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CABSALPINIA BONDUC (L.) ROXB. SEED OIL: LIPID COMPOSITION ASSESSMENT

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ABSTRACT:

Thirty different fatty acids of various classes were extracted from the non-polar fraction of the seeds of *Caesalpinia bondue* (L.) Roxb. (Caesalpiniaceae). Eight among them were found to be saturated while 22 were unsaturated acids, which included nine monoenoic, five dienoic, seven triennia and one polyenoic acid. Unsaturated fatty acids were present in a large proportion (65.76%) than the saturated ones (34.24%). Spectroscopic methods (GLC and GC-MS) were used for their characterization and identification. Hexadecadienoic acid occurred in the highest proportion (21.17%). The single polycnoic acid (tetracosapentaenoate) was rare and present in traces (0.95%).

INTRODUCTION

Caesalpinia bonduc (L.) Roxb., which belongs to the family Caesalpiniaceae (Gibbs, 1974), is a valuable medicinal plant and its different parts such as bark, leaves, roots and seeds are utilized in traditional system of medicine. The roots are very effective as an antiperiodic and antispasmodic agent (Chopra *et al.,* 1956), while the bark is useful as an anthelmintic and febrifuge. The leaves are found to be a useful remedy as an emmenagogue (Baquar, 1989). The seeds have a reputation for various pharmacological actions like antiperiodic, antipyretic, febrifuge and asthmatic (Dhar *et al.,* 1968; Nadkarni and Nadkarmi, 1976).

The biological significance of naturally occurring fatty acids revealed that they are necessary for the animal metabolism and storage of energy. A great majority of fatty acids were found in fats and oils in the form of triglyccrides. Dietary fatty acids influence the utilization and metabolism of carbohydrates, proteins, minerals and vitamins. They also serve as a carrier for fat-soluble vitamins such as, D, E and their

precursors (Chow, 1989). Another important character of fatty acids is to influence immune response, as finoleic acid has an effect on lymphatic system and immune function (Satiyavati *et al*., 1976; Dewille, 1979). The humeral response could be improved by the high consumption of essential fatty acids (Sharma and Singh, 1972; Wagner *et al.,* 1982). Keeping the above mentioned facts, the seed oil of *Caesalpinia bonduc cer*tainly merits closer examination, as no work was carried out on this aspect of the plant. Therefore, in this communication an attempt was made to display the fatty acid composition of the seeds of *C. bonduc*.

MATERIALS AND METHODS

The seeds of *Caesalpinia bonduc* (6.5 kg) were collected from Gulshane-Iqbal, Malir and Gadap, areas at and near Karachi and identification was brought about by Dr. Saood Omer, Department of Botany, University of Karachi.

Extraction:

The seeds *were* ground in a homogenizer and soaked in methanol (MeOH) for 60 days at room temperature (Fig.1). A thick gummy residue (95 g) was obtained after evaporating McOH under reduced pressure. The gummy mass was subjected to fractionation, and 1-120 and ethyl acetate (EtOAc) were added to it in equal proportions (1:1) to separate the lipophilic components. The EtOAc was evaporated under reduced pressure and the thick residue so obtained (45 g) was subjected to SiO2 column chromatography. Different fractions were collected using n-hexane as an eluent.

Esterification:

All the oily fractions obtained from the column were esterified with diazomethane. Approximately 0.5 mg of each fraction was dissolved in diethyl ether (Et2O) and 0.5 mL of diazomethane was added to it. The reaction mixture was kept overnight at room temperature (28°C) and thereafter evaporated under reduced pressure.

Gas chromatography mass *spectrometry:*

The unknown fatty acid fractions were analysed first by gas liquid chromatography (GLC) along with methyl ester standards on a Shimadzu GC-9A model gas chromatograph, equipped with a Shimadzu C-R6A chromatopac integrator. The column length was 2 m, whereas inner diameter of the column was 3 mm and outer diameter 5 mm. The column material used was GP 3% SP-2310/ 2% SP-2300 on 100/120 chromosorb WAW. The column initial temperature was 150°C, while final temperature was 250°C with a rate of increase of 8°C/min. The detector as well as

injector temperature was 300°C, nitrogen flow rate was 30 mL/min. Later on the acids were analysed by gas chromatography-mass spectrometry (GC-MS), which was per-formed on a Hewlett Packard GC with a 11/73 DEC computer system and a 1.2 m x 4 mm packed glass capillary column, coated with gas chrome Q (100-120 mesh, GV 101, 1%). The column temperature was programmed from 70°C to 250°C with a rate of in-crease of 8°C/min. The carrier gas helium (He) flow rate was 32 mL/min., injector temperature was 250°C.

