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Aphrodisiac activity of oils from *Anacardium occidentale* L. seeds and seed shells

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Abstract

Oils from the seeds and seed shells of *Anacardium occidentale* L. were obtained by the use of a soxhlet extractor. Phytochemical analysis of the seed-oil revealed the presence of saponins, alkaloids, flavonoids, steroids and terpenoids, while tannins were absent. The seed-oil was later tested on male albino rats for sexual behavior which resulted to significant increase in mount and intromission frequencies, and decrease in mount latency which are considered as the indices of both libido and potency. Also, the seed shell oil of *Anacardium occidentale L.* was tested on both male and female albino rats for toxicity which demonstrated mortalities at various doses (0.10, 0.60 and 1.10 ml). Results of this study revealed that the seeds and seed shells of *Anacardium occidentale L.* have pharmacological and toxicological attributes. Thus, the seed oil of this plant should be used to manage impotency in male humans, while its seed shell oil should be used to kill mice in our homes by application on foods eaten by mice.

Keywords: seed-oil; seed-shell oil; aphrodisiac; Phytochemicals; toxicant; albino rats; *Anacardium occidentale*

Introduction

The cashew *Anacardium occidentale L.* is a tree in the flowering plant family, *Anacardiceae.* The plant is native to North-Eastern Brazil where it is called by its Portuguese name caju (the fruit) or cajueiro (the tree). What appears on the tree to be the true fruit of cashew is an oval pear-shaped pseudo fruit or false fruit that develops from the receptacle of cashew flower (Varghese and Pundir, 1964). Within the true fruit is a single seed, the cashew nut. The seed or nut is surrounded by a double shell containing a caustic phenolic resin (Tyman and Morris, 1989). Cashew plant is modest in its soil requirements and can adapt to varying soil conditions without impairing productivity. It grows best on deep friable well drained sandy loams without a hard pan, and thrives on pure sandy soils although mineral deficiencies are likely to occur. It is a tropical plant that thrives at high temperatures. Areas

where temperatures range from 20 to 30 ◦C with an annual precipitation of 1000mm-2000mm are ideal for cashew growing. It needs a climate with a well defined dry season of at least three months to produce the best yield.

Though cashew plant was initially used to prevent soil erosion, it is now mainly grown because of its great economic and medicinal values (Aiyadurai, 1963). Its commercial importance is due to the richness of its fruit and nut in nutrient that constitutes of 47% fat, 21% protein and 22% carbohydrate, vitamins and all essential amino acids especially thiamine(Rajesh et al., 2009). Apart from the use of the fruit and nut of cashew plant as food, there are medicinal and other properties of these parts of the plant. The fruit is eaten to treat scurvy and diarrhoea. It is also effective in preventing cholera and can be used as remedy to neurological pain and rheumatic fever (http://www.fao.org/inpho/content/documents/vlibrary/ac306e/ac306e04.htm, accessed on 20/03/2011). The seeds are consumed orally to cure impotency and as aphrodisiac (Evan, 2001; Onilude, 2010). Cashew nuts have various health advantages as they are significant sources of iron (essential for red blood cell function and enzyme activity), magnesium (promotes energy release and bone growth), phosphorus (builds bones and teeth) and zinc (essential to digestion and metabolism). In addition, the nuts contain significant amounts of phytochemicals with antioxidant properties that protect the human body from cancer and heart disease (Aiyadurai, 1963).

In tropical medicine, cashew nut shell liquid (CNSL) is used to treat leprosy, elephantiasis, psoriasis, ringworm, diabetes, warts and corns (Meylan et al., 1999).The liquid also has innumerable applications in polymer based-industries, such as friction linings, paints, vanishes, laminating resins, rubber compounding resins, polyurethane based polymers, surfactants, epoxy resins, wood preservatives, insecticides and fungicides (Mathew et al., 2006; Risfaheri et al., 2009). After extracting CNSL, cashew nut shell can be used in the manufacture of agglomerates. Together with the testa, it may be used either in the manufacture of dyestuff or to provide durability to hammocks. It offers much scope and varied opportunities for the development of other tailored polymers. (http://www.fao.org/inpho/content/documents/vlibrary/ac306e/ac306e04.htm, accessed on 20/03/2011).

