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The Phytochemical and Structural Make-Up of Regrown and Original Tree Barks Used in Ethnomedicine

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Abstract: The increasing consumption of Phytomedicine in Nigeria has led to a continuous use of plant parts. There is therefore an uncontrolled harvesting of plant parts from the wild. There are many trees showing various degrees of debarking while some regrown barks were equally re-debarked. The pattern of debarking and regrowth and its implication on the quality of tree barks in phytomedicine and the danger it poses has not yet been evaluated. This study was therefore, undertaken to compare the phytochemical and structural make-up of both the initial and regrown tree barks of some plant species that were frequently debarked for various phytomedicinal purposes following standard methods. The primary formed bark and regrown bark screened for phytochemicals viz., alkaloids, tannins, flavonoids, anthocyanins, saponins, steroids, terpenes, coumarins, mucilage and combined anthracene derivatives showed the presence of these phytochemicals at various degrees for a particular bark type and plant species. Among the studied plants, there was none of the primary formed and regrown barks that had the same set of phytochemicals. Difference were observed in the type of phytochemical present in the bark type. *Khaya senegalensis* had the most varied phytochemicals in the original and regrown barks, followed by *Alstonia boonei* while *Mangifera indica* had the least. It was however, observed that tannin was present in all the tree bark categories tested while cyanogenic derivatives were absent in all the bark samples. It was noted that the presence of some phytochemicals found in some original barks were absent in the regrown. In the least, a few secondary metabolites found absent in the original barks, were present in the regrown tree barks.

Key words: Secondary metabolites · Tree bark · Phytomedicine · Regrown bark · Bark fissures

INTRODUCTION

Various defenses against tree bark consumption by animals have evolved and these include production of secondary metabolites [1-3], which however appears inadequate when human beings harvest tree barks indiscriminately without giving it a thought of being grown or planted. It has been pointed out that tree stem and bark are very sensitive components of perennial trees and that their potential resources if well protected will only be used in crucial situations as needed by various human groups.

Tree bark is an important component of African traditional medicine as herbal medicine is still the main source of health care for the majority of Africans and in particular Nigerians. As Nigerian population grows, tree debarking becomes more rampant and the trade of tree barks has become more established and commercialized by market traders. In recent years the advent of new incurable diseases has increased the demand for medicinal plants, including tree barks. Apart from the medicinal uses of plant barks, in many countries, AIDS the pandemic disease has also increased the demand for bark as a cheap source of material to make coffins to bury the dead of the poorest people. Major markets in Nigeria are already composed of all types of preferred tree barks. In Malawi, medicinal plants are harvested for export to South Africa and this trade is likely to extend to some other African countries. Currently, the pressure in Malawi is such that local extirpation of two medicinal tree species is already reported from Liwonde Forest and this is mainly due to excessive bark harvesting. Zambia has a relatively low

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population density yet many trees have had their barks and roots harvested. The harvesting continues despite the prosecution of illegal harvesters. The uncontrolled harvesting of bark could result in: local annihilation of plant species; compromised ecological processes in the forest; reduction in forest incomes for owners, collectors, traders and traditional doctors; the loss of useful medicines and compromised health security of the general population. The difficulties of quantifying growth and replenishment rates of tree barks are even greater as very little scientific work has been done on bark recovery after harvesting.

A study conducted by Fasola and Egunyomi [4] and Delvaux, [5] and Delvaux *et al*. [6] have identified reliable, scientific and participatory methods for quantifying bark regrowth and optimising harvesting strategies. It did not however take cognizance if the different phytochemicals present at harvesting are equally present at regrowth. Tree bark screening may therefore be essential so as to know the implications of debarking to plant secondary metabolites adjudged to be responsible for curative effects in phytomedicine.

MATERIALS AND METHODS

Phytochemical Screening: Portions of primary formed bark were debarked and the regrown bark from the same tree stand were separately dried and powdered for phytochemical analysis following standard methods. The primary formed bark and regrown barks were screened for phytochemicals such as Alkaloids, Tannins, Flavonoids, Anthocyanins, Saponins, Steroids, Terpenes, Coumarins, Mucilage and Combined Anthracene derivatives as described by Obadoni and Ochuko [7] and Trease and Evans [8].