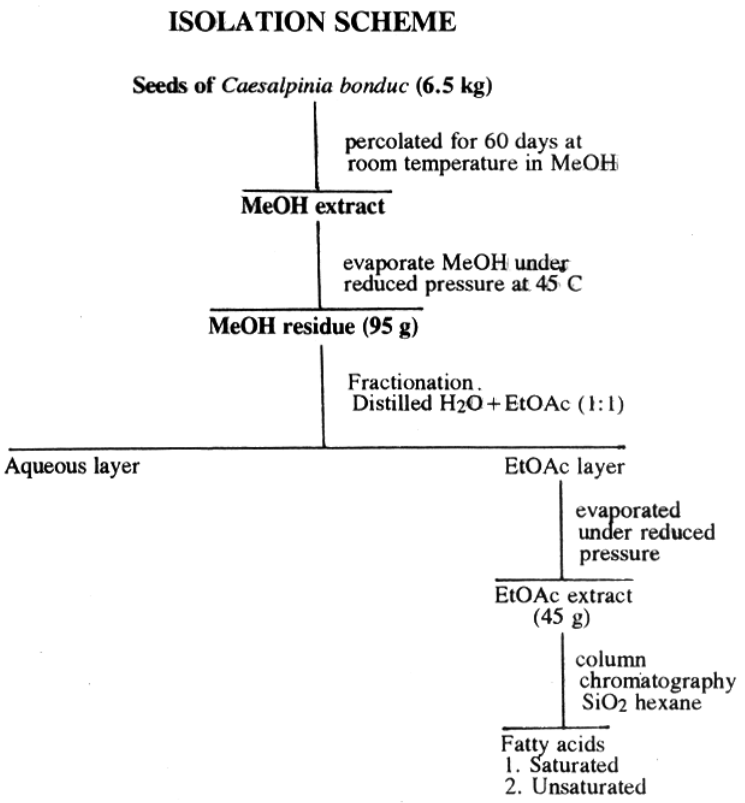

Fig. 1: Scheme for the extraction of fatty acids from seed oil of *Caesalpinia bonduc.*

Identification:

Consequently the fatty acids were identified by matching their GC-mass spectra with those reported in Literature (Godbole et al., 1928; Tumin, 1928; Olszewski and Bankowski, 1955; Daultabad et al., 1988; Kulkarni a *al.,* 1992). The significant ions in the mass spectra of the isolated fatty acids as their methyl esters are given below:

Methyl tridecatrienoale: GC-MS: *m/z* (rel. int. %) 222 (M^+, $C_{14}H_{22}O_2$, 20%), 191 (M^+-31, 4%), 179 (M^+-43, 15%), 165 (M^+-57, 4%), 151 (15%), 137 (35%, 123 (20%), 109 (10%), 95 (32%), 82 (100%).

Tridecadienoate: 224 (M^+, $C_{14}H_{24}O_2$, 10%), 167 (M^+-57, 2%), 153 (5%), 139 (8%), 125 (15%), 97 (63%), 83 (87%), 69 (100%).

9-Tridecenoate: 226 (M^+ $C_{l4}H_{26}O_2$, 15%), 194 (M^+ -32, 2%), 183 (M^+ -43, 5%), 169 (M^+-57, 7%), 155 (6%), 141 (8%), 127 (4%), 113 (12%), 99 (18%), 85 (45%), 71 (84%), 57 (100%).

2,4,5-Tetradecatrienoate: 236 (M^+, $C_{13}H_{24}O_2$, 12%), 205 (M^+-31, 18%), 193 (M^+-43, 16%), 179 (M^+-57, 32%), 165 (80%), 148 (5%), 134 (2%), 120 (4%), 106 (15%), 92 (4%), 78 (26%), 64 (100%).

2,6,10-Pentadecatrienoate: 250 (M^+, $C_{16}H_{28}O_2$, 14%), 193 (M^+-57, 10%), 179 (8%), 167 (2%), 165 (7%), 151 (3%), 137 (10%), 123 05%), 109 (25%), 95 (25%), 81 (20%), 58 (100%).

