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Full Length Research Paper

Lipid content and antimicrobial activity of some Egyptian Fabaceae (Leguminosae) plants

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In this study, lipid contents and antimicrobial activity of *Saraca indica* L. and *Enterolobium cyclocarpum* Jacq. (family *Fabaceae*) were evaluated. The gas liquid chromatography (GLC) analysis of the *n*-hexane extracts of both plants revealed total saturated fatty acids of 59.14 and 62.44%, respectively. Within the saturated fraction, the major fatty acid was palmitoleic acid ($C_{16.0}$), the content of which both plants was 36.55 and 38.89%, respectively. The monounsaturated fatty acid contents of *S. indica* and *E. cyclocarpum* is 40.83 and 37.51%, respectively. Concerning the monounsaturated fatty acids, palmitoleic acid ($C_{16:1}$) was present in larger percentages of 28.30 and 28.99%, respectively, than the others. Both lipid fractions of *S. indica* and *E. cyclocarpum* showed moderate to good antimicrobial activity against Gram positive and Gram negative bacteria.

Key words: *Saraca indica, Enterolobium cyclocarpum,* lipid, gas liquid chromatography (GLC), antimicrobial.

INTRODUCTION

Fabaceae (Leguminosae) is a family of flowering plants comprising about 269 genera and 5100 species and it is one of the largest plant families in the world (Mabberley, 1997). Leguminosae is well suited with respect to chemical components. Lipids from some more common Leguminosae have been investigated to some extent, other legume lipids have not been studied in any great detail, because of their low lipid content and limited or negligible use for oil purposes (Gunstone et al., 1972; Kleiman, 1988; Chowduryet al.,, 1984; Chowdury and Banerjii, 1995; Ucciani, 1995; Grela and Gunter, 1995).

Omega 3 fatty acids are polyunsaturated fatty acids which have been associated with many health benefits (Freeman, 2000). Linoleic acid is needed for a normal immune response and an essential fatty acid deficiency impairs B and T cell-mediated responses (Meydani et al., 1991). Polyunsaturated fatty acids (PUFAs) function as major nutrients, constituents of cell membranes and precursors of various signal molecules (Needleman et al., 1986; Sakuradani et al., 1999). They are important in both the medical and pharmaceutical fields, as they are involved in the human inflammatory response, blood pressure regulation, cholesterol metabolism, and infant retinal and brain development (Horrobin, 1992; van Gool et al., 2003).

In this study, the lipid content of *Saraca indica* and *Enterolobium cyclocarpum* were evaluated for antimicrobial activity and chemical gas liquid chromatography (GLC) analysis.

MATERIALS AND METHODS

Plant

Fresh leaves of *S. indica* L. and *E. cyclocarpum* Jacq. (Griseb), were collected on April, 2008 from El-Orman garden (Giza, Egypt) and identified by Professor Dr. Abdel Megeed Ali, head of plant

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taxonomy in Agriculture Research Center (Dokki, Egypt).

Chemical analysis

Preparation of the lipoidal matter

About 100 g of the air-dried powder from each plant were exhaustively extracted separately with *n*-hexane. The solvent was evaporated under reduced pressure and at a temperature up to 40°C till constant weight. The yield of the lipoidal matter was 1.4 and 1.8 g from *S. indica* and *E. cyclocarpum*, respectively. One gram of the *n*-hexane extracts of each were separately treated with chloroform:methanol (2:1) to extract the lipids. The combined *non*-lipids were removed by washing each extract three times with methanol:water (1:1). The chloroformic layers were then filtered over anhydrous sodium sulfate and the solvent was evaporated under vacuum at 40°C.

Saponification of the lipoidal matter

The lipid samples under investigation were separately saponified overnight with ethanolic potassium hydroxide (KOH, 20%) at room temperature. The saponified extracts were concentrated to third their volumes. The cooled reaction mixtures were diluted with an equal volume of distilled water and exhaustively extracted with ether. The ethereal extracts were washed several times with distilled water until free of alkalinity and filtered over anhydrous sodium sulfate (Vogel, 1975). After evaporation of the solvent to dryness, the residues [435 (43.5%) and 455 mg (45.5%)] of *S. indica* and *E. cyclocarpum*, respectively, were kept for studying the unsaponifiable matter.

The alkaline aqueous solutions remaining after extraction of the unsaponifiable matter were acidified with hydrochloric acid (5 N) to liberate the free fatty acids, followed by extraction several times with ether. The ether extracts were washed three times with distilled water then filtered over anhydrous sodium sulfate (Vogel, 1975). The residues [355 (35.5%) and 385 mg (38.5%)] of *S. indica* and *E. cyclocarpum*, respectively, were kept for studying the fatty acid contents.

Preparation of fatty acid methyl esters (FAME)

The fractions containing free fatty acids of each plant, as well as, the standard fatty acids were separately dissolved in a small amount of anhydrous methanol and were methylated by the addition of diazomethane ethereal solution drop wise until gas evolution ceased and the mixtures acquired a pale yellow color indicating the addition of excess diazomethane. The reaction mixtures were left for 10 min and ether was evaporated under nitrogen stream at room temperature (Vogel, 1975). The residues were kept in desiccators for GLC analysis.