Anatomical Cell Components: Longitudinal, transverse and tangential sections of primary formed bark and the regrown barks were produced using Reichert Sledge Microtome at 10 micrometer thickness. The sections were stained in Safranin O for 3minutes, rinsed in water twice and dehydrated in series of grades of Ethanol (50,70 80,90 and 100%). Dehydrated sections were treated in absolute Xylene to clear the sections and to provide medium miscible with DPX mountant. Cleared sections were mounted in DPX on clean slide and labeled. Cell (phloem and cortex) measurements were done using micrometer (stage and eye piece micrometer) at the transverse and longitudinal planes. This was done in triplicate and subjected to statistical analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The primary formed bark and regrown bark screened for their phytochemicals *viz.,* alkaloids, tannins, flavonoids, anthocyanins, saponins, steroids, terpenes, coumarins, mucilage and combined anthracene derivatives showed presence of these phytochemicals at various degrees for a particular bark type and plant species. Among the studied plants, there was none of the primary formed and regrown bark that had the same set of phytochemicals but was different in the least of such phytochemicals tested with an exception of *Mangifera indica* which varied in the leucoanthocyanins (Table 1). Differences were observed in the type of phytochemical present in a particular bark type. As an example, *Khaya selegalensis* was the most varied phytochemicals in the original and regrown barks. It had the presence of steroids, coumarines and combined anthracene of C-Heterosides Agenines in the original bark while these were absent in the regrown bark. This was followed by *Alstonia boonei* while *Mangifera indica* had the least. It was however, observed that tannin was present in all the tree bark categories tested while cyanogenic derivatives were absent in all the bark samples. It was noted that the presence of some phytochemicals found in some original barks were absent in the regrown. In the least, a few phytochemicals found absent in the original barks, were present in the regrown tree barks. Among the studied phytochemicals are alkaloids which are large group of organic compounds found in plants. They are mainly derived from amino acids and contain nitrogen. Alkaloids are water soluble, usually bitter in taste and physiologically active. The occurrence of some compounds may be related to the uses of plants and for the treatment of different ailments as alkaloids have significant therapeutic values and form the ingredients for many important medicines. The presence of alkaloids in water and alcoholic extracts of the dry samples of some plants justify their use therapeutically. This may be supported by Pelletier [9] who reported that plants containing alkaloids can relief pain. The occurrence of alkaloids may explain the usefulness of some plants in reducing high blood pressure. Useful alkaloids such as rhynchophylline were reported by Jeffery and Harborne [10] to help in improving cardiac conditions by reducing blood pressure, increasing circulation and inhibiting the accumulation of arteriosclerosis plague and blood clots.

In ethnomedicine, saponins are used as anti-inflammatory, analgesic, antipyretic, antitumor; antiulcer and antifungal. They are soap-like foaming when shaken in aqueous solution and this implies that the plant part(s)

Table 1: Antinutritional analysis of some original and regrown tree barks

			Group of Compounds							
			Tannins							
Plant Species	Bark Type	Alkaloids	Gallic	Catechin	Flavonoids	Anthocyanins	Leucoanthocyanins	Quinonic derivatives	Saponins	Steroids
Mangifera indica	Original	-	+	+	+	+	-	ND	-	-
	Regrown	-	+	+	+	+	+	-	-	-
Alstonia boonei	Original	-	-	+	-	-	+	-	-	-
	Regrown	+	-	+	-	-	+	-	-	-
Albizia lebbeck	Original	-	-	+	+	-	+	-	+3	+
	Regrown	-	-	+	+	+	+	-	+3	-
Khaya senegalensis	Original	-	-	+	+	+	+	-	+3	+
	Regrown	-	-	+	+	-	+	-	+3	-
Azadirachta indica	Original	-	-	+	+	+	+	-	+3	-
	Regrown	-	-	+	-	-	+	-	+3	-

		Group of Compounds								
									Combined Anthracene derivatives	
Plant Species	Bark Type	Terpenes	Cardiac glycosides	Coumarins	Mucilage	Reducing compounds	Cyanogenic derivatives	Antracene derivatives	O-Heterosides a genines	C-Heterosides a genines
Mangifera indica	Original	-	-	+	+	+	-	-	-	-
	Regrown	-	-	+	+	+	-	-	-	-
Alstonia boonei	Original	-	-	-	+	+	-	-	-	-
	Regrown	+	ND	-	-	+	-	-	-	+
Albizia lebbeck	Original	+	-	-	-	+	-	-	-	+
	Regrown	-	ND	-	-	+	-	-	+	-
Khaya senegalensis	Original	-	-	+	-	+	-	+	-	+
	Regrown	+	-	-	+	+	-	+	-	-
Azadirachta indica	Original	-	-	+	+	+	-	-	-	-
	Regrown	-	ND	+	+	+	-	+	-	-

+: Present -: Absent ND: Not determined

can be useful in the preparation of detergents or can serve as useful additives. They are grouped structurally by the composition of one or more hydroplilic glycoside moieties combined with a lipophilic triterpene derivatives. Some plant saponins enhance nutrient absorption and aid in animal digestion while some others from various plant sources have been known as against possible carcinogens. Jun *et al*. [11] expressed possible blockage of carcinogenesis initiative stage by soybean saponins. In the same vein, Zha *et al*. [12] observed the anti-inflammatory effect of soya saponins through suppression of nitric oxide production in LPS- stimulated cells and by attenuation of NF-KB-mediated nitric oxide synthase expression.

