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EVALUATION OF THE CHEMICAL COMPOSITION OF *DACRYODES EDULIS* AND *RAPHIA HOOKERI* MANN AND WENDL EXUDATES USED IN HERBAL MEDICINE IN SOUTH EASTERN NIGERIA

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Abstract

The phytochemical contents and medicinal values of *Dacroydes edulis* and *Raphia hookeri* exudates were investigated. Phytochemical screening of the plant showed that they contain the presence of bioactive compounds comprising saponins (2.08-3.98mg $100g^{-1}$), alkaloids (0.28-0.49 mg $100g^{-1}$), tannins (0.47-0.72 mg $100g^{-1}$), flavonoids (0.26-0.39 mg $100g^{-1}$), and phenolic compounds (0.01-0.05 mg $100g^{-1}$). The carbohydrates, lipids and protein content were 77.42-78.90%, 2.02-4.185% and 16.63-18.38% respectively. The exudates are a good source of water soluble vitamins; ascorbic acid (7.04-26.40 mg $100g^{-1}$), niacin (3.12-4.00 mg $100g^{-1}$), riboflavin (0.14-0.54 mg $100g^{-1}$) and thiamine (0.15-0.22 mg $100g^{-1}$),). Both plants exudates are good sources of minerals such as Ca, Mg, P, Fe, Zn, Cu and Mn while Cr and Co were trace. These results indicate that exudates can be potential sources of feedstock for the pharmaceutical industry.

Key words: Exudates, *Dacroydes edulis*, *Raphia hookeri*, Bioactive compounds, Vitamins, Pharmaceutical agents.

Introduction

Over the years, man has acquired extensive knowledge regarding the utilization of plants around him as food and medicine. These plants exhibit a wide range of biological and pharmacological activities such as anti-inflammatory, diuretic, laxative, antispasmodics, anti-hypertensive and anti-microbial functions. These functions are performed due to the chemical constituents comprising sugars, lipids, protein, vitamins, minerals and phytochemicals. The raffia palm (*Raphia hookeri* Mann and Wendl) Palmae is probably the most diversely useful plant of Nigeria. It is one of the most important sources of forest food species in Southern Nigeria (Akachuku 2001). It occurs within the fresh water swamp forest. *R. hookeri* grows to height of 9m and is adapted to life with its roots in water logged soil, by possessing breathing roots. It has a crown of dark green pinnately divided leaves, which may be over 9m in length. The trunk is more fibrous than woody but is used for beams (Gledhill, 1972, Akachuku 2001). The leaves provide thatching material, building poles (from the mid-rib), fibers for matting and piassava of which base brooms are made (Akachuku 2001, Gledhill, 1972). Thousands of tones of Raphia palm wine are produced yearly in Nigeria. The Urhobo of Jeremi Clan in Delta State of Nigeria produce local gin "Ogogoro" through a process of distillation from palm wine sap (Akachuku 2001, Otedoh 1972, Ogbonna 2000, 1972). Wine palm is a source of beverage (Palm wine) and many other products, which are economically important in Southern Nigeria and other West African countries (Akachuku 2001). Palm wine is very rich in ascorbic acid especially when freshly prepared from the tree.

Palm wine plays vital roles in many ceremonies in Southern Nigeria. Guests at weddings, births celebrations, coronations and funeral wakes are served with generous amounts. Palm wine is often infused with

medicinal herbs to remedy a wide variety of physical complaints. As a token of regard and respect to the deceased ancestors, many drinking sessions in Igbo land begin with a small amount of palm wine spilled on the ground as a libation to appease the gods (Akachuku 2001). These palms produce exudates along with the palm wine and are extensively and intensively used in native medicine.

