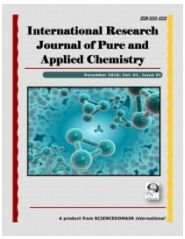

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Isolation and Characterization of the Chemical Constituents of the Stem Bark of *Parinari polyandra* Benth

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Authors' contributions

This work was carried out in collaboration between all authors. Author KOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GAO and ATA managed the analyses of the study. Author UMB managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The young and old stock stem barks of *Parinari polyandra* Benth. were air-dried, chopped into smaller pieces and cold extracted exhaustively with ethanol. The phytochemical screening of the crude extracts was carried out using standard procedures while the young stem bark crude extract was partitioned with n-hexane. The crude extract and the partitioned fractions were concentrated and fractionated using column chromatography packed with Si-gel and alumina and eluted with appropriate solvent systems accordingly. In order to obtain pure compounds, partially purified fractions were further purified using Preparative Thin Layer Chromatography. The preliminary phytochemical test carried out on the crude extracts showed the presence of alkaloids, flavonoids, tannins, saponins, polyphenols, reducing sugars and cardiac glycosides. The structures of the isolated compounds were determined by using data obtained from GC-MS spectrum. The most abundant compound, oleic acid, isolated from the stem bark extracts of *P. polyandra* Benth. is a healthy source of fat in the diet and it has also been implicated to be responsible for the hypotensive effects of olive oil.

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1. INTRODUCTION

Since ancient times mankind has been using plant source to alleviate or cure diseases. Plants constitute a source of novel chemical compounds which are of potential use in medicine and other applications. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such asleaves, flowers, bark, seeds, fruits, root, etc. The therapeutic effects of plant materials typically result from the combination of these secondary products [1].

Parinari polyandra Benth. (Family- Chrysobalanaceae), a medicinal plant found mostly in the tropical regions including Nigeria, Ghana, Cameroon, Senegal, Sudan among others is used for the treatment of several diseases that include diabetes, malaria, inflammation and high blood pressure. The coconut water extract of *Parinari polyandra* Benth. seeds has been reported to have antidiabetic, anti-hyperlipedimia and anticholesterolemia potentials [2]. The cold preparation of a hot water infusion of the stem bark of *Parinari polyandra* Benth. is used in the treatment of diabetes mellitus [3] while in experimental rats, the methanol stem bark extract of *P. polyandra* Benth. Showed hypoglycemic activity [4]

The ethanolic fruit extract of *P. polyandra* Benth. may predispose to hyperlipidemia and electrolye imbalance leading to hypercalcaemia and high risk of raised blood pressure in pregnant rabbits [5]. *Spondias mombin* and *Parinari polyandra* Benth. have glucose lowering effects on alloxan-induced diabetic rats and the study also revealed the possible hepatotoxic effect of co-administration of the two in unorthodox traditional management of diabetes in Nigeria [6].

Oil content, fatty acid composition and protein content of *Parinari polyandra* Benth were investigated [7,8] and it was revealed that the seed oil is not edible because of its high content of free fatty acids and relatively high concentration of eleostearic acid; a polyunsaturated fatty acid with a significant drying property [7].

However, a phytochemical study carried out in 1985 on 31 species of *Parinari* showed a predominance of flavonoids and glycosides based on myrcetin, quarcetin and kaempferol [9]. Other phytochemical studies of *Parinari* species led to the isolation and identification of flavonoids, fatty acids and their glycosides [9,10].

The purpose of this study is to identify the constituent compounds present in the young and old stem bark extracts of *P. polyandra* Benth. and discusss their relevance to the medicinal uses of the plant. The old and the young stem bark of Parinari polyandra Benth. have different morphology and the idea was to investigate the difference in their chemical constituents using the results obtained from Thin Layer Chromatography (TLC) and GC-MS.

2. MATERIALS AND METHODS

2.1 Equipment and Chemicals

A QP2010 PLUS, GC interfaced with a BG mode analytical mass spectrometer was used. Helium was used as carrier gas. Initial column temperature was 120ºC, held for 5 min and

increased at 5ºC /min to 230ºC and held for 5 min. For the MS, electron impact ionization was carried out at 70 eV. Identification of the constituent compounds was by the Chem-Office software along with the MS library.