Materials and Methods

Extraction

The extraction of CNSL was carried out using a Soxhlet extractor and n-hexane as solvent. 350ml of n-hexane was poured into the round bottom flask of the soxhlet apparatus. Subsequently, 20g of crushed cashew nut shell was introduced into the thimble and fitted into the soxhlet extractor. The apparatus was assembled. The solvent in the set-up was heated to 68^0c and the vapor produced was subsequently condensed by water flowing in and out of the extraction set-up. This process of heating and cooling continued until a sufficient quantity of CNSL was obtained for about 4hours. At the end of the extraction, the thimble was removed while the remaining solvent in the extractor was recharged into the round bottom flask for the process to be repeated. Finally, the set-up was then re-assembled and heated to recover the solvent from the oil (Mathew et al., 2006). This same procedure was used for the extraction of oil from the seeds of *Anacardium occidentale L.*

Qualitative phytochemical analysis

Test for tannins

Few drops of 1% lead acetate were added to 5ml of the oil extract in a test tube. A yellow precipitate was formed which indicated the presence of tannins.

Test for saponins

The oil extract was diluted with 2 ml of distilled water and it was agitated in a test tube for about 15 minutes. The formation of 0.1cm layer of foam showed the presence of saponins.

Test for flavonoids

Few drops of dilute sodium hydroxide were added to 1ml of the oil extract in a test tube. An intense yellow color was formed which turned colorless on addition of few drops of dilute acid indicating the presence of flavonoids.

Test for alkaloids

The oil extract (2ml) was added to 2ml of HCl. To the acidic medium, 1 ml of Dragendroff's reagent was added. An orange or red precipitate was immediately formed which indicated the presence of alkaloids.

Test for steroids

To 1ml of the oil extract in a test tube, 10 ml of chloroform was added. Equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turned red, whereas the sulphuric acid layer turned yellow with green fluorescence. This indicated the presence of steroids.

Test for terpenoids

Two ml of the oil extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (1 ml) was carefully added to form a layer. A reddish brown coloration was formed at the interface to show positive results for the presence of terpenoids.

Test for cyanogenic glycosides

Small quantity of the oil extract was put in a test tube. 1.5mL of distilled water and 6 drops of chloroform were added and the mixture stirred with a rod. The test tube was stoppered with a cork containing a strip of picrate-impregnated paper hanging down from the stopper, and incubated at ambient temperature for 2 hours. A color change of the paper, from yellow to brown-red, indicated the release of HCN by the plant. If there was no release of HCN within 2 hours, indicating a negative test, the tube was left at ambient temperature for 24 and 48 hours, so that it could be re-examined. A brown-red coloration within 2 h indicated

the presence of cyanogenic glycoside and the respective hydrolytic enzyme, and the plants were considered cyanogenic in the field. A brown-red color appearing within 48 hours indicated that the cyanogenic glycoside spontaneously released HCN without the action of enzyme. No color change after 48 hours indicated that the test was negative for cyanogenic glycoside.

Quantitative phytochemical analysis

Test for alkaloids

The powdered sample (5g) of cashew nut seed was weighed into a 250 ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for 4hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.

Test for flavonoids

The powdered sample (10g) was extracted twice with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman No.1 filter paper. The filtrate was later transferred into crucibles, evaporated to dryness on a water bath to a constant weight.

Test for saponins

The powdered sample (20g) was added to 100ml of 20% aqueous ethanol and kept in a shaker for 30 minutes. The samples were heated on a water bath for 4 hours at 55°C. The mixture was then filtered and the residue re-extracted with another 200ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40ml over water bath at 90°C. The concentrate was transferred into a 250ml separating funnel, extracted twice with 20ml diethyl ether. Diethylether layer was discarded, while aqueous layer was retained and 60ml of nbutanol was added to it. Then n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath and after evaporation the samples were dried in an oven (40°C) to a constant weight. The saponin content was calculated as percentage of the initial weight of sample taken.

Test for tannins

Distilled water (50ml) was added to 500mg of the sample in a 500 ml flask and kept in a shaker for 1 hour. The resultant mixture was filtered into a 50 ml volumetric flask and made up to the mark. 5 ml of the filtrate was pippetted into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 605 nm within 10 minutes.

Test for phytates

The powdered sample (4.0g) of cashew nut seed was soaked in100ml of 2% hydrochloric acid for five hours and was filtered. 25ml of the filtrate was taken into a conical

flask and 5.0ml of 3% ammonium thiocyanate solution was titrated with a standard solution of iron (III) chloride until a brownish-yellow color persisted for five minutes.