The local pear (*Dacryodes edulis* G. Don Lam) Burseraceae grows mostly in the tropics. It grows up to 18m in height and exudes an odoriferous gummy substance from injured or excised portion of the stem (Ekpa, 1993). It is cultivated in most rural communities by the peasant farmers for its fruits. The fruit is red, turning blue-black when ripe with unpleasant turpentine smell. The fruit is oval in shape and matures within the months of May and June. The fruit consist of large seeds, surrounded by thin mesocarp. The pulp is boiled, roasted or eaten raw as a dessert fruit. The pulp may also be boiled or roasted to form a kind of butter (Irvine, 1961, Leakey 1999). The leaves are pinnate with leaflets measuring 3 to 4 cm by 2-3 cm. The leaflets are glabrous, narrowly oblong and elliptic (Sofowara, 1993)

Resins or exudates occur in the genus and the resin from some species is used in African medicine (Leakey 1999). The stem bark yields a resin or exudates, which is also primitive oil. The resin is medicinal and is applied to cure skin diseases such as ringworms, craw-craw and wounds. They are also used to treat parasitic organism like ticks and jiggars (Hutchinson, *et al* 1993). The exudates are used in food and cosmetic industry as thickeners flavors, stabilizers and as emulsifying agents in drugs and cosmetics (Ekpa, 1993). Exudates from *D. edulis* when applied in lotions and creams stabilize emulsion, add smooth to the skin and form protective coating on the skin. The exudates are used in traditional medicine as antibacterial agent and as incense. It is believed that the smoke and sweet smell from the exudates when burning wades off evil spirit (Sofowara, 1993).

Recently, exudates from *R. hookeri* and *D. edulis* have been used in cosmetics (Ekpa, 1993). In Nigeria, research has not been extensively conducted for the improvement and refining technology in upgrading the quality of exudates into high-grade oils that can be harnessed for pharmaceuticals. Considering the importance of the exudates, which are commonly used in herbal medicine, it is necessary to investigate the properties of exudates from *R. hookeri* and *D. edulis* as a pharmaceutical agent.

The present study was undertaken to evaluate the chemical constituents of exudates from *R. hookeri* and *D. edulis* and to consequently assess their potential usefulness as food supplements or pharmaceutical raw material for drug formulation.

Materials and Methods

The experiment was carried out in the Department of Chemistry, Michael Okpara University of Agriculture, Umudike Nigeria, in June 2004. Fresh leaves and fruits of *R. hookeri* and *D. edulis* Voucher No RH 206 and DE 311 respectively were collected from Ekwelu Village, Araim Usaka Ikwuano Local Government, Abia State, Nigeria. They were botanically identified by Dr. A. Nmeregini of the Taxonomy Unit of the Department of Forestry, of this University.

Excised portions were made on the stems of the plants in the village and the exudates were collected daily during the morning hours for one week. (From 20th June to 27th June 2004). The exudates (450g of *R. hookeri* and 320g of *D. edulis*) were collected in amber bottles and stored in a refrigerator. Total Nitrogen (N) content was determined by the use of an apparatus (Micro-Kjeldahl: MD 55, Singapore). The protein content was calculated as N X 6.25. Crude fat (ether extract), crude fiber and ash content were determined according to the methods of Association of Analytical Chemist (AOAC) (1984). Total carbohydrates were estimated as the remainder after accounting for ash, crude fiber, protein and fats (Muller and Tobin, 1980). The gross food energy was estimated according to the methods of Osborne and Voogt (1978), by using the equation:

FE = (%CP X 4) + (%CHO X 4) + (%Fat X 9)

Where:

FE= Food energy (In gm calories)

Cp = Crude protein, CHO = Carbohydrates

The minerals, calcium sodium, potassium, magnesium and trace elements (iron, zinc, cobalt, copper, chromium and manganese) all of which were determined according to the method of Shahidi *et al*., (1999). Phosphorus content of the digest was determined calorimetrically according to the method described by Nahapetain and Bassiri (1995). Alkaloids and phenols were determined according to the methods of Harborne (1973) while tannin was determined using the method of Van-Burden and Robinson (1981). Saponins content was determined using the method of Obadoni and Ochuko (2001). Flavonoids were determined according to the method of Boham and Kocipai (1994). The B-complex vitamins (Thiamine, riboflavin and niacin) were determined according to the

methods of SKALAR Analyzers (2000) while ascorbic acid (Vitamin C) was determined using the method of Baraket *et al*. (1993).

