with *A. spinosus* (138.21  $\pm$  0.42 mmol/L), but when co-administered, the plasma cholesterol concentration (126.81  $\pm$  1.24 mmol/L) was lower than that of the rat treated with *A. spinosus* alone. No significant difference was observed in the plasma triglyceride concentration of rat treated with *A. spinosus* alone (67.48  $\pm$  0.96 mmol/L) and that which was co-administered with *A. spinosus* and vitamin C (66.08  $\pm$  4.03 mmol/L). The observed triglyceride concentration in both groups though lower than that of the group placed on high dietary fat without subsequent treatment (101.30  $\pm$  6.68 mmol/L) was significantly higher ( $p \leq 0.05$ ) than the normal control value (58.96  $\pm$  0.36 mmol/L). Separate administration of vitamin C also reduced the plasma triglyceride level

below that of rats placed on high fat diet without treatment, however, the observed value of 92.16  $\pm$  3.70 mmol/L was still higher than that of the group treated with *A. spinosus*.

Table 3 also indicates that high fat diet significantly ( $p \leq 0.05$ ) increased the plasma LDL-cholesterol above the normal control value but lowered the plasma HDL-Cholesterol significantly ( $p \leq 0.05$ ). There was no significant difference in LDL- concentration for all treatments. The observed plasma HDL-cholesterol concentration of rat separately treated with vitamin C and *A. spinosus* (73.29  $\pm$  0.56 and 86.88  $\pm$  5.41 mmol/L, respectively) were not different from each other and were also not different from that of rats co-administered with *A. spinosus* and vitamin C (74.75  $\pm$  1.26 mmol/L) and the normal control value (79.19  $\pm$  0.77 mmol/L). However, the observed values in all these groups of rats were significantly ( $p \geq 0.05$ ) higher than that of the rats placed on high fat diet but without treatment (30.87  $\pm$  0.04 mmol/L). The result of VLDL- cholesterol concentration followed the same pattern, however the observed VLDL-cholesterol concentration of 23.14  $\pm$  1.03 mmol/L in rats treated with vitamin C alone was significantly higher than that of the rats treated with *A. spinosus* (13.51  $\pm$  0.05

**Table 3.** Effect of treatment on plasma lipid profile.

Group	Treatment	Cholesterol (mmol/L)	Triglyceride (mmol/L)	LDL-cholesterol (mmol/L)	HDL-cholesterol (mmol/L)	VLDL-cholesterol (mmol/L)
1 (Normal control)	SRF	133.94 ± 0.95 <sup>a</sup>	58.96 ± 0.36 <sup>a</sup>	31.95 ± 1.48 <sup>a</sup>	79.19 ± 0.77 <sup>a</sup>	13.10 ± 0.07 <sup>a</sup>
2 (Test control)	HF	224.56 ± 9.03 <sup>b</sup>	101.30 ± 6.68 <sup>b</sup>	42.43 ± 1.21 <sup>b</sup>	30.87 ± 0.04 <sup>b</sup>	22.09 ± 1.01 <sup>b</sup>
3 (Test 1)	HF + AS	138.21 ± 0.42 <sup>a</sup>	67.48 ± 0.96 <sup>c</sup>	33.84 ± 5.51 <sup>b</sup>	86.88 ± 5.41 <sup>a</sup>	13.51 ± 0.05 <sup>a</sup>
4 (Test 2)	HF + As + VC	126.81 ± 1.24 <sup>c</sup>	66.08 ± 4.03 <sup>c</sup>	38.85 ± 0.82 <sup>b</sup>	74.75 ± 1.26 <sup>a</sup>	13.29 ± 0.61 <sup>a</sup>
5 (Test 3)	HF + VC	158.18 ± 4.08 <sup>d</sup>	92.16 ± 3.70 <sup>b</sup>	38.46 ± 4.23 <sup>b</sup>	73.29 ± 0.56 <sup>a</sup>	23.14 ± 1.03 <sup>b</sup>

Results presented are mean ± SEM (n=8). Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan's multiple range test (DMRT). Value in the same column with similar superscript are not significantly different (p>0.05) from each other. SRF = Standard Rat Feed, HF = High fat diet, AS = *Amaranthus spinosus*, VC = Vitamin C, MDA = Malondialdehyde, SOD = Superoxidedismutase, GSH = Reduced glutathione.

mmol/L) alone and that of rats co-administered with *A. spinosus* and vitamin C (13.29 ± 0.61 mmol/L).