Test for Hydrogen cyanide

The powdered sample (10g) of cashew nut seed was soaked in a mixture of 200ml of distilled water and 10ml of orthophosphoric acid. The mixture was left for 12hours to release all bound hydrocyanic acid. Anti-bombing agents were added and the solution distilled until 150ml of the distillate collected. 20ml of the distillate was taken into a conical flask and diluted with 40ml of distilled water, 8.0ml of 6.0mol/dm_3 ammonium hydroxide and 2.0ml of 5% (w/v) potassium iodide solutions were added. The mixture was titrated with 0.02mol/dm_3 silver nitrate using a micro burette until a faint but permanent turbidity was obtained. (1ml of turbidity was obtained. (1ml of 0.02mol/dm $AgNO_3$=1.08mgHCN).

Mounting behavior test

Albino rats weighing between 200-250g and aged between 3-4months were selected for the study. The rats were housed in separate cages (males and females) and kept under normal conditions of temperature and light. The animals were allowed free access to food and fresh tap water for some short period. The male rats were later starved within the period that the experiment took place. They were randomly allocated into five groups of 3 rats each, and were dosed in their respective groups. Group 1 rats (3) were each given an oral dose of 500µg/ml, 1,000µg/ml and 1,500µg/ml of Viagra taking into consideration their body weights. Group 2 rats (3) were each given food and tap water only. Group 3 rats (3) were each given oral dose of 0.50ml of the oil extract of cashew nut seed. Group 4 rats (3) were each given oral dose of 1.0ml of the oil extract of cashew nut seed. Group 5 rats (3) were each given oral dose of 1.50ml of the oil extract of cashew nut seed. During the study male rats dosed as above in the morning, were kept for 30 minutes interval after which female rats were then introduced to them. The manifestation of male sexual behavior was observed with the aid of a digital camera, and results were recorded over a period of 2 hours and expressed as mount latency (ML), the time interval from when a female is introduced to a male to the first mount by the male; mount frequency (MF), the number of mounts without intromission from the time of introduction of the female until ejaculation; and intromission frequency (IF), the number of intromissions from the time of introduction of the female until ejaculation.

Acute toxicity test

To determine acute toxicity, doses of 0.10, 0.60 and 1.10 ml/kg (b.w) respectively of the liquid extract of cashew nut shell were administered orally to 3 groups of animals each consisting of 3 animals each. The albino rats were observed continuously for 1hr for any gross behavioral changes and deaths, if any, and intermittently for the next 6 hrs and then again at 48hrs after dosing. Survival animals were kept under observation for 7 days. The behavior parameters observed were asthenia, anorexia convulsion, hyperactivity, sedation, diarrhoea and increased respiration.

Results and Discussion

The results of qualitative analysis on oil extract of *Anacardium occidentale L*. seed samples are shown in Table1. The results obtained showed the presence of alkaloid,

Table 1. Phytochemical analysis results for cashew nut seed oil and cashew nut seed

Phytochemicals	Inferences (cashew nut seed oil)	% Composition (cashew nut seed)
Alkaloids	++	8
Saponins	++	12
Flavonoids	++	3
Steroids	+++	NC
Terpenoids	+++	NC
Tannins	-	NC
Phytates	NC	0.007
Hydrogen cyanide	NC	0.020

Key: + = slightly present; ++ = averagely present; +++ largely present;
- = completely absent; and NC = not carried out

saponins, flavonoids, steroids and terpenoids and the absence of tannins. These results are in accordance with the reports of Tedong et al., 2006 that *A. occidentale L.* contained the presence of alkaloids, and saponins. The phytochemicals found in the seeds of this plant which are also in other plants have been reported to have cytotoxic and pharmacological attributes (Mbatchou et al., 2011a and b). This is an indication that oils from the seeds of *Anacardium occidentale L.* contained antimicrobial agents.

Results for quantitative analysis of *Anacardium occidentale L.* seeds are presented in Table 1. Saponins showed the highest concentration of 12% followed by alkaloids (8%), flavonoids (3%), hydrogen cyanide (0.02%) and phytates (0.007%). A variety of herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the protective effect of fruits and vegetables has been attributed to phytochemicals, which are non-nutrient plant compounds.

Saponins have been implicated as possible bioactive agent responsible for aphrodisiac effect in *Tribulus terrestris* extract and this could be the reason why cashew nut seeds are consumed in Brazil, Columbia and India to increase libido or enhance sexual drive (Gauthaman et al., 2002). It has been reported that saponins can affect animal performance and metabolism in a number of ways including erythrocyte haemolysis, reduction of blood and liver cholesterol, depression of growth rate, inhibition of smooth muscle activity and reduction in nutrient absorption (Cheeke, 1971). Saponins have been found to be potentially useful for the treatment of hyperglycaemia (Olaleye, 2007). Saponins inhibit Na^+ efflux by the lockage of the entrance of the Na^+ out of the cell. This leads to higher Na^+ concentration in the cells, activating a Na^+-Ca^{2+} anti porter in cardiac muscle. The increase in Ca^{2+} influx through this anti porter strengthens the contractions of heart muscle (Schneider and Woliling, 2004).

