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Comparative Analysis of Anthraquinones from Five Medicinal Plants

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Abstract:
The present study was conducted to evaluate the comparative analysis of anthraquinones from five selected medicinal plants both qualitatively and quantitatively. Anthraquinones present in the leaf extract was compared with that of the stem bark extract to show which has the highest anthraquinones. The result of the preliminary qualitative phytochemical screening of all the plant parts reveals the presence of anthraquinones with the exception of A. senegalensis. Quantitative analysis of the extract divulged that the stem bark (g/100g) extract of Khaya senegalensis had the highest contents of anthraquinones (2.72 ± 0.02) when compared to the leaf extract. However, the leaf extract of Vernonia amygdalina (1.62 ± 0.02), Azadirachta indica (1.23 ± 0.06), Senna podocarpa (1.08 ± 0.02), and Annona senegalensis (1.05 ± 0.00) stem bark extract have high amount of anthraquinones present. The result showed that Annona senegalensis, Azadirachta indica, Khaya senegalensis, Senna podocarpa and Vernonia amygdalina contains anthraquinones a pharmaceutical ingredient and provides evidence for their use in traditional medicine.

Keywords: *anthraquinone, phytochemical screening, medicinal plants.*

1. Introduction

Anthraquinones are a class of natural compounds that consists of several hundreds of compounds that differ in the nature and positions of substituent groups (Schripsema *et al.,* 1999). This class of compounds contains derivatives that consist of the basic structure of 9, 10 anthraquinone (Bajaj, 1999). Anthraquinones can be divided into alizarin and emodin types based on two main biosynthetic pathways. The alizarin types are formed through chorismate/δ-succinylbenzoic acid pathway and only have one of the rings unsubstituted (Koblitz, 1988; Van der Berg and Labadie, 1989). These anthraquinones are found in the family of plants known as Rubiaceae (*Rubia, Morinda, Galium* and *Cinchona*) (Korunaglo *et al.,* 1992). The emodin types are formed through the polyketide pathway (acetate-malonate pathway) and have both rings substituted. Anthraquinones are widely applied in medicine, food and the dye industry. In the pharmaceutical industry, the natural and synthetic derivatives of 9, 10 anthraquinones includes many important drugs collectively called anthracenediones, they include laxatives (such as dantron, emodin, aloe emodin and some of the *senna* glycosides), antimalarials (such as rufigallol) and antineoplastics used in the treatment of cancer (such as mitoxantrone, pixantrone and the antracyclines) (Chan *et al*., 2011). Anthraquinones have also been used in constipation; they are reported to possess antiviral, antibacterial and antifungal properties (Heman and Lalita, 2012; Koyama *et al.,* 2008).

This study evaluates and compares five medicinal plants (*Annona senegalensis, Azadirachta indica, Khaya senegalensis, Senna podocarpa and Vernonia amygdalina*) for their qualitative and quantitative anthraquinones properties in the leaf and stem bark extract *Annona senegalensis* is a small tree of height 2-6m tall popularly known as "Wild custard apple" belongs to the family *Annonaceae*. In Nigeria it is known as "Gwandar daji" in Hausa, "Ewe-aso" in Yoruba and "Uburuocha" by the Igbo speaking people (Kayode *et al.,* 2009 and Orwa *et al.,* 2009). It has enjoyed enormous reputation because of its high medicinal values and ethnomedicinal uses. It is widely spread in the savannah areas and near worm, diarrhea, snakebite, headache and respiratory infections; the leaves are used for treating pneumonia while the gums forming from the bark are used in sealing cuts and wounds (Orwa *et al*., 2009). Extract from the plant is found to possess antimalarial, antiulcer, analgesic, anti-inflammtory, antibacterial and anthelmintic (Suleiman *et al*., 2008; Alawa *et al*., 2003; Apak and Otila, 2006). Larvicidal, cytotoxic properties and trypanocidal properties of the extract were also reported (Magadula *et al.,* 2009).

Azadirachta indica popularly known as Neem is an evergreen robust tree belonging to the family *Meliaceae*. It is found mostly in the tropics and sub-tropical areas of the world (Africa and Asia). Neem has a brown dark gray bark and a dense rounded pinnate leaves.

Its fruits are yellow when ripe with garlic like aroma (Ogbadoyi *et al.,* 2007). Some of the pharmacological uses of Neem (leaves, stem bark, fruits and roots) includes; analgesic activity, antihelminthic activity, antiulcer activity, antifilarial activity, antifungal activity, antinematodal activity, antipyretic activity, antitumor activity, antispermatogenic activity, hypoglycaemic activity, immunomodulator activity, hypercholesterolmic activity, abortifacient activity, antiyeast activity, insecticidal activity and diuretic activity (Manal *et al.,* 2015). It is also used in treating diabetes, cancer, heart disease, herpes, allergies, hepatitis and several other diseases (Brototi and Kaplay, 2008).