GLC analysis of the unsaponifiable matter:

An aliquot (2 µl) of 2% chloroformic solution of the unsaponifiable matter of each plant under investigation was analyzed by GLC technique together with 2% chloroformic solution of available authentic. GLC analysis of unsaponifiable matter was performed on Pye UNIC Gas Liquid Chromatography, series 304 equipped with flame ionization detector (FID). A coiled glass column (UNICAM Pro GC, 2.8 m × 4 mm) packed with diatomite (100 to 120 mesh) and coated with 3% OV-17 was used in the separation (Principal Laboratory, Faculty of Agriculture, Cairo University).

Identification of the hydrocarbons and sterols was carried out by comparing the relative retention times of the peaks with those of the pure available authentics. Quantitative estimation was done by peak area measurement using a computing integration. Results of GLC analysis of unsaponifiable matter of the *n*-hexane extracts of *S. indica* and *E. cyclocarpum* are shown in Table 1.

GLC analysis of the FAME

An aliquot (2 µl) of 2% chloroformic solution of the FAME of each plant under investigation was analyzed by GLC technique together with 2% chloroformic solution of available authentics (Farag et al., 1986). GLC analysis of FAME was carried out similar to the unsaponifiable matter except that a coiled glass column (UNICAM Pro GC, 1.5 m × 4 mm) packed with diatomite (100 to 120 mesh) and coated with 10% polyethylene glycol adipate (PEGA) was used (Principal Laboratory, Faculty of Agriculture, Cairo University).

Identification of the fatty acids was carried out by comparing the relative retention times of the peaks with those of the pure available authentics. Quantitative estimation was done by peak area measurement using a computing integration. Results of GLC analysis of FAME of the *n*-hexane extracts of *S. indica* and *E. cyclocarpum* are shown in Table 2.

Antimicrobial activity

The antimicrobial activity of the lipid part of *S. indica* and *E. cyclocarpum* was determined by using a modified Kirby-Bauer disk diffusion method (Bauer et al., 1966). 100 µl of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of approximately 108 cells/ml for bacteria or 105 cells/ml for fungi (Pfaller et al., 1988). Then, 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method (NCCLS, 1993, 1997). Plates are inoculated with filamentous fungi as *Saccharomyces cerevisiae* at 25°C for 48 h; Gram positive bacteria as *Bacillus subtilis, Staphylococcus aureus*; Gram negative bacteria as *Escherichia coli*, *Pseudomonas aeruginosa*, were incubated at 35 to 37°C for 24 to 48 h and yeast as *Candida albicans* incubated at 30°C for 24 to 48 h (Bauer et al., 1966).

Impregnated discs with 10 µl of DMSO were used as negative control. Standard discs impregnated with tetracycline (antibacterial agent) and amphotericin B (antifungal agent) served as positive control for antimicrobial activity, respectively. Blank paper disks (Schleicher & Schuell, Spain) with a diameter of 8 mm were impregnated with 10 µl of the tested concentration of the stock solution and were placed on agar plates, the diameters of the inhibition zones were measured in millimeters.

Minimum inhibitory concentration (MIC)

S. aureus and *P. aeruginosa* were tested for the MIC against *S. indica* and *E. cyclocarpum*, respectively, using agar dilution method. Stationary phase cultures of each bacteria were prepared at 37°C and were used to inoculate fresh 5 ml culture to an OD_{600} (optical density at 600 nm) of 0.05 ml. The 5 ml cultures were then incubated at 37°C until an OD_{600} (optical density at 600 nm) of 0.10 ml was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6×10^5 CFU/ml. Serial dilutions from the lipid extracts (0 to 320 µg/ml) were prepared and

Table 1. Results of GLC analysis of the unsaponifiable fractions of the *n*-hexane extracts of *S. indica* and *E. cyclocarpum*.

Peak number	Component	RR_t*	Relative percentage	
			S. indica	***E. cyclocarpum***
1	*n*-tetradecane (C_{14})	0.57	11.46	2.69
2	*n*-pentadecane (C_{15})	0.58	2.81	1.53
3	*n*-hexadecane (C_{16})	0.59	5.44	1.33
4	*n*-octadecane (C_{18})	0.71	20.94	19.73
5	*n*-nonadecane (C_{19})	0.72	0.59	1.26
6	*n*-eicosane (C_{20})	0.95	0.68	1.22
7	*n*-docosane (C_{22})	1.00	0.93	8.25
8	*n*-tetracosane (C_{24})	1.03	3.58	10.95
9	*n*-hexacosane (C_{26})	1.06	0.00	1.48
10	*n*-heptacosane (C_{27})	1.10	7.21	2.77
11	*n*-octacosane (C_{28})	1.13	4.27	1.47
12	*n*-nonacosane (C_{29})	1.14	22.67	2.39
13	Stigmasterol	1.16	4.35	4.78
14	*β*-sitosterol	1.17	6.81	3.68
15	*n*-triacontane (C_{30})	1.19	5.58	28.79
16	*n*-dotriacontane (C_{32})	1.22	0.22	6.76
Percentage of total hydrocarbons			97.54	99.08

*RR_t: Relative retention time to n-docosane (R_t = 38.3 min).