The presence of flavonoids in Table 1 shows that the seed will be good for the management of cardiovascular diseases and oxidative stress, since flavonoids are biologic antioxidants. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrile. An imbalance between antioxidants and reactive oxygen species

results in oxidative stress, leading to cellular damage (Burlon and Ingold, 1984). Oxidative stresses have been linked to cancer, aging, atherosclerosis, inflammation, ischemic injury and neuro degenerative diseases (Palozza, 1998).

Flavonoids help provide protection against these diseases by contributing along with antioxidant vitamins and enzymes to the total antioxidant defense system to the human body. Many plants containing alkaloids and flavonoids have diuretic, antispasmodic, anti-inflammatory and analgesic effects, (Owoyele et al., 2002). In the traditional Nigerian and Brazilian pharmacopoeia, the stem-bark of *A. occidentale* L. is known for its inflammatory effects (Mota et al., 1985; Ojewole, 2004). This confirms its use internally and externally for the treatment of certain illnesses like diabetes, weakness, muscular debility, urinary disorders, diarrhoea, fungal infection, asthma, eczema and psoriasis (Dare et al., 2011).

A study done at Children's Hospital and Research Center Oakland, in collaboration with scientists at Heinrich Heine University in Germany proved that epicatechin, quercetin and luteolin types of flavonoids inhibited the development of fluids that result in diarrhoea by targeting intestinal cystic fibrosis membrane conductance regulators (Schuier et al., 2005). The presence of flavonoids in the seed of *Anacardium occidentale L*. reveals that it can be used for the treatment of diarrhoea.

However, alkaloids and flavonoids inhibit certain mammalian enzymatic activities such as those of phosphodiesterase, prolonging the action of cyclic-AMP. Alkaloids also affect glucagons and thyroid stimulating hormones (Okaka et al., 1992).

Studies have been carried out to determine the levels of phytates in different foods in many countries. For instance, a study in India showed that the phytate contents of foods ranged from 480 to 520mg/100g (Pushpanjali and Santosh, 1995). The phytate contents of Korean foods ranged from 191.7 to 973.3mg/100g for cereals, and from 508.5 to 1371.8mg/100g for legumes (Joung et al., 2004), while in Indonesia the contents ranged from 8 to 319 mg/100g for cereals, and from 24 to 1018 mg/ 100g for legumes (Sanny et al., 2007). The phytate content of cashew nut seeds determined in this research was 0.007% which is 6.78mg/100g. This value falls below the levels mentioned under the listed countries. High concentrations of phytates usually found in seeds must be considered as potential risk; against the backdrop that phytates can act as a chelating agent on some important nutritional ions such as zinc, calcium, magnesium, and iron (Erdman, 1979).

Terpenoid is a heart-friendly phytochemical constituent which helps to reduce diastolic blood pressure and lowers the sugar level in blood (Hawkins and Ehrlich, 2006). *A. occidentale L.* is one of the over 700 plants described to be beneficial in the treatment of diabetes mellitus (Day, 1995). The presence of terpenoids in cashew nut oil extract justifies why decoction of dried kernel is used for the treatment of diabetes mellitus.

Plant steroids are collectively known as phytosterols (Roberts, 1971). They are mainly restricted to plant membranes and may function there as cholesterol does in animal membranes (Goodwin and Mercer, 1983). It should be noted that steroidal compounds are of importance and interest in pharmacy due to their relationship with compounds such as sex h-

Table 2. Results for the effect of cashew nut seed oil on the sexual behavior of male albino rats.

Doses (ml)	Mount latency	Mount frequency	Intromission frequency
0.50	2.67± 0.58	24.33± 7.50	7.33± 4.04
1.00	2.17± 0.76	25.33± 3.51	8.67± 2.08
1.50	1.67± 0.58	28.00± 3.61	10.33± 2.92
+ve control (500µg/ml)	0.83± 0.29	37.67± 3.06	12.67± 2.52
-ve control	0.00	0.00	0.00

Key: ± = Standard error mean; +ve control = sildenafil citrate aphrodisiac drug 500µg/ml ≡ 0.5ml;
-ve control = neither oil extract nor sildenafil citrate aphrodisiac drug was administered.

ormones (Okwu, 2001). The presence of steroids in the seed of *Anacardium occidentale L*. is an indication that it contains compounds which are related to sex hormones.