Khaya senegalensis popularly known as African mahogany belong to the family *Meliaceae* known as "Madaci" and "Ago" by the Hausa and Igala ethnic groups of the northern and central parts of Nigeria respectively. It is a very large tree and regarded as one of the most popular medicinal plants in African traditional remedies. Some of the medicinal properties of *K. senegalensis* recorded in literatures are; the decoction of the bark is extensively used as an antimalarial agent, treatment of stomach disorder, urinogenital diseases, worm infestation, and treatment of trypanosomiasis (Omar *et al.,* 2003). It is reported to be effective agent as a gastrointestinal nematocide. Other medicinal uses include antifungal effects, its antimicrobial effect as an antiprotozoal agent and anti-sickling effect (Ademola *et al.,* 2004).

Senna podocarpa is a glabrous shrub of about 5m high. It belongs to the family *Caesalpinioideae.* The plant is found locally on old farm lands in both western and northern parts of Nigeria. *S. podocarpa* has been used memorial past in the fold medicines for the treatment of varieties of diseases among the Igbo and Yoruba speaking tribes in Nigeria. Some of the medicinal uses are for the treatment of skin diseases (such as eczema, scabies and ringworm), the leaves are employed for their antigonorrhoeal and purgative properties, guinea-worm and sore healing remedy among the Igbos (Trease and Evans, 1996). The decoction of the leaves, roots and flowers is given for the treatment of veneral diseases in women. Extract obtained from the plant materials have been used scientifically as antifungal, antiviral and anticancer. The leaf infusion is also given as a mild laxative and in large doses as a purgative (Akanmu, 2005).

Vernonia amygdalina Del known as bitter leaf is a rapid regenerating shrub soft wooded of 2 - 10m tall with petiolate leaves of around 6mm in diameter. *V. amygdalina* belongs to the family *Compositae.* It is known for its medicinal properties since memorial times, the medicinal property of *V. amygdalina* arise when zoopharmacologist found that a sick chimpanzee with empty stomach sucked pith and juice from the unsavory *Vernonia* plant stalk (which was not a common diet) enhanced body fitness, increased strength or appetite and reduced constipation or diarrhea especially during rainy season (Ijeh, 2011). *Vernonia amygdalina* is an important formulation to treat various diseases such as malaria, anthrax infection, worm infection in cattle and chicken, measles, diabetes mellitus, cough, bronchitis, aprophylaxis, schistosomiasis (Gbolade, 2009; Moshi *et al*., 2009 and Sanibure *et al*., 2009). Some ethnomedicinal uses of *V. amygdalina* are that leaves, twig and root in Northern Nigeria are used in the treatment of parasite infection, ringworm, typhoid fever, headache, constipation, pile (Haemorrhoids) and stomach ache (Ajibesin *et al*., 2008). Abo *et al.,* (2008) reported that the leaves and root of *V. amygdalina* are used in Ethiopia in the treatment of gastroenteritis, diarrhea, dysentery, hepatitis, and worm infection. Agyare *et al.,* (2004) reported that the leaves are used in the treatment of schistosomiasis, infertility and amenorrhoea in South Africa. The stem and roots are also used in India in the treatment of measles, amoebiasis, influenza, mastitis infection and HIV. Other uses include; antimalarial, antiviral activity, anticancer and cytotoxic effect, antimutagenic activity, anticoagulant and antithrombic activities and analgesic (reduced pain) and antiseptic effect, antioxidant and liver protection (Inanya *et al.,* 2010).

2. Materials and Methods

2.1. Plant Collection and Identification

Fresh leaves and stembarkof *Annona senegalensis, Azadiraachta indica, Khaya senegalensis, Senna podocarpa* and *Vernonia amygdalina* were collected from nearby forest around Moddibo Adama University of Technology, Yola. The plants were identified and authenticated by a Botanist in the department of Forestry, Moddibo Adama University of Technology, Yola. The leaves and stem bark of each of the plant material was cleaned and air-dried at room temperature. Each sample was grounded into a fine powder using laboratory mortar and pestle. The powdered samples were stored in an air-tight container and protected from light. The containers were kept in desiccators until use.

2.2. Extract of the Plant Material

Air-dried and powdered plant materials of twenty gram (20g) each was macerated with 100ml of 70% ethanol. The extraction process was repeated three times. The macerated extract was combined, filtered and evaporated to dryness using rotary evaporator at temperature (40-50^{o}C) to yield maceration crude extract.