Table 2. Results of GLC analysis of the fatty acid methyl esters of the saponifiable fractions of the *n*-hexane extracts of *S. indica* and *E. cyclocarpum*.

Peak number	Component (FAME)	RRt*	Relative percentage	
			S. indica	***E. cyclocarpum***
1	Capric $C_{10:0}$	0.42	3.49	3.68
2	Lauric $C_{12:0}$	0.46	0.00	3.38
3	Myristic $C_{14:0}$	0.85	18.72	15.88
4	Palmitic $C_{16:0}$	1.00	36.55	38.89
5	Palmitoleic $C_{16:1}$	1.09	28.30	28.99
6	Stearic $C_{18:0}$	1.34	0.38	0.61
7	Linoleic $C_{18:2}$	1.84	11.98	7.57
8	Linolenic $C_{18:3}$	2.38	0.55	0.95
Percentage of total unsaturated (FAME)			40.83	37.51
Percentage of total saturated (FAME)			59.14	62.44

*RR_t: Relative retention time to palmitic (R_t = 2.87 min).

mixed with 5 ml of the standardized bacteria suspension, then they were added to the plates and incubated for 24 h at 37°C. The colony forming units (CFU) were counted for each dilution (NCCLS: M7 - A4, 1997).

RESULTS AND DISCUSSION

The lipid contents of the fresh collected leaves of *S. indica* and *E. cyclocarpum* were extracted by n-hexane. The obtained extracts were saponified with ethanolic KOH, the unsaponifiable contents were extracted by ether, while the alkaline aqueous solutions remaining were acidified with hydrochloric acid (5 N) to liberate the free fatty acids. The chemical compositions of unsaponifiable and the methylated fatty acids (by diazomethane) for both plants of *S. indica* and *E. cyclocarpum* were analyzed by GLC.

The GLC analysis of the unsaponifiable matter revealed

Table 3. The antimicrobial activity of *S. indica* and *E. cyclocarpum.*

Microorganism	Inhibition zone diameter (mm/µg)			
	Tetracycline (antibacterial)	Amphotericin B (antifungal)	*S. indica*	*E. cyclocarpum*
B. subtilis ATCC 6633	33	-	13	11
S. aureus ATCC 5141	31	-	13	13
E. coli ATCC 10536	35	-	13	12
P. aeruginosa NTCC 6750	32	-	14	12
C. albicans	-	18	0.0	0.0
S. cerevisiae	-	15	0.0	0.0

Table 4. Minimum Inhibitory Concentration, MIC of *S. indica* and *E. cyclocarpum*.

Sample	MIC (µg/ml)	
	***S. aureus* ATCC 5141**	***P. aeruginosa* NTCC 6750**
S. indica	18.6	-
E. cyclocarpum	-	16.9
Tetracycline	0.032	0.028

a high relative percentage of hydrocarbons in the unsaponifiable fractions of the *n*-hexane extracts of *S. indica* and *E. cyclocarpum* being 99.08 and 97.54%, respectively. The major constituents in the unsaponifiable fractions of both plants were *n*-nonacosane (22.67%) and *n*-triacontane (28.79%), respectively. *β*-Sitosterol and stigmasterol were the only sterols identified in both samples, where *β*-sitosterol was predominant in *S. indica* (6.81%), while stigmasterol was predominant in *E. cyclocarpum* (4.78%). The GLC analysis of the FAME revealed a high relative percentage of total saturated *S. indica* and *E. cyclocarpum* being 59.14 and 62.44%, respectively. Palmitic acid is the major constituent of the identified saturated fatty acids in both plants with relative percentage of 36.55 and 38.89%, respectively.

The total relative percentage of unsaturated (FAME) *S. indica* and *E. cyclocarpum* is 40.83 and 37.51%, respectively, and the major constituent of the identified unsaturated fatty acids is palmitoleic acid with relative percentage of 28.30 and 28.99%, respectively.

The results of the this study, as far as unsaturated fatty acid content is concerned, showed that the main components in the oils are palmitic-oleic type fatty acids, while the previous leguminous studies (Sengupta and Basu, 1978; Hamberg and Fahlstadius, 1992; Liu et al., 1995), reported that the saturated and particularly unsaturated FAME contents of Fabaceae seed oils are closely allied to each other and that the main components in the oils are linoleic-oleic type fatty acids.

Antimicrobial evaluation

Both lipid fractions of *S. indica* and *E. cyclocarpum* were subjected for antimicrobial test against some selected pathogenic bacteria and fungi. The fractions of both plants showed moderate to good antimicrobial activity against Gram positive and negative bacteria, but no antifungal activity (Table 3).

From Table 3, the lipid fraction of *S. indica* fraction showed good inhibition zoon activity against *S. aureus*, and the lipid fraction of *E. cyclocarpum* showed good antimicrobial activity against *P. aeruginosa*. The MIC of *S. indica* against *S. aureus* is 18.6 µg/ml; while the MIC of *E. cyclocarpum* against *P. aeruginosa* is 16.9 µg/ml (Table 4).

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