Table 4 shows that high fat diet significantly (p ≥ 0.05) increased the atherogenic indices (atherogenic risk index of plasma, AIP and coronary risk ratio, CRR). However, in rats placed on high fat diet and separately treated with *A. spinosus*, the AIP (0.81 ± 0.01) and CRR (1.60 ± 0.03) were not significantly different (p ≥ 0.05) from those of the normal control value (0.77 ± 0.01 and 1.68 ± 0.01, respectively). Similarly, the observed AIP (0.89 ± 0.05) and CRR (1.70 ± 1.10) in rats co administered with *A. spinosus* and vitamin C, were not different from that of the normal control value. Although the AIP and CRR was reduced during treatment with vitamin C alone (when compared with the normal control value), the observed values were significantly higher than those observed in the normal control group.

## DISCUSSION

Cardiovascular disease is currently one of the

world's leading causes of death (Ikewuchi and Ikewuchi, 2009). One of the major risk factors for the development of cardiovascular disease is dyslipidemia (American Diabetic Association, 2004; Shen, 2007). Dyslipidemia usually involve elevated plasma levels of triglycerides, total cholesterol, LDL and VLDL-cholesterol and a low level of HDL-cholesterol (Howard, 1987; Lakin and Lipsky, 2003; American Diabetic Association, 2004). Our result indicates that lard is a dietary fat capable of predisposing to cardiovascular disease. This is indicated by increased plasma cholesterol, triglyceride, LDL-cholesterol and VLDL-cholesterol and a reduce HDL-cholesterol observed in the study when rats were placed on lard supplemented diet. Lard is an animal fat which contains saturated fatty acid. Over the years, studies had reported an association between intake of dietary saturated fatty acids (SFA) and serum cholesterol levels (Mensink et al., 2003; Lukas and George, 2014) Therefore, any nutritional and pharmacologic intervention that improves or normalizes abnormal lipid metabolism may be useful in reducing the risk of cardiovascular diseases. Data from our study

thus support this assertion.

Presently, several drugs are available for the management of dyslipidemia. However, there is renewed interest in the use of herbal products (Mudhaffar, 2013; Assmann et al., 2008). This is partly due to the perceived safety of herbal drug and poverty level particularly among the rural populace. Report from the present study indicates that *A. spinosus* leaves contain phytochemicals capable of ameliorating symptoms of cardiovascular disease. Rats treated with the extract while subsisting on high fat diet showed a reduced level of triglyceride, plasma total cholesterol, plasma LDL-cholesterol and VLDL-cholesterol and an increased level of plasma HDL-cholesterol when compared with those not treated with the extract. A high plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases (Dobiasova, 2004; McBride, 2007) and is often associated with hypertension, abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus (Ostlund and Lin, 2007; McBride, 2007).

Our study demonstrated a reduction in triglyceride level occasioned by *A. spinosus* in rats

**Table 4.** Effect of Treatment on Atherogenic and Coronary Risk Indices.

Group	Treatment	AI	CRR
1 (Normal control)	SRF	0.77 ± 0.01	1.68 ± 0.01
2 (Test control)	HF	3.45 ± 0.05	7.45 ± 0.12
3 (Test 1)	HF + AS	0.81 ± 0.01	1.60 ± 0.03
4 (Test 2)	HF + AS + VC	0.89 ± 0.05	1.70 ± 0.10
5 (Test 3)	HF + VC	1.26 ± 0.06	2.16 ± 0.05

Results presented are mean ± SEM (n=8). Mean values were compared using one-way ANOVA. Level of significance was evaluated using Duncan's multiple range test (DMRT). Value in the same column with similar superscript are not significantly different ( $p > 0.05$ ) from each other. SRF = Standard Rat Feed, HF = High fat diet, AS = *Amaranthus spinosus*, VC = Vitamin C, MDA = Malondialdehyde, SOD = Superoxidedismutase, GSH = Reduced glutathione, AI = Atherogenic Index, CRR: Coronary Risk Index.

placed on high fat diet. We also observed that along with triglyceride and total cholesterol, *A. spinosus* also caused significant reduction in plasma LDL-cholesterol and VLDL-cholesterol and an increase in HDL-cholesterol, suggesting that it is a potent agent in reducing risk of cardiovascular disease. This is in conformity with reports from previous study which indicated that an elevated LDL-cholesterol concentration in plasma is atherogenic (Rang et al., 2005; Lichtennstein et al., 2006; Martirosyan et al., 2007), whereas, a high HDL-cholesterol level is cardioprotective (Miller and Miller, 1977; Assmann et al., 2008).