Statistical Analysis

All measurement were replicated three times and the standard deviations determined by using turkey's student t-test at $P<0.05$ was applied to assess the difference between the means (Steel and Torrie, 1980).

Results

The phytochemical content of the exudates from *R. hookeri* and *D. edulis* is shown in **Table 1.** The saponin content was very high on both exudates with *R. hookeri* containing 3.98mg $100g^{-1}$ and *D. edulis* had 2.08 mg $100g^{-1}$ of saponins. Tannin content was more in *R. hookeri* having 0.72 mg $100g^{-1}$ of tannins and exudates from *D. edulis* contained 0.47 mg $100g^{-1}$ of tannin. High quantity of alkaloids was found in the exudates from *R. hookeri,* which contains 0.49 mg $100g^{-1}$ while *D. edulis* contained 0.28 mg $100g^{-1}$ of alkaloids. *R. hookeri* exudates have more phenol (0.05mg $100g^{-1}$) while *D. edulis* contained (0.01 mg $100g^{-1}$). *D. edulis* contained 78.90% carbohydrate while *R. hookeri* had 77.42% of carbohydrate (Table 2). Exudates from *R. hookeri* contained 4.18% of lipids while *D. edulis* contained 2.02% of lipids. Exudates from *R. hookeri* contained 2.10 mg $100g^{-1}$ of calcium while *D. edulis* contained 0.06 mg $100g^{-1}$ of calcium. Also the exudates from *R. hookeri* contained 1.58 mg $100g^{-1}$ of magnesium. The magnesium content in the exudates from *D. edulis* was 0.22 mg $100g^{-1}$. The concentrations of iron in the exudates were relatively high with *D. edulis* containing 3.12 mg $100g^{-1}$ if iron, *R. hookeri* contained 0.40 mg $100g^{-1}$. The concentration of zinc, manganese, copper, chromium and colbalt were relatively low (Table 3).

Table 1: Phytochemcial composition of exudates from *Raphia hookeri* and *Dacroyodes edulis* on dry weight basis (mg $100g^{-1}$)

Phytochemical	*R. hookeri*	*D. edulis*
Tannins	0.72 ± 0.10^{a}	0.47 ± 0.20^{a}
Saponins	3.98 ± 0.22^{b}	2.08 ± 0.10^{b}
Alkaloids	0.49 ± 0.10^{a}	0.28 ± 0.03^{a}
Phenols	0.05 ± 0.20^{a}	0.01 ± 0.20^{a}
Flavonoids	0.26 ± 0.02^{a}	0.39 ± 0.11^{a}

Data are means ± standard deviation of triplicate determinations (mg $100g^{-1}$). Means followed the same superscript in each column are not significant ($P<0.05$)

Table 2: Proximate composition of exudates from *Raphia hookeri* and *Dacroydes edulis* on dry weight basis %

Constituents	*R. hookeri*	*D. edulis*
Moisture %	58.60 ± 0.11^{a}	79.58 ± 0.10^{a}
Crude protein (N x 2.65%)	16.63 ± 0.20^{a}	18.38 ± 0.20^{a}
Crude fibre %	0.05 ± 0.10^{a}	0.03 ± 0.10^{a}
Crude lipids	4.18 ± 0.10^{a}	2.02 ± 0.22^{a}
Ash %	1.72 ± 0.10^{a}	0.69 ± 0.02^{a}
Carbohydrates	77.42 ± 0.11^{a}	78.90 ± 0.11^{a}
Food energy (Cal g^{-1})	413.83	407.30

Data are means ± standard deviation of triplicate determinations (%). Means followed the same superscript in each column are not significant ($P<0.05$)

Table 3: Mineral composition of exudates from *Raphia hookeri* and *Dacroydes edulis* on dry weight basis (mg $100g^{-1}$)