***n*-Pentadecanoate**: 256 (M^+, $C_{16}H_{32}O_2$, 15%), 225 (M+-31, 5%), 213 (M+-43, 10%), 199 (M^+-57, 20%), 185 (10%), 171 (25%), 157 (18%), 143 (20%), 129 (5%), 115 (15%), 101 (2%), 87 (45%), 73 (100%).

6,10,14-Hexadecatrienoate: 264 (M^+, $C_{17}H_{28}O_2$, 10%), 221 (M^+ -43, 4%), 207 (M^+-57, 2%), 199 (5%), 180 (3%), 157 (5%), 111 (27%), 97 (54%), 83 (82%), 69 (100%).

Hexadecadienoate: 266 (M^+, $C_{17}H_{30}O_2$, 10%), 223 (M^+-43, 5%), 209 (M^+-57, 20%), 195 (15%), 181 (2%), 167 (4%), 153 (5%), 139 (8%),125 (18%), 111 (34%), 97 (60%), 83 (84%), 69 (100%).

9-Hexadecenoate: 268 (M^+, $C_{17}H_{32}O_2$, 8%), 236 (M^+-32, 2%), 225 (M^+- 43, 10%), 211 (M^+-57, 8%), 194 (M^+-74, 6%), 180 (7%), 166 (5%), 152 (12%), 138 (8%), 124 (4%), 110 (2%), 96 (25%), 82 (42%), 71 (100%).

***n*-Hexadecenoate**: 270 (M^+, $C_{17}H_{32}O_2$, 25%), 239 (M^+-31, 8%), 227 (M^+- 43, 6%),

213 (M^+-57, 2%), 199 (4%), 185 (6%), 171(2%), 157 (3%), 143 (15%), 129 (6%), 115 (2%), 101 (6%), 87 (50%), 74 (100%).

Heptadecatrelnoate: 278 (M^+, $C_{18}H_{30}O_2$, 5%), 235 (M^+-43, 5%), 221 (M^+- 57, 2%), 207 (8%), 193 (15%), 179 (8%), 165 (2%), 151 (7%), 137 (35%), 123 (20%), 109 (22%), 95 (32%), 81 (100%).

9,12-Heptadecatreinoate: 280 (M^+, $C_{38}H_{32}O_2$, 10%), 248 (M^+-32, 8%), 206 (M^+-74, 12%), 192 (15%), 178 (12%), 164 (35%), 150 (12%), 136 (2%), 122 (4%), 108 (5%), 94 (6%), 80 (7%), 73 (100%).

Heptadecenoate: 282 (M^+, $C_{18}H_{34}O_2$, 8%), 251 (M^+-31, 6%), 239 (M^+- 43, 10%), 225 (M^+-57, 2%), 208 (M^+ -74, 4%), 194 (12%), 180 (14%), 166 (12%), 152 (15%), 138 (25%), 124 (10%), 110 (13%), 96 (32%), 82 (65%), 71 (100%).

***n*-Hexadecanoate**: 284 (M^+, $C_{18}H_{36}O_2$, 14%), 253 (M^+-31, 2%), 241 (M^+- 43, 6%), 227 (M + -57, 2%), 213 (2%), 199 (4%), 185 (2%), 171 (1%), 157 (12%), 143 (7%), 129 (3%),115 (5%), 101 (50%), 88 (100%).

9,12,15-Octadecatrienoate: 292 (M^+, $C_{19}H_{32}O_2$, 65%), 261 (M^+ -31, 10%), 235 (M^+-57, 5%), 218 (M^+-74, 2%), 199 (12%), 185 (14%), 171 (10%), 157 (6%), 143 (30%), 129 (16%), 115 (15%), 101 (12%), 89 (75%), 69 (100%).

9,12-Octadecatrienoate: 294 (M^+, $C_{19}H_{34}O_2$, 18%), 262 (M^+-32, 10%), 220 (M^+-74, 6%), 178 (5%), 164 (9%), 150 (12%), 136 (14%), 122 (7%), 108 (26%), 94 (58%), 80 (82%), 67 (100%).

9-Octadecenoate: 296 (M^+, $C_{19}H_{36}O_2$, 10%), 264 (M^+-32, 8%), 253 (M^+-43, 4%), 239 (M^+-57,10%), 226 (2%), 208 (4%),180 (1%), 166 (5%), 155 (4%), 141(6%), 124 (8%), 110 (10%), 96 (15%), 82 (45%), 71(103%).