TLC was carried out using Silica gel 60 F_{254} plates (Merck).Column chromatography was carried out on neutral alumina of 70-300 mesh. All the solvents were redistilled before used and reagents which were obtained in high purity were used without further purification.

2.2 Collection of Plant Material

The young and old stem barks of *Parinari polyandra* Benth. were collected behind the Senate Building, University of Ilorin, Ilorin, Nigeria. They were identified and authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The Voucher with Specimen number 807 is available at the Unit for reference.

2.3 Preparation of Plant Material

The plant sample were air dried at room temperature and then ground to fine powder using pestle and mortar and then stored in an appropriate container until required for use.

2.4 Extraction of Plant Material

1 kg each of the pulverized young and old stem barks were soaked separately in re-distilled ethanol at room temperature for three days. The crude extracts obtained were decanted and filtered and the marcs discarded. The crude extracts obtained were later concentrated to dryness in a vacuo.

2.5 Preliminary Phytochemical Screening

The two extracts were screened for the presence of some secondary metabolites using standard procedures [11,12,13].

2.6 Partitioning of Crude Extract

300 ml of re-distilled n-hexane was added to the young crude extract in a separating funnel. The mixture was shaken and the n-hexane partitioned fraction was obtained.

2.7 Isolation of Components

19 mg of the concentrated n-hexane partitioned fraction of the young stem bark extract was applied on the Si-gel PTLC plate, thinned and air-dried. The plate was developed with n-hexane/dichloromethane (2:1) and viewed under the UV-lamp at short wavelength (254 nm). The zones were marked accordingly and attention was paid to the prominent zones. The observed zones were then scraped, eluted with DCM, filtered and concentrated to obtain the isolates. The TLC analyses of the isolated components were carried out and the retention factor (R_f) values were recorded. The components of the old stem bark extract (5g) were isolated by subjecting it to Column Chromatography CC) packed (with alumina (neutral) and eluted with ethanol. The bright yellow solution obtained was concentrated and treated with activated charcoal. The elution was monitored by TLC. Each time 50 ml was collected in a

flask and identical eluates (TLC monitored) were combined, concentrated and subsequently kept in a clean sample bottle prior to further analysis.

3. RESULTS

3.1 Phytochemical Screening Result

The result of preliminary phytochemical screening is summarized in Table 1 below.

Table 1. Phytochemical constituents of the young and old stem bark extract of *Parinari polyandra* Benth

Constituents bark	Young stem bark	Old stem bark
Alkaloids	++	++
Flavonoids	++	++
Saponins	++	++
Tannins	++	++
Cardiac glycosides	-	+
Polyphenols	++	++
Reducing sugars	++	++
Terpenoids	-	-

++ = Moderate Amount; + = Trace Amount; - = Absence of Constituents

3.2 Isolation and Characterization Result

3.2.1 Young stem bark extract

The partitioning of the crude extract with n-hexane substantially reduced the complexity of the crude extract. The compounds isolated from the young stem bark of *Parinari polyandra* Benth. were characterized using data obtained from GC-MS spectrum. The gas chromatogram of the constituent compounds from the young stem bark extract of *P. polyandra* Benth is shown in Fig. 1 and the constituent compounds having significant abundances with their corresponding mass spectra data are reported in Table 2.