Mineral	*R. hookeri*	*D. edulis*
Macro element		
Calcium	2.10 ± 0.10^{b}	0.06 ± 0.20^{b}
Magnesium	1.58 ± 0.22^{b}	0.22 ± 0.10^{b}
Phosphorus	0.75 ± 0.02^{b}	0.20 ± 0.02^{a}
Potassium	0.17 ± 0.10^{b}	0.03 ± 0.10^{b}
Sodium	0.19 ± 0.11^{b}	0.01 ± 0.11^{b}
Micro element		
Iron	0.40 ± 0.10^{b}	3.12 ± 0.10^{b}
Zinc	0.58 ± 0.11^{a}	0.63 ± 0.10^{a}
Cobalt	0.11 ± 0.10^{a}	0.26 ± 0.20^{a}
Copper	$0.13a \pm 0.20^{a}$	0.52 ± 0.22^{a}
Chromium	trace	0.23 ± 0.10^{b}
Manganese	$0.25a \pm 0.11^{a}$	$0.45a \pm 0.20^{a}$

Data are means ± standard deviation of triplicate determinations (mg 100 g^{-1}). Means followed the same superscript in each column are not significant ($P<0.05$)

Table 4: Vitamin composition of exudates from *R. hookeri* and *D.s edulis* on dry weight basis (mg 100 g^{-1})

Vitamin	*R. hookeri*	*D. edulis*
Ascorbic acid	7.04 ± 0.11^{b}	26.40 ± 0.10^{b}
Niacin	3.12 ± 0.30^{a}	4.00 ± 0.20^{a}
Riboflavin	0.14 ± 0.10^{a}	0.54 ± 0.10^{a}
Thiamine	0.15 ± 0.22^{a}	0.22 ± 0.20^{a}

Data are means ± standard deviation of triplicate determinations. Means followed the same superscript in each column are not significant ($P<0.05$)

Discussion

The highest phytoconstituents of plants exudates is the presence of saponins. Saponins are produced by plants to stop the bacterial and fungal attack. Some of the general characteristics of saponins include formation of foams in aqueous solution, (Okwu and Emenike 2006). Saponins natural tendency to ward off microbes makes them good candidates for treating fungal and yeast infections. These compounds served as natural antibiotics, helping the body to fight infections and microbial invasions (Okwu 2004, 2005, Okwu and Emenike 2006). These compounds appear to greatly enhance the effectiveness of certain vaccines. Plant saponins help humans to fight fungal infections, combat microbes and viruses, boost the effectiveness of certain vaccines and knock out some kinds of tumor cells particularly lung and blood cancers (Stray 1998). They also lower blood cholesterol thereby reducing heart disease. The most outstanding and exciting prospect for saponins is how they inhibit or kill cancer cells. They may also be able to do it without killing normal cells, as is the mode of some cancer fighting drugs. Cancer cells have more cholesterol-type compounds on their membranes than normal cells. Saponins therefore bind cholesterol and thus interfere with cell growth and division (Okwu 2005, Okwu and Emenike 2006).

Other phytochemicals relevant in the pharmaceutical formulations from exudates include tannins, alkaloids and flavonoids. Tannins have astringent properties; hasten the healing of wounds and inflamed mucous membranes (Okwu 2004). In traditional medicine exudates from *D. edulis* when applied to open wounds, ringworms and craw-craw caused a significant increase in cell proliferation (wound healing). This suggests that the damaged tissue is effectively provided with nutrients to facilitate healing while undergoing treatment with exudates. This also supported the use of exudates from *D. edulis* in traditional medicine for the treatment of wounds and parasitic skin disease (Hutchinson, *et al*., 1993, Sofowara, 1993).

