

Original Research Article

ISOLATION AND CHARACTERIZATION OF THE CHEMICAL CONSTITUENTS OF THE STEM BARK OF *PARINARI POLYANDRA* BENTH.

ABSTRACT

The young and old stock stem barks of *Parinaripolyandra*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 proceduress while theyoung stem bark crude extract was partitioned with n-hexane. The crude extract and the partitioned fractions were concentrated and fractionated using column chromatography (CC) 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 (~~prep.TLC~~). 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.

Keywords: *Parinaripolyandra*Benth., GC-MS, olive oil, oleic acid, hypotensive.

INTRODUCTION

Since ancient times mankind has been using plant source to alleviate or cure diseases. Plantsconstitute a source of novel chemical compounds which are of potential use in medicine and otherapplications. 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 typicallyresult from the combination of these secondary products [1]

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

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

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

55 However, a phytochemical study carried out in 1985 on 31 species of *Parinari*
 56 ~~revealed that they~~ showed a predominance of flavonoids and glycosides based on myrcetin,
 57 quercetin and kaempferol [9]. Other phytochemical studies of *Parinari* species led to the
 58 isolation and identification of flavonoids, fatty acids and their glycosides [9, 10].

59 The purpose of this study is to identify the constituent compounds present in the
 60 young and old stem bark extracts of *P. polyandra*Benth. and discuss their relevance to the
 61 medicinal uses of the plant.



MATERIALS AND METHODS

Equipment and Chemicals

UNDER PEER REVIEW

69 A QP2010 PLUS, GC interfaced with a BG mode analytical mass spectrometer was
70 used. Helium was used as carrier gas. Initial column temperature was 120°C, held for 5 min
71 and increased at 5°C /min to 230°C and held for 5 min. For the MS, electron impact
72 ionization was carried out at 70 eV. Identification of the constituent compounds was by the
73 Chem-Office software along with the MS library.
74 TLC was carried out using Silica gel 60 F₂₅₄ plates (Merck). Column chromatography was
75 carried out on neutral alumina of 70-300 mesh. All the solvents were redistilled before used and
76 reagents which were obtained in high purity were used without further purification.

78 Collection of Plant Material

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

84 Preparation of Plant Material

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

88 Extraction of Plant Material

89
90 1 kg each of the pulverized young and old stem barks were soaked separately in re-
91 distilled ethanol at room temperature for three days. The crude extracts obtained were
92 decanted and filtered and the marcs were discarded. The crude extracts obtained were later
93 concentrated to dryness in a vacuo.

95 Preliminary Phytochemical Screening

96
97 The two extracts were screened for the presence of some secondary metabolites using
98 standard procedures [11], [12] and [13].

104 Partitioning of crude extract

300ml 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.

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/DCM (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 the latter 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 and concentrated and subsequently kept in a clean sample bottle prior to further analysis.

DCM in full.

Eluting with ethanol is wrong!!

RESULTS

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 *Parinaripolyandra* Benth.

Constituents	Young Stem Bark	Old Stem Bark
Alkaloids	++	++
Flavonoids	++	++ →
Saponins	++	+
Tannins	++	++
Cardiac Glycosides	-	+
Polyphenols	++	++
Reducing sugars	++	++
Terpenoid	-	-

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

147

148

149 ISOLATION AND CHARACTERIZATION RESULT

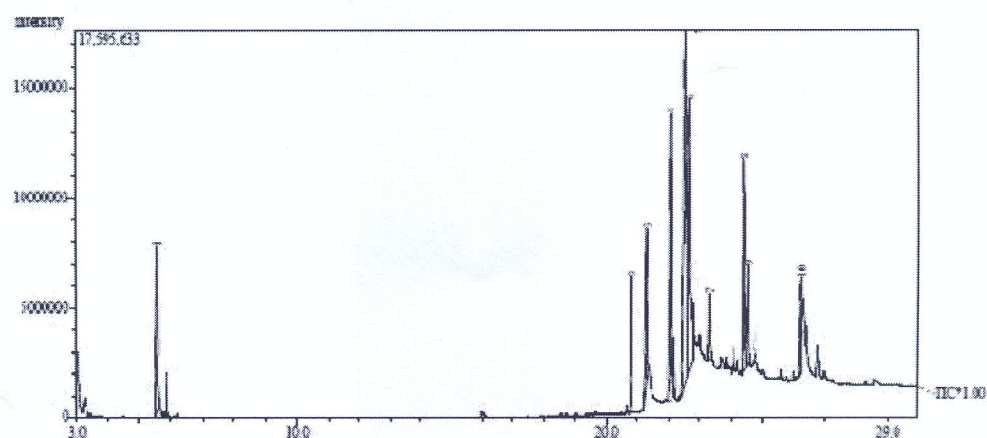
150

151 Young Stem Bark Extract

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

158

159



160

161 **Figure 1 :** The gas chromatogram of the constituent compounds from the young stem bark
 162 extract of *Parinaripolyandra*Benth.

163

164

165

166

167

168

169

170

171

172 **Table 2: Percentage composition of the chemical constituents of the young stem bark**
 173 **extract of *Parinaripolyandra* Benth.**

174

175

Compound	R.I.	% 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

Every table
should be
self
Explanatory

R.I = ????