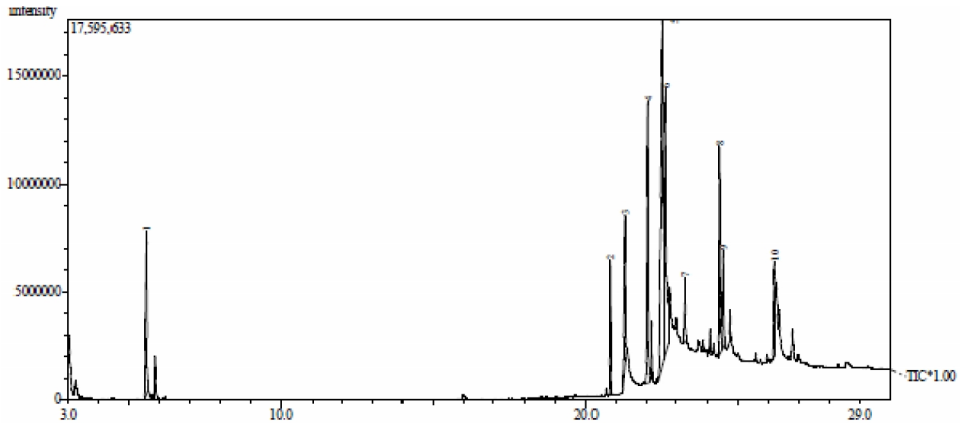

Fig. 1. The gas chromatogram of the constituent compounds from the young stem bark extract of *Parinari polyandra* Benth

Table 2. Percentage composition of the chemical constituents of the young stem bark extract of *Parinari polyandra* Benth

Compound	Retention index	% Composition	Mass spectra data
14-methyl pentadecanoate	1814	3.79	28, 41, 57, 74, 87, 101, 115, 129, 143,171,185, 199, 213, 227, 239, 270
Hexadecanoic acid	1968	8.05	27, 41, 43, 60, 73, 85, 98, 115, 129,143, 157, 171, 185, 213, 227, 256
9-octadecenoic acid, methylester	2085	12.89	27, 41, 55, 69, 74, 87, 98, 123, 137,180, 199, 222,264, 296.
Oleic acid	2175	31.01	7, 41, 55, 69, 83, 97,98,125, 151, 264
Octadecanoic acid	2167	18.21	27,41, 43, 60, 73, 85, 98, 115, 129, 143, 171, 185, 199, 213, 241, 255,284
9-octadecenal	2007	7.85	39, 41, 55, 69, 81, 95,121, 135, 149, 248

It is obvious from Table 2 that the two major compounds obtained from the young stem bark extract of *P. polyandra* Benth. are oleic acid (31.01%, R_T22.535) and octadecanoic acid (20.59% , R_T 22.632) both constituting 51.60% of total. Four other significant compounds constituting 32.58% of total are 14-methyl pentadecanoate (3.79%, R_T 20.795), hexadecanoic acid (8.05%, R_T 21.287), 9-octadecenoic acid, methyl ester, (12.89%, R_T 22.046), 9-octadecenal (7.85%, R_T 24.392). All the six compounds add up to 84.18% of total.

3.2.2 Old stem bark extract

The gas chromatogram of the compounds isolated from the old stem bark extract of *Parinari polyandra* Benth. is shown in Fig. 2 and the constituent compounds having significant abundances with their corresponding mass spectra data are reported in Table 3.

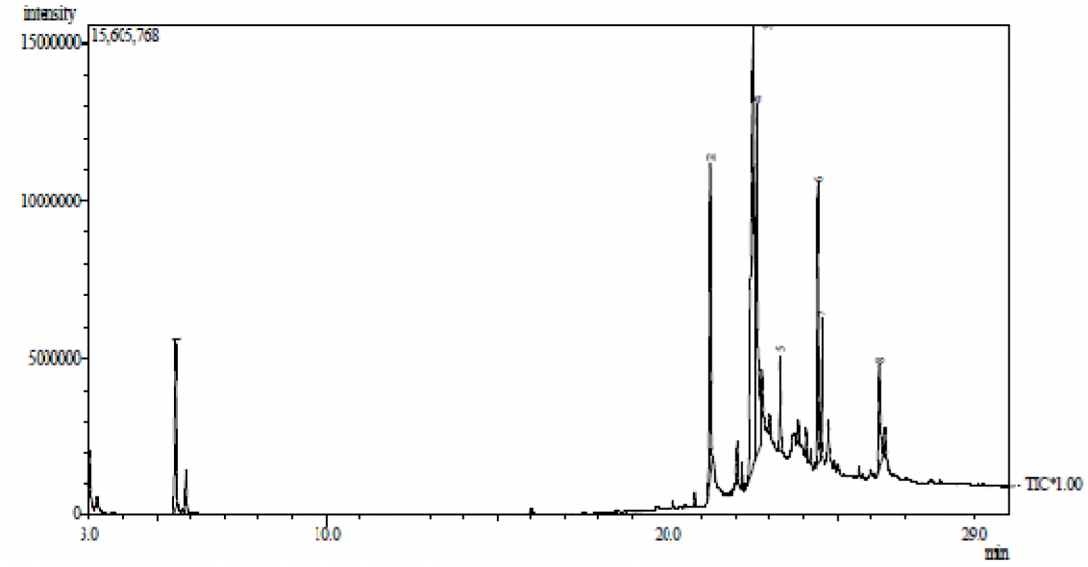

Fig. 2. The gas chromatogram of the essential oil from the old stem bark extract of *Parinari polyandra* Benth