***n*-Octadecanoate**: 298 (M^+, $C_{19}H_{38}O_2$, 15%), 267 (M^+-31, 4%), 255 (M^+-43, 7%), 241 (M^+-57, 5%), 227 (2%), 213 (7%), 199 (8%), 185 (3%), 171 (2%), 157 (1%), 143 (13%), 189 (8%), 115 (5%), 101 (6%), 87 (54%), 83 (40%), 74 (100%).

***n*-Eicosanoate**: 326 (M^+, $C_{21}H_{42}O_2$, 8%), 295 (M^+-31, 6%), 283 (M^+-43, 8%), 296 (M^+-57, 12%), 252 (M^+-74, 10%), 239 (10%), 225 (12%), 211 (6%), 197 (7%), 183 (6%),169 (2%), 155 (11%), 144 (8%), 127 (15%), 113 (18%), 99 (25%), 85 (45%), 71 (100%).

Heneicosenoate: 338 (M^+, $C_{22}H_{42}O_2$, 12%), 307 (M^+-31, 5%), 295 (M^+-43, 11%),

281 (M^+-57, 15%), 267 (8%), 253 (4%), 139 (8%), 225 (2%), 211(1%), 197 (8%), 183 (4%), 169 (5%), 155 (12%), 141 (15%), 127 (10%), 113 (18%), 99 (15%), 85 (60%), 71 (98%), 57 (100%).

***n*-Heneicosanoate**: 340 (M^+, $C_{22}H_{44}O_2$, 12%), 297 (M^+-43, 10%), 283 (M^+-57, 12%), 241 (8%), 227 (12%), 199 (8%), 177 (8%), 157 (12%), 143 (6%), 129 (11%), 115 (15%), 101(20%), 87 (65%), 73 (85%), 57 (100%).

11-Drcosenoate: 352 (M^+, $C_{23}H_{44}O_2$, 25%), 309 (M^+-43, 12%), 295 (M^+-57, 10%), 281 (8%), 267 (8%), 253 (8%), 239 (9%), 225 (8%), 211 (8%), 197 (8%), 183 (8%), 155 (10%), 141 (10%), 127 (14%), 113 (15%), 99 (20%), 85 (60%), 71 (97%), 57 (100%).

***n*-Docosanoate**: 354 (M^+, $C_{23}H_{46}O_2$, 10%), 311 (M^+-43, 8%), 297 (M^+-57.4%),280 (M^+-74, 4%), 266 (6%), 252 (6%), 238 (12%), 224 (2%), 210 (6%), 196 (14%), 182 (4%), 168 (5%), 154 (5%), 97 (45%), 83 (52%), 69 (84%), 57 (100%).

Tricosenoate: 366 (M^+, $C_{24}H_{46}O_2$, 8%), 323 (M^+-43, 2%), 309 (M^+-57, 5%), 295 (10%), 281 (6%), 267 (2%), 253 (1%), 239 (4%), 225 (5%), 211 (4%), 197 (6%), 83 (6%), 169 (7%), 155 (6%), 141 (6%), 113 (8%), 111 (14%), 99 (15%), 97 (20%), 85 (50%), 71 (85%), 57 (100%).

***n*-Tricosanoate**: 368 (M^+, $C_{24}H_{48}O_2$, 10%), 337 (M^+ -31, 5%), 325 (M^+-43, 4%), 311 (M^+ -57, 6%), 297 (4%), 279 (20%), 255 (5%), 239 (5%), 221 (15%), 211 (10%), 185 (12%), 171 (4%), 167 (40%), 149 (60%), 129 (15%), 101 (10%), 87 (40%), 74 (100%).

Telracosapentaenoate: 372 (M^+, $C_{25}H_{40}O_2$, 6%), 341 (M^+-31, 8%), 329 (M^+-43, 12%), 315 (M^+ -57, 15%), 298 (M^+-74, 8%), 221 (20%), 207 (15%), 191 (6%), 111 (30%), 97 (42%), 83 (50%), 71 (82%), 57 (100%).

Tetracosadienoate: 378 (M^+, $C_{25}H_{46}O_2$, 25%), 346 (M^+ -31, 10%), 335 (M^+-43, 30%), 321 (M^+-57, 12%), 304 (5%), 266 (2%), 205 (10%), 149 (80%), 121 (25%), 93 (44%), 73 (100%).

15-Tetracosenoate: 380 (M^+, $C_{25}H_{48}O_2$, 8%), 349 (M^+-31, 6%), 337 (M^+-43, 5%), 323 (M^+-57, 10%), 309 (8%), 281 (4%), 267 (4%), 267 (4%), 253 (4%), 239 (2%), 211 (1%), 197 (5%), 183 (5%), 169 (5%), 141 (5%), 113 (8%), 85 (50%), 71 (82%), 57 (100%).