Pure isolated plant alkaloids and their synthetic derivatives are used as a basic medicinal agent for its analgesic, antispasmodic and antibacterial properties (Okwu 2004). Alkaloids exhibit marked physiological activity when administered to animals. Most of the plants used in the cure of diseases have been reported to contain traces of alkaloids. For instance *Azadirachta indica* (Meliaceae) is employed in the treatment of malaria and fever in Nigeria folk medicine. The plant contains gedunin, which has anti-malaria properties (Adesegun and Coker 2001). The roots of *Eurycoma longifolia* Jack (Simaroubaceae) contain canthin-6-one alkaloid, a quassinoid eurycomanone and 7-methy-b-carboline-1-proprionic acid. However, only the last two showed anti-malarial activity (Kardono *et al*., 1991; Adesegun and Coker, 2001). *Cinchona* bark consists of various species, races and hybrids of *Cinchona*. These plants produce quinine-type alkaloids. Quinine is used for treatment of malaria. Other alkaloids like quinidine are employed for the prophylaxis of cardiac *arrhythmias* and for the treatment of arterial fibrillation (Trease and Evans, 1983; Adesegun and Coker 2001).

The presence of alkaloids in the exudates from the *R. hookeri* and *D. edulis* investigated suggests that the exudates investigated have medicinal properties. The biological functions of flavonoids include protection against allergies, inflammation, free radicals scavenging, platelets aggregation, microbes, ulcers, hepatoxins, viruses and tumors (Okwu and Omodimiro 2005, Okwu 2005, Okwu and Emenike 2006). Quercetin is found to be the most active of the flavonoids reported in literature. Many medicinal plants owe much of their efficacy and activity as a result of their high quercetin content (Okwu and Emenike 2006). Quercetin has demonstrated significant anti-inflammatory activity because of their direct inhibition of several initial process of inflammation. For example, it inhibits both the manufacture and release of histamine and other allergy inflammatory mediators (Okwu 2005, Okwu and Omodamiro 2005). Furthermore, quercetin exerts potent antioxidant activity and vitamin C sparing action (Okwu 2005). Quercetin forms the glycosides quercetrin and rutin together with rhamnose and rutinose respectively. Quercetin may have positive effects in combating or helping to prevent cancer, arthritis, cataracts, allergies or inflammations as well as respiratory diseases such as bronchitis and asthma (Okwu and Emenike 2006)

Phenols protect plants from oxidative damage and perform the same functions for humans (Okwu 2005). The outstanding phytonutrient feature of phenols is their ability to block specific enzymes that causes inflammations. They also modify the prostaglandin pathways, thereby protecting platelets from clumping (Okwu and Omodamiro 2005).

Plants exudates are complex mixture of branched polysaccharides that contains galactose, rhamnose, gluconic acid, arabinose residues, mucilages and gums. Mucilage is a substance derived from sugar with a jelly-like consistency. It has emollient (soothing), anti-inflammatory and mild laxative effect (Roger, 2002). Mucilages are quite useful in all inflammatory dysfunctions of the digestive systems; esophagitis, gastritis, gastric ulcer, proctitis (inflammation of the rectum) anal fissures and hemorrhoids (Roger, 2002). They have emollient and anti-inflammatory actions when applied on the skin. This however, supported the findings of Ekpa (1993) who reported that exudates from *R. hookeri* and *D. edulis* could be incorporated into creams and lotions for respiratory disease. Mucilages sooth irradiated mucosal membranes in conditions like laryngitis or tracheitis and clam cough (Roger, 2002). This however supported the findings of Igoli *et al.* (2005) who reported that *D. edulis* stem bark is used in treating cough in Igede traditional medicine in Nigeria. *R. hookeri* and *D. edulis* contain mucilages and saponins which give them soothing, anti-inflammatory and wound healing properties. The use of lotions and shampoos incorporated with exudates from *R. hookeri* and *D. edulis* is highly regarded in cosmetics (Ekpa 1993). This is because the exudates effectively and efficiently combat skin dryness. It stabilizes the emulsion and increases the viscosity, assists in imparting spreading properties, adds a smooth feel to the skin thereby forming protective coating on the skin (Ekpa, 1993).

The lipids are gummy and can be used in tablets coating. This oil can be ointments and creams. The exudates can also be comparable with gum arabic. This implies that they can be used as suspension agent for calamine suspension, kaolin suspension and cold liver oil emulsion due to their high emulsifying and stabilizing properties (Osborn and Dakay 1991).