2.3. Phytochemical Screening

2.3.1. Identification of Anthraquinones in Plant Extract

The freshly prepared extracts were qualitatively tested for the presence of anthraquinones using Borntrager's reaction. Three (3ml) of the aqueous extract of each sample was mixed with 3ml of benzene and filtered followed by the addition of 5ml of 10% ammonia solution to the filtrate. The mixture was shaken. A pink-red or violet colour in ammonical (lower) phase indicated the presence of anthraquinones.

2.3.2. Determination of Total Anthraquinone Content in 70% Ethanol Extracts

The freshly prepared 70% ethanolic extract was separately analyzed for total anthraquinone content using UV spectrophotometer at 515nm. Each of the sample extract was analyzed in triplicate and the total anthraquinone content was reported as mean ± SEM.

3. Results

3.1. Phytochemical Screening

Table 1 showed the presence and absence of anthraquinones in both the leaf and stem bark ethanolic extract of the plants while table 2 showed the quantitative anthraquinone estimation (g/100).

Plants	Leaf extract	Stem bark extract
Annona senegalensis	-	+
Azadirachta indica	+	+
Khaya senegalensis	+	+
Senna podocarpa	+	+
Vernonia amygdalina	+	+

Table 1: Qualitative Phytochemical Screening of the leaf and stem bark of five medicinal plants
Key: Present = +, Absent = -

Plants	Leaf extract (g/100)	Stem bark extract (g/100)
Annona senegalensis	ND	$1.05 \pm 0.00^{\#}$
Azadirachta indica	$1.23 \pm 0.06^{\#}$	0.82 ± 0.01
Khaya senegalensis	0.63 ± 0.01	$2.72 \pm 0.02^{**\#}$
Senna podocarpa	$1.83 \pm 0.02^{*\#}$	0.42 ± 0.02
Vernonia amygdalina	1.62 ± 0.02	$2.10 \pm 0.02^{\#}$

Table 2: Quantitative Anthraquinones Composition of Five Selected Medicinal Plants
Values are in mean ± SEM;
Key: ND= Not Detected
$^{\#}$ = significant ($p<0.05$) high compared to the same extract of the plant in the same row
* = significant ($p<0.05$) high compared to leaf extract of different plants in the same column
$^{*\#}$ = significant ($p<0.05$) high compared to stem bark extract of different plants in the same column

3. Discussion

Comparative analysis of five medicinal plants for preliminary phytoconstituent of anthraquinones showed anthraquinone is present (+) in all the selected medicinal plants except for *Annona senegalensis* leaf extract in which anthraquinone is absent (-) these was contrast to the result obtained by Ayola *et al.,* (2008) who reported that anthraquinone was absent in the leaves of *Vernonia amygdalina*. Further quantitative determination of anthraquinones in the leaf and stem bark of the five medicinal plants revealed that the ethanolic stem bark extract of *Khaya senegalensis* and *Vernonia amygdalina* has the maximum content of anthraquinone (2.72 ± 0.02) and (2.10 ± 0.02) respectively these agreed with the study by Ebong *et al*.,2008 who studied the antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (*A. Juss*) (Neem) and *Vernonia amygdalina*. It could also be depicted from the result that *Azadirachta indica, Sennna Podocarpa* and *Vernonia amygdalina* ethanolic leaf extract showed significant quantities of anthraquinones (1.23 ± 0.06, 1.83 ± 0.02 and 1.62 ± 0.02) respectively while the stem bark ethanolic extract of *Annonia senegealensis* (1.05 ± 0.00) also reveals a noticeable amount of anthraquinones which agrees with the study by Ayola *et al.,* (2008) on the phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. He reported that *Azadirachta indica, Sennna Podocarpa* and *Vernonia amygdalina* ethanolic leaf extract showed significant quantities of anthraquinones The presence of the anthraquinones in the leaf and stem bark extract of the selected medicinal plants provides evidence to the reason why it is employed in traditional medicine and its use locally. Evidence from literatures has shown that the selected medicinal plants have been used since memorial and hence provides evidence to its usage.

4. Conclusion

The study showed that *Khaya senegalensis* and *Vernonia amygdalina* has high anthraquinone content in the stem bark extract with *Annona senegalensis* stem bark extract in a noticeable quantity. Further analysis of the leaf extract showed that *Azadirachta indica, Senna podocarpa* and *Vernonia amygdalina* are also in significant quantities. This study therefore suggests that the medicinal plants may be considered for its future benefits.

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