Pentacosenoate: 394 (M^+, $C_{26}H_{50}O_2$, 15%), 363 (M^+-31, 5%), 351 (M^+ -43, 6%), 337 (M^+-57, 3%), 323 (6%), 309 (4%), 295 (8%), 281 (7%), 267 (8%), 253 (8%),

239 (8%), 225(2%), 211(2%), 197(6%), 183(6%), 169(8%), 155(8%), 141(8%), 127.

Nonacosatrienoate: 446 (M^+, $C_{30}H_{54}O_2$, 4%), 415 (M^+-31, 2%), 403 (M^+-43, 5%), 389 (M^+-57, 5%), 375 (4%), 303 (2%), 275 (4%), 247 (2%), 207 (5%), 197 (6%), 163(6%), 149(6%), 135(6%), 111 (15%), 97 (20%), 85 (50%), 71 (86%), 57 (100%).

RESULTS AND DISCUSSION

It has been revealed through literature survey that no work was published so far on fatty acid components of *Caesalpinia bonduc.* However, some fatty acids have been isolated from other species of the genus. The fatty acids such as crepeynic, dehydrocrepeynic, lignoceric, linoleic, myristic, oleic, palmitic and stearic acids were reported from C. bonducella Fleming (Godbole *et al*, 1928; Tumin, 1928; Kulkarni *et al*, 1992); whereas octadecenoic and octadecadienoic acids were found to be present in *C. codana* (Iacq.) Willd. and *C. decapetala* (Godbole *et al*., 1928). While linoleic, myristic, oleic, olinie, palmitic and stearic acids (Daulatabad *et al*., 1988), as well as linoleic, oleic, palmitic and stearic acids were described from *C. pulcherrima* (L.) Swartz (Southan, 1994).

The non-polar fraction of the methanolic extract from the seeds of *Caesalpinia bonduc* resulted in the isolation of thirty fatty acids, out of which eight were found to be saturated and twenty two unsaturated (Table 1). The unsaturated fatty acids included nine monoenoic, five dienoic, seven trienoic and only one polyenoic acid. Unsaturated fatty acids were present in a larger proportion (65.76%) than the saturated ones (34.24%). They have been identified by methylene splitting and other characteristic fragmentation pattern as well as on the basis of differences in their relative retention time.

Table 1

Fatty acids analysed as methyl esters in the seed oil of *Caesalpinia bonduc*

Systematic name	Common name	Molecular formula	Mol. wt.	Relative % age
Saturated fatty acid methyl esters:				
n-Pentadecanoate	Pentadecylate	$C_{16}H_{32}O_2$	256	2.06
n-Hexadecanoate	Palmitate	$C_{17}H_{34}O_2$	270	19.10
n-Heptadecanoate	Margarate	$C_{18}H_{36}O_2$	284	1.22
n-Octadecanoate	Stearate	$C_{19}H_{38}O_2$	298	5.28
n-Eicosanoate	Arachidate	$C_{21}H_{42}O_2$	326	1.41

Table continue...