Tannins help in growth regulation and also protect the plants from predators. They are water soluble, astringent, bitter and contain sufficient hydroxyls and other suitable groups to form strong complexes with protein and other macromolecules. They are medically used as hemostatic, antidiarrhea and antihemorrhoid. They help to heal burns, control bleeding and infections. They form protective layer over exposed tissue of wound to keep the wound from infections. They have been used for immediate relief of sore throats, dysentery, fatigue and skin. Tannins can cause regression of tumors already present in tissue though excessive use over time can also cause tumors in healthy tissue. Tannins have also demonstrated potential antiviral, antibacterial and antiparasitic effects. There is no bark type among the studied that did not show the presence of tannin though they may not contain the both types of tannin screened. The presence of tannin in anycase supports their use ethnomedically. The work of Banso and Adeyemo [13] exhibited the antibacterial activities of tannin against some microorganisms.

Flavonoids are also a class of water soluble, plant secondary metabolites. They are pigments of vibrant colours to plants. They also help protect the plants from microbes and insect attacks. They are plant nutrients that when consumed in the form of fruits and beverages are non toxic and beneficial to the human body having been linked to numerous health benefits. Recent research indicates that flavonoids can be nutritionally helpful by triggering enzymes that reduce the risk of certain cancers, heart disease and age related degenerative diseases. Flavonoids may also help to prevent tooth decay and reduce the occurrence of common ailments such as flu. Flavonoids function as antioxidants and antioxidants are compounds that protect cells against damaging effects of reactive oxygen. Epidemiological studies have shown that flavonoids intake is inversely related to mortality from coronary heart disease and the incidence of heart attacks. However, the capacity of flavonoids to act as antioxidants depends upon their molecular structure. It prevents the oxidation of low density lipoproteins and there by reducing the risk of developing atherosclerosis [14]. Though in the further work of Lotito *et al*. [15], he stressed how flavonoids are extensively metabolized in humans for its anti-inflammatory and antioxidant properties. Over 5,000 naturally occurring flavonoids have been characterized from various plants. The most important dietary sources are tea, soybean and fruits. The important fruits are apple and citrus fruits. From the result of the phytochemical screening, flavonoids are present in all the tree barks studied except in *Alstonia boonei* and the regrown bark of *Azadiracta indica*. This implies that some tree barks have the properties identified with flavonoids and may be useful medicinally as anticancer, antiviral, antiallergy, antiplatelet, anti-inflammatory and antioxidant.

Phenol an aromatic organic compound used medicinally as a disinfectant for sterilizing wounds, surgical dressing and instruments. It is the preferred chemical for embalming bodies for studies because of its ability to preserve tissues for a prolonged period of time. It is also used in preparation of cosmetics including sunscreen [16], hair dyes and skin lightening preparation [17]. The role of phenol on dermal toxicity was stressed by Kovacic and Somanathan [18]. Likewise, the studies of Allemann and Baumann [19] confirmed how phenol compounds act as potent free radical scavengers. This was also corroborated by Pessego *et al*. [20] by presenting phenolic compounds as potential antinitrosating agents.

Terpenes, though not present in both the original and regrown barks of the same plant but where present, terpenoids have antimicrobial activities; as many antibacterial and antifungal properties have been due to terpenoids [21]. The plants screened are used ethnomedicinally by the Hausa and Fulani tribes of Northern Nigeria as a remedy for several human and animal ailments that include stomach ache, diarrhea, dysentery, wound and cancer [22]. The antimicrobial activities of terpenoids may possibly be through membrane disruption of the micro-organisms by their lipophilic compounds.