Atherogenic indices are powerful indicators of the risk of heart disease and have been successfully used as an additional index when assessing cardiovascular (CV) risk factors: the higher the value, the higher the risk of developing cardiovascular disease and *vice versa* (Frohlich and Dobiasova, 2003; Dobiasova, 2004; Brehm et al., 2004; Takasaki, 2005). AIP values of  $-0.3$  to  $0.1$  are associated with low,  $0.1$  to  $0.24$  with medium and above  $0.24$  with high CV risk (Dobiasova, 2006). Observation from our study indicates that high fat diet increases atherogenic indices (AI and CRR) suggesting that high fat diet predisposes to cardiovascular diseases. *A. spinosus* was observed to significantly reduce atherogenic indices (CRR and AIP). The effect produced by separate administration of the extract was not different from that observed with co-administration of the extract and vitamin C. Although separate administration of vitamin C was also observed in our study to also reduce atherogenic indices, the effect was however not as pronounced as it was with *A. spinosus*. In an attempt to be able to investigate the role of oxidative stress in the development of CVD and the possible mechanism by which *A. spinosus* ameliorates the disorder, we assessed the oxidative status of the heart during treatment. Result from the study indicate that rats placed on high fat diet showed increased MDA level, decreased catalase activity and decrease reduced glutathione concentration.

Administration of *A. spinosus* during treatment however prevented increase in malondialdehyde level and also boosted the antioxidant status. Thus, this suggests that the extract is capable of reducing oxidative stress induced by high dietary fat.

The imbalance between ROS production and the cellular antioxidant defense system resulting from oxidative stress has been implicated in cell damage, necrosis and apoptosis via oxidation of lipids, proteins and DNA (Elahi et al., 2009) and provoke also endothelial dysfunction, infiltration and activation of inflammatory cells (Hulsmans et al., 2012). Studies have reported that oxidative stress has a central role in the pathogenesis of atherosclerosis and that it is indeed a critical feature in atherogenesis (Liao et al., 1994; Elahi et al., 2009; Lee et al., 2012). An increased generation of ROS in the vascular wall and a reduction of nitric oxide (NO) bioavailability lead to endothelial dysfunction in atherogenesis (Lee et al., 2012). ROS cause damage to cellular structures within the vascular wall, and trigger several redox-sensitive transcriptional pathways, shifting the cell towards a proatherogenic transcriptomic profile. Animal models of atherosclerosis demonstrate the involvement of ROS in atherosclerosis by the accumulation of lipid peroxidation products (Liao et al., 1994). This is supported by the report from our study when we observed increased MDA level with increased fat diet. ROS and reactive nitrogen species (RNS) produced by the endothelium promote oxidative modification of low density lipoprotein in the phase that precedes the transfer into the subendothelial space of the arterial wall, where they initiate atherosclerosis (Stocker and Keaney, 2004). Data from our study thus suggest that *A. spinosus* contains bioactive component which has antioxidative potential and thus is able to mop up these ROS and prevent subsequent damage to the cellular macromolecules.

Studies on the stem bark extract of *A. spinosus* identified the betalains isolated from the bark of the plant

as amaranthin, isoamaranthine, hydroxycinnamates, rutin, quercetin and kaempferol glycosides (Stintzing et al., 2004; Ashok et al., 2008). Betalains are well known for their antioxidant, anticancer, antiparasitosis and antiviral properties (Hussain et al., 2008). Our study thus suggests the antioxidant properties of *A. spinosus* as a basis for its cardioprotective effect. This agrees with previous reports on cardiovascular protective effect of *Vaccinium meridionale* (Yasmin et al., 2013), *Phyllanthus emblica* and *Piper rostratum* (Wattanpitayakul et al., 2005) and methanolic extract of *Amaranthus viridis* (Saravanan et al., 2013).

## Conclusion

Our study showed antihyperlipidaemic effect of *A. spinosus*, indicating a possible protective mechanism of the plant against the development of atherosclerosis and coronary heart disease in high fat fed rat. The study also lends support to the antioxidative potential of the plant and suggests that this is the mechanism by which it serves as a cardioprotective agent. Provided animal to human extrapolation is allowed, our result suggests that the plant may be recommended as base medicine for use in managing cardiovascular diseases and in the development of drug against cardiac related disorder.

## Conflicts of interest

Authors have none to declare.

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The background of the journal cover features a close-up of several sliced citrus fruits, likely lemons or oranges, arranged in a circular pattern. A magnifying glass is positioned over the center, focusing on one of the slices. The overall color palette is warm, dominated by oranges, yellows, and browns.

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