Systematic name	Common name	Molecular formula	Mol. wt.	Relative % age
n-Heneicosanoate	Heneicosoate	$C_{22}H_{44}O_2$	340	1.25
n-Docosanoate	Behenate	$C_{23}H_{46}O_2$	354	2.65
n-Tricosanoate	Tricosoate	$C_{24}H_{48}O_2$	368	1.27
Unsaturated fatty acid methyl esters:				
Monoenoic acids:				
9-Tridecenoate	Decylacrylate	$C_{14}H_{26}O_2$	226	1.67
9-Hexadecenoate	Palmitoleate	$C_{17}H_{32}O_2$	268	0.69
Heptadecenoate	Heptadecylenate	$C_{18}H_{34}O_2$	282	0.93
9-Octadecenoate	Oleate	$C_{19}H_{36}O_2$	296	1.06
Heneicosenoate	Heneicosenoate	$C_{22}H_{42}O_2$	338	2.28
11-Docosenoate	Cetoleate	$C_{23}H_{44}O_2$	352	1.62
Tricosenoate	Tricosenoate	$C_{24}H_{46}O_2$	366	1.22
15-Tetracosenoate	Selacholeate	$C_{25}H_{48}O_2$	380	1.14
Pentacosenoate	Pentacosenoate	$C_{26}H_{50}O_2$	394	0.56
Dienoic acids:				
Tridecadienoate	Tridecadienoate	$C_{14}H_{24}O_2$	224	3.20
Hexadecadienoate	Hexadecadienoate	$C_{17}H_{30}O_2$	266	21.17
9,12-Heptadecadienoate	Heptadecadienoate	$C_{18}H_{32}O_2$	280	1.62
9,12-Octadecadienoate	Linoleate	$C_{19}H_{34}O_2$	294	6.16
Tetracosadienoate	Tetracosadienoate	$C_{25}H_{46}O_2$	378	3.03
Trienoic acids:				
Tridecatrienoate	Tridecatrienoate	$C_{14}H_{22}O_2$	222	1.51
2,4,5-Tetradecatrienoate	Tetradecatrienoate	$C_{15}H_{24}O_2$	236	1.65
2,6,10-Pentadecatrienoate	Pentadecatrienoate	$C_{16}H_{26}O_2$	250	2.29
6,10,14-Hexadecatrienoate	Hiragonate	$C_{17}H_{28}O_2$	264	1.91
Heptadecatrienoate	Heptadecatrienoate	$C_{18}H_{30}O_2$	278	4.58
9,12,15-Octadecatrienoate	Octadecatrienoate	$C_{19}H_{32}O_2$	292	5.91
Nonacosatrienoate	Nonacosatrienoate	$C_{30}H_{54}O_2$	446	0.53
Polyenoic acid:				
Tetracosapentaenoate	Tetracosapentaenoate	$C_{25}H_{40}O_2$	372	0.95

Out of all the detected fatty acids hexadecadienoate had the highest relative percentage of occurrence (21.17%). The seed oil of *Caesalpinia coriaria* contained linoleic acid (31.3%) as major acid (Daulatabad *et al*., 1988). Usually palmitate was found to be present in the highest amount in the non-polar fractions of the plant extracts, but in C. bonduc its relative percentage was 19.10%. It was detected as 18.0% in the seed oil of *C*. *coriaria* (Daulatabad *et al.,* 1988), which is more or less the same amount. Polyenoic acids occur very rarely in higher plants, same was the case here i.e. only one polyenoic acid (tetracosapentaenoate) was found in trace amount in *C. bonduc* (0.95%).

Among saturated fatty acids, palmitate was detected in the highest amount (19.10%), whereas stearate had the relative percentage of 5.28% (Table 1). Stearic acid was present in appreciable amount (12.9%) in the seed oil of C. *coriada* (Daulatabad *et al,* 1988). Behenate and pentadecylate were present in 2.65% and 2.06% respectively in *C. bonduc*, while margarate, arachidate, heneicosoate and tricosoate had the relative percentages of 1.22%, 1.41%, 1.25% and 1.27%, respectively. Among nine monoenoic acids palmitoleate, heptadecylenate and pentacosenoate were present in trace amounts i.e. 0.69%, 0.93% and 0.56% respectively; whereas decylacrylate, oleate, heneicosenoate, cetoleate, tricosenoate and selacholeate were detected in small quantities i.e. 1.67%, 1.06%, 2.28%, 1.62%, 1.22% and 1.14%, respectively. The seed oil of *C. coriaria* also contained oleic acid (4.9%) in small amounts (Daulatabad *et al.,* 1988). Among five dienoic acids, hexadecadienoate was present in the highest amount (21.17%), whereas linoleate (6.16%), tridecadienoate (3.20%), tetracosadienoate (3.03%) and heptadecadienoate (1.62%) were also present. Among seven trienoic acids the highest relative percentage was of octadecatrienoate (5.91%), whereas heptadecatrienoate (4.58%), pentadecatrienoate (2.29%), tridecadienoate (1.51), tetradecatrienoate (1.65%) and hiragonate (1.91%) were also present and nonacosatrienoate occurred in trace amount (0.53%). These fatty acids are being reported for the first time from this species.

The unsaturated fatty acids are useful for humans and animals as these are not synthesized by them, *they* are supplemented from vegetable food. Beside this aspect, unsaturated fatty acids have an effect on hyperlipidemia by lowering the levels of triglycerides and cholesterol. The hyperlipidemia is related to the development of atherosclerosis and coronary heart disease (Ross and Harker, 1976). It may be indirectly interpreted that the seed oil of *Caesalpinia bonds c* can be compared with the corn oil or soybeen oil in its contents of unsaturated fatty acids.

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