Quinones, though not detected, they are important naturally occurring pigments widely distributed in nature and are known to demonstrate various physiological activities as antimicrobial and anticancer [23]. Among such is anthraquinone derivatives. The occurrence of anthraquinones in a few plants is an indication that they can be used as a mild laxatives. According to Evans [24], anthraquinones act on gastrointestinal tract to increase the peristalsis action. Anthraquinone is an aromatic compound, a plant chemical formed on anthracene (a triple benzene ring) skeleton. Anthraquinones are used as laxatives, antimalarials and antineoplastics used in the treatment of cancer. Though despite its usefulness, a prolonged use of natural anthraquinone as laxative and abuse leads to melanosis coli [25, 26] and electrolyte imbalance. The presence of anthraquinone though sparsely present, may therefore have laxative effects.

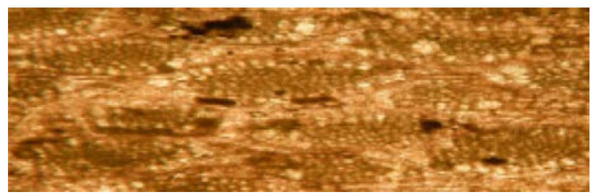

Plate 1a: Longitudinal section of *Khaya senegalensis* cortex original bark x 200

Plate 1b: Longitudinal section of *Khaya senegalensis* cortex regrown bark x 200

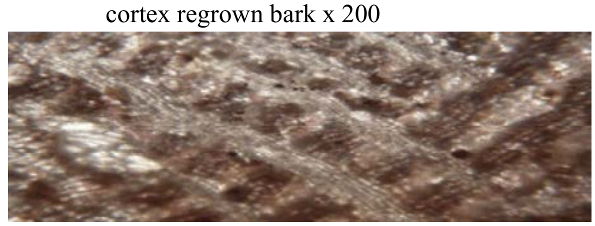

Plate 2a: Transverse section of *Khaya senegalensis* cortex original bark x 200

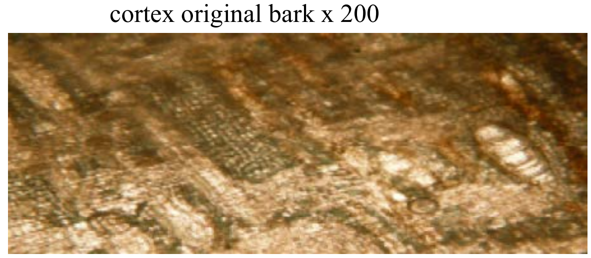

Plate 2b: Transverse section of *Khaya senegalensis* cortex regrown bark x 200

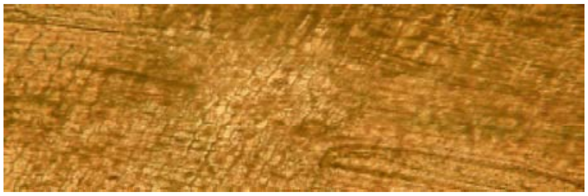

Plate 3a: Tangential section of *Vitex doniana* phloem original bark x 200

Plate 3b: Tangential section of *Vitex doniana* phloem regrown bark x 200

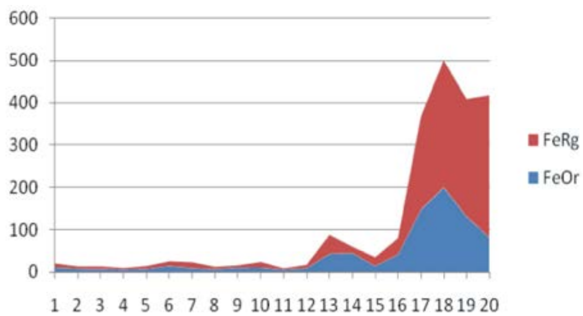

Fig. 1: Showing different cell sizes of the original and regrown barks of *Ficus exasperata*

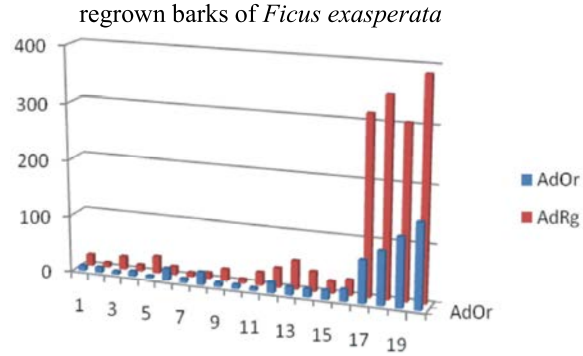

Fig. 2: Various tissues showing cell sizes of the original and regrown barks of *Adansonia digitata*