Table 3. Percentage composition of the chemical constituents of the old stem bark extract of *Parinari polyandra* Benth

Compound	Retention index	% Composition	Mass spectra data
hexadecanoic acid, ethyl ester	1978	11.68	28, 41, 57, 74, 87, 101, 115, 129, 143, 171, 185, 199, 213, 227, 239, 270
Oleic acid	1275	37.88	27, 41, 55, 69, 83, 97, 98, 125, 151,264
Octadecenoicacid	2167	21.61	27, 41,43, 60, 73,85,98, 115, 129, 143, 171, 185,199,213, 241,255,284
Cis, cis-linoleic acid	2183	9.46	29,41, 55, 67,81, 95, 109, 123, 137,280
Glycidol stearate	2366	4.01	27,41, 43, 60, 73, 85, 98, 115, 129, 143, 171, 185, 199, 213, 241, 255, 284

It is obvious from Table 3 that the two major compounds present in the old stem bark extract of *P. polyandra* Benth. are oleic acid (37.88%, R_T 22.535) and octadecanoic acid (21.61% , R_T 22.632) both constituting 59.49% of total. Three other significant compounds constituting 25.27% of total are hexadecanoic acid, ethyl ester(11.68%, R_T 21.284), cis, cis-linoleic acid (9.46%, R_T 24.397) andglycidol stearate (4.01%,R_T 24.519). All the five compounds add up to 84.76% of total.

4. DISCUSSION

Preliminary phytochemical screening of the young and old stem bark extracts revealed the presence of phytoconstituents including alkaloids, flavonoids, tannins, saponins, polyphenols, cardiac glycoside and reducing sugars (Table 1). Both extracts showed identical phytoconstituents except for the cardiac glycoside that was conspicuously absent in the young stem bark extract. This result exposes the fact that the plant has quite a number of chemical constituents which may be responsible for its many pharmacological actions. Although their specific roles were not investigated in this study, it has been reported that most active principles in plants are frequently flavonoids, steroids, glycosides, terpenoids, quinones and alkaloids [14].

The constituent compounds isolated from the young and old stem bark of *Parinari polyandra* Benth. as analyzed by GC-MS are long chain carboxylic acids (saturated and unsaturated) and their derivatives including aldehydes and esters.

It is now pertinent to identify the possible roles of these constituent compounds in the curative properties attributed to the plant by herbal medical practitioners. Oleic acid is an unsaturated fatty acid present in several plants and being unsaturated is considered as a healthy source of fat in the diet. Many fatty acids are known to have antibacterial and antifungal properties [15]. It has also been reported that oleic acid content is responsible for the hypotensive effects of olive oil [16]. Hexadecanoic acid and octadecanoic acid are among fatty acids known to have antibacterial and antifungal activities. [17,18].

Linoleic acid is an essential fatty acid that the body cannot synthesize from other food components [19]. A diet only deficient in linoleate causes mild skin scaling, hair loss [20] and poor wound healing in rats [21]. Fatty acid methyl esters are used indirectly in a wide range of food, pharmaceutical, cosmetic and industrial applications. Epoxy fatty acids have been reported to have fungicidal properties in rice [22].

5. CONCLUSION

The old stem bark extract of *Parianri polyandra* Benth. was found to be rich in fatty acid compositions when compared to the young stem bark extract and the variations in their composition may be as a result of climatic condition, temperature and rainfall effect. There is no doubt that the stem bark extracts (young and old) of this plant are reservoirs of potentially useful chemical compounds which can serve as drugs, provide newer leads and clues for modern drug design.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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