Table 3 shows the macro and micro-elements in the exudates from *R. hookeri* and *D. edulis*. The exudates contained appropriate amount of macro elements comprising calcium, phosphorus, potassium and magnesium. Exudates from *R. hookeri* contained 2.10 mg $100g^{-1}$ of calcium while *D. edulis* contained 0.06 mg $100g^{-1}$ of calcium. Also the exudates from *R. hookeri* contained 1.58 mg $100g^{-1}$ of magnesium. The magnesium content in the exudates from *D. edulis* was 0.22 mg $100g^{-1}$. Normal extra cellular calcium concentrations are very important for blood coagulation (Okaka and Okaka 2001). A lack of calcium or phosphorus in the diet causes a disease known as rickets (Fliedner and Teichman 1965) and osteoporosis (Hunt *et al*. 1980; Okwu and Emenike 2007) disease normally results due to lack of calcium. In osteoporosis condition, the bone mass is so decreased that adequate mechanical support can no longer be provided and sustained, spontaneous fractures often results (Hunt *et al*, 1980; Okwu and Emenike 2007). The enormous concentration of calcium and phosphorus in the exudates from *R. hookeri* is very

significant because calcium is known to enhance the qualities of bone and teeth (Okwu and Ekeke 2003). It is well documented (Macral *et al*, 1993, Okwu and Ekeke 2003) that the surface enamel is more resistant to carries attack than the surface dentine. This is attributed to the content of fluoride and other trace elements such as zinc, copper, iron; cobalt and manganese, which are thought to protect the surface enamel from demineralization (Macral *et al*., 1993; Olabanji *et al*. 1996; Okwu and Ekeke 2003). However, iron is known to be an important element in human body. It is a component of hemoglobin. It helps in oxygen transport. Iron together with hemoglobin and ferrodoxin play vital roles in man's metabolism. It is worthy of note that magnesium, zinc, sodium, phosphorus and calcium are present in the exudates from *R. hookeri* and *D. edulis*. The combination of these elements together with fluoride may have therapeutic, protective and preventive roles in teeth (Olabanji *et al*., 1996; Okwu and Ekeke 2003). Exudates from *R. hookeri* and *D. edulis* can be used as a pharmaceutical agent in the formulation of toothpaste. This implies that Ca, Zn, Fe, Mg and other mineral elements are essential components for human health. Mineral deficiencies such as Ca, Zn and Fe are the major health problems in developing countries particularly for infants. (Zhao, 2007). Zn or Fe deficiency causes poor growth, impaired immune function and consequently delayed mental development. Exudates from *R. hookeri* and *D. edulis* can therefore act as mineral supplement.

The vitamin content showed that *D. edulis* contained 26.40 mg $100g^{-1}$ and *R. hookeri* had 7.04 mg $100g^{-1}$ of ascorbic acid. The exudates also contained the B-vitamins such as niacin, riboflavin and thiamine (**Table 4**). Deficiency of any of the vitamins can cause widespread clinical symptoms. Lack of ascorbic acid impairs the normal formation of intracellular substances through out the body, including collagen, bone matrix and tooth dentine. A striking pathological changes resulting from this defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intracellular substance. Consequently, the clinical manifestation of scurvy from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints and defect in skeletal calcification can be related to the association of ascorbic acid and normal connective tissue metabolism (Hunt *et al*., 1980). This function of ascorbic acid also accounts for its requirement for normal wound healing. Ascorbic acid is essential to prevent diseases associated with connective tissue and to improve and immune functions (Zhao, 2007). A deficiency of riboflavin does not result to any disease and one is apt therefore to under estimate its importance. The symptoms are inflammations of the tongue, lesions at the eyes and lips, congestion of conjunctiva blood vessels and desquamation of the skin. *R. hookeri* and *D. edulis* apart from being important food crops also produces other non-timber natural products. They are therefore economic trees, which can greatly improve the income of the peasant farmers and provide raw materials for the pharmaceutical industries. If judiciously taped, extracted and processed, exudates could provide raw materials for the pharmaceutical industries in the country.

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