The anatomical structure of the primary formed bark and newly-regrown bark was not too different in the transverse and longitudinal sections of inner bark for both the cortex and the phloem of many of the plant species but major difference was observed in the transverse and longitudinal sections of outer bark of the primary and the regrown tree barks. The outer bark of the primary bark was made up of dead cells with thickened cell wall made up of cellulose, pectin and/or lignin while the deadness and thickness of the regrown was dependent on the age of the plants. The photomicrograph slides (Plates 1a - 3b)

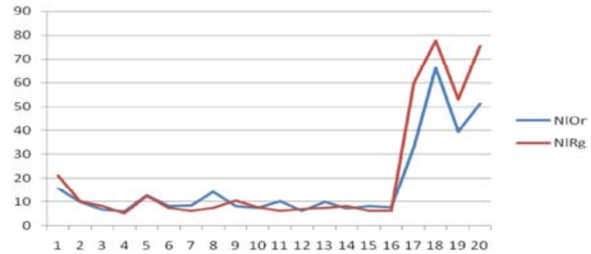

Fig. 3: Different tissue types indicating cell sizes of the original and regrown barks of *Newbouldia laevis*

1. TSPDCx - T.S parenchyma diameter cortex
2. TSPLPh - T.S parenchyma diameter phloem
3. TSPLCx - T.Sp arenchyma diameter length cortex
4. TSPLPh - T.S parenchyma diameter phloem
5. LSPDPh - L.S parenchyma diameter phloem
6. LSPDCx - L.S parenchyma diameter cortex
7. LSPLPh - L.S parenchyma length phloem
8. LSPLCx - L.S parenchyma length cortex
9. TgPDPh - Tg parenchyma diameter phloem
10. TgPDCx - Tg parenchyma diameter cortex
11. TgPLPh - Tg parenchyma length phloem
12. TgPLCx - Tg parenchyma length cortex
13. FDPh - Fibre cell diameter phloem
14. FDCx - Fibre cell diameter cortex
15. FThPh - Fibre cell thickness phloem
16. FThCx - Fibre cell thickness in cortex
17. FLPhIn - Fibre cell phloem length inner
18. FLPhOu -Fibre cell phloem length outer
19. FLCxIn - Fibre cell cortex length inner
20. FLCxOu - Fibre cell cortex length outer

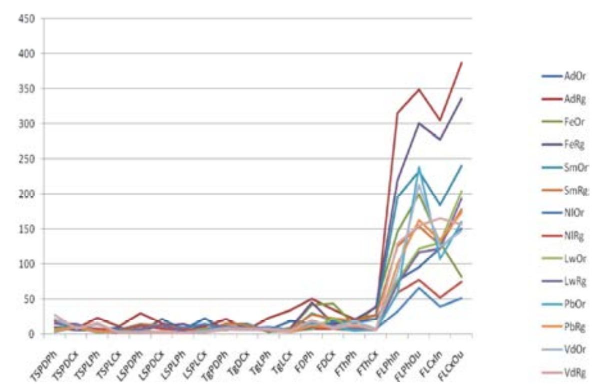

Fig. 4: Various tissues showing cell sizes of the original and regrown barks of some trees

Adansonia digitata original bark - AdOr
Adansonia digitata regrown bark - AdRg
Ficus exasperata origina bark - FeOr
Ficus exasperata regrown bark - FeRg
Spondias mombin original bark - SmOr

Spondias mombin regrown bark - SmRg
Newbouldia laevis original bark - NlOr
Newbouldia laevis regrown bark - NlRg
Lannea welwtchii original bark - LwOr
Lannea welwtchii regrown bark - LwRg
Parkia biglobosa original bark - PbOr
Parkia biglobosa regrown bark - PbRg
Vitex doniana original bark - VdOr
Vitex doniana regrown bark - VdRg

showed the debarked plant cells and the regrown plant cells, while the cell sizes of many were measured and shown in Figures 1- 4 as illustrated. The parenchyma cell sizes ranged from 3-35 µm, whereas the fibre length was up to 400 µm in some species. The fibre length showed a great significant difference in the regrown and the original tree barks. It was distinctly observed that fibre length of regrown barks are longer than the primary formed bark. This was probably to hasten the healing of the wound while the excessive fibre length could be related to the uses of some of the plants.

CONCLUSIONS

This work has implication for plant diversity conservation since it has revealed that debarked tree species may not contain the same chemical composition and therefore need to be protected. This work will in no doubt enrich the science and practice of phytomedicine and the need to ensure sustainable tree bark harvest as it is obvious that the regrown and the original bark varied in chemical make-up. As there has been virtually no previous study on this subject, findings from this work will be used to advise collectors of plant parts with a view to a sustainable use of tree barks.

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