176

177 It is obvious from Table 2 that the two major compounds obtained from the young stem bark
 178 extract of *P. polyandra* Benth. are oleic acid (31.01%, R_T 22.535) and octadecanoic acid
 179 (20.59%, R_T 22.632) both constituting 51.60% of total. Four other significant compounds
 180 constituting 32.58% of total are 14-methyl pentadecanoate (3.79%, R_T 20.795), hexadecanoic
 181 acid (8.05%, R_T 21.287), 9-octadecenoic acid, methyl ester, (12.89%, R_T 22.046), 9-
 182 octadecenal (7.85%, R_T 24.392). All the six compounds add up to 84.18% of total.

183

184 Old Stem Bark Extract

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

188

189

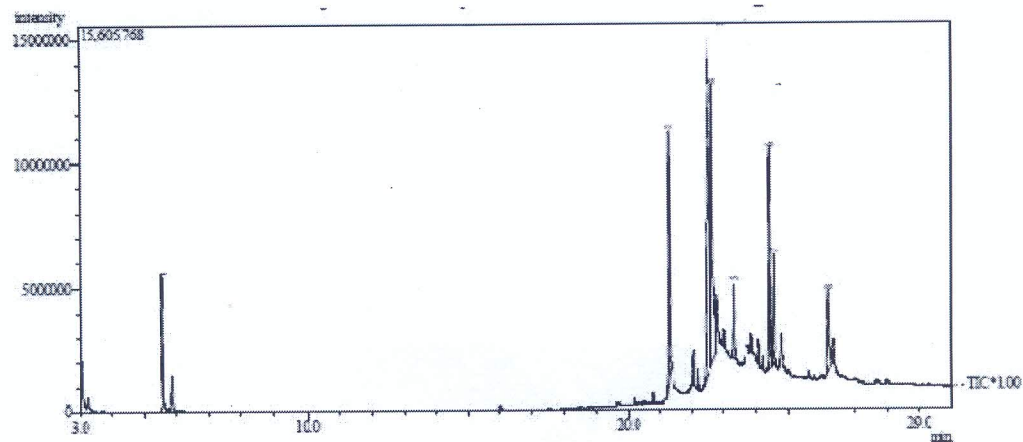


Figure 2: The gas chromatogram of the essential oil from the old stem bark extract of *Parinaripolyandra*Benth.

Table 3: Percentage composition of the chemical constituents of the old stem bark extract of *Parinaripolyandra*Benth.

Compound	R.I.	% 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

R.I. = ???? See previous Comments

200 It is obvious from Table 3 that the two major compounds present in the old stem bark extract
201 of *P. polyandra* Benth. are oleic acid (37.88%, R_T 22.535) and octadecanoic acid (21.61%, R_T
202 22.632) both constituting 59.49% of total. Three other significant compounds constituting
203 25.27% of total are hexadecanoic acid, ethyl ester (11.68%, R_T 21.284), cis, cis-linoleic acid
204 (9.46%, R_T 24.397) and glycidol stearate (4.01%, R_T 24.519). All the five compounds add up
205 to 84.76% of total.

206

207

DISCUSSION

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

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

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

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

232

Conclusion must be
simply a straight forward
You did
not do this.

CONCLUSIONS

- The preliminary phytochemical studies suggest that the pharmacological potentials of the *Parianaripolyandra* Benth. can be attributed to the presence of alkaloids, flavonoids, polyphenols, saponins, tannins and reducing sugars,
- The old stem bark extract of *Parianripolyandra* 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.

REFERENCES

- [1] Tonthubthimthong P., Chuaprasert S., Douglas P., Luewisutthichat W. (2001). Supercritical CO₂ extraction of *nimbin* from neem seeds, an experimental study. *J. Food Eng.*, 47: 289-293.
- [2] Ighodaro O.M., Omole J.O., Adejuwon A.O. and Odunaiya A.A. (2012), Effects of *ParinariPolyandra* Seed Extract on Blood Glucose Level and Biochemical indices in Wistar Rats, *International Journal of Diabetes Research*, 1(4): 68-72.
- [3] Vongtau, H.O., Osunkwo U.A., Okwuasaba F., Gamanniel K.S., and Wambebe C. (1997). Potential antidiabetic activity of extracts of *Parinaripolyandra*. *J. Pharm. Res. Dev.*, 2: 33-37.
- [4] Vongtau, H.O., Abbah J., Ngazal, I.E., Kunle, O.F., Chindo, B.A and Otsapa, P.B. (2004) Anti-nociceptive and anti-inflammatory activities of the methanolic extract of *Parinaripolyandra* stem bark in rats and mice. *J. Ethnopharmacol.*, 90: 115-121.
- [5] Abolaji O.A., Adebayo A.N. and Odesanmi O.S., (2007) Nutritional qualities of three medicinal plants: *Xylopiaspp*, *Blighiaspp* and *parinaripolyandra*, *Pakistan Journal of Nutrition*, 6, 665-668.
- [6] Emeka. E.J. Iweala and Funmilayo D. Oludare, 2011. Hypoglycemic Effect, Biochemical