Results in table 2 represent the effect of cashew nut seed oil on the sexual behavior of male albino rats. Sildenafil citrate was used as the standard drug of reference. The significant increase in the indices of sexual vigor that is mount and intromission frequencies, and the significant decrease in mount latency compared to the negative control are indications of the aphrodisiac potential of *Anacardium occidentale L.* oil extract from seeds. Also, other parameters such as genital sniffs and penile licks were observed when the extract was administered indicating a direct effect of the extract on libido or sexual drive. Comparing the negative control to oil extract administered, 0.50, 1.0, and 1.50ml; mount latency decreased by 36.38%, 29.56% and 22.75% respectively. In addition, the oil extract significantly increased mount frequency by 21.10%, 22.0% and 24.28%, and intromission frequency by 18.79%, 22.23% and 26.49% respectively in a dose dependent manner. The mount and intromission frequencies are considered as the indices of both libido and potency (Rosen and Ashton, 1993; Ratna-Sooriya and Dharmasiri, 2000). The standard drug was found to produce significant reduction in mount latency (ML) as compared to the oil extract, while a highly significant increase was found in mount and intromission frequencies vis- a-vis the standard drug (sildenafil citrate). This is also an evidence of the sexual function improving effect of the oil extract of *Anacardium occidentale L*. seeds. Among the phytochemicals identified, saponins showed the highest concentration of 12%. Studies have linked the saponin component of plants in enhancing aphrodisiac properties due to its androgen increasing property. Saponins present in the oil extract of this plant might have assisted in stimulating an increase in the body natural endogenous testosterone levels by raising the level of leutinizing hormones (LH). This LH released normally by the pituitary gland helps to maintain testosterone levels; as LH increases, so does the testosterone (Gauthaman et al., 2002). The increase in testosterone seemed to have translated into the male sexual competence observed in this study. Furthermore, phytochemical analysis revealed steroids. Thus, the resultant aphrodisiac effectiveness of the oil extract might also be attributed to steroids (Tajuddin et al., 2004). In the present study, the oil extract from the seed was found to be devoid of any general conspicuous short term toxicity. Toxicity test for the oil extract of the seed recorded 0% mortality rate and no signs of toxicity after 14days of administration of the extract. Long term toxicity remains to be investigated.

Contrary, when 0.10ml of the liquid extract of cashew nut husk was administered, deaths were recorded on the 3rd day. Doses of 0.10, 0.60 and 1.10 ml of the cashew nut shell liquid extract administered orally to 3 groups of the albino rats, each consisting of 3 animals each, recorded mortalities of 66.6, 100 and 100% respectively. At 0.60ml, all the animals died on the next day after the administration of the liquid extract. Also at 1.10ml 2 hours after the administration of the extract, all the rats died. In addition, at every level of the extract dose the rats showed symptoms such as anorexia, asthenia, reduction in motor activity, increased respiration and syncope. However, the symptoms were more pronounced at a dose of 1.10ml. To confirm the toxic nature of any plant product, one has to consider several factors that can alter its toxicity profile, including the growth stage, and the maturity of the plant, the specific part (s) of the plant (such as leaves, roots, bark, flowers, seeds etc..) used, the storage conditions of the product (freshly collected or stored for long time), the seasonal variation in the relative abundance of phytochemicals (Jaouad et al., 2004). Qualitative tests of *Anacardium occidentale L.* liquid extract from seed revealed the presence of alkaloids, saponins, steroids, terpenoids and flavonoids. The toxic activity of *Anacardium occidentale L.* liquid extract of the seed shell may be related to its alkaloid content. Importantly, more than 350 species which contain alkaloids have been shown to display a wide spectrum of toxicological activities (Schuppan et al., 1999; Stedman, 2002; Pageaux and Larrey, 2003). Larrey (1997) have also reported that some galenic preparations are hepatotoxic because of their alkaloid content. Results of this study revealed that the seed oil of *Anacardium occidentale L.* increased the sexual libido and potency of male albino rats, and have provided scientific evidence to support the acclaimed role of the plant's seed as an aphrodisia in traditional medicine. Also, the seed shell liquid exhibited toxicological activity on albino rats. The seed oil of *Anacardium occidentale L.* should be drunk to improve sexual libido and potency in male humans, while the liquid extract from the seed shell of this same plant should be applied on food stuffs that are fed on by mice to intoxicate mice that cause destruction of items in our homes.

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