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Fatty Acid Profile and Biological Activities of the Aerial Parts of *Desmodium elegans* Dc

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Abstract: The present study was aimed at investigating the chemical composition and biological activities of the n-hexane fraction obtained from the aerial parts of *Desmodium elegans*. As a routine exercise for such studies, the fatty acids were transformed *in situ* to the corresponding methyl esters and subjected to gas chromatographic-mass spectrometric analysis. A total of 19 fatty acid methyl esters (FAMEs) have been identified. The predominant components are palmitic acid (59.01%), stearic acid (12.33%), behenic acid (7.48%), arachidic acid (4.20%), tetracosanoic acid (3.84%), tricosanoic acid (2.37 %) and margaric acid (2.36) %). The same n-hexane fraction was also tested for Brine shrimp cytotoxicity, antibacterial and insecticidal activity. The experimental data revealed that the fatty acids are non-toxic, moderately antibacterial against Klebsiella pneumonia, Staphylococcus aureus and Bacillus subtils, while moderately insecticidal against *Callosbruchus analis*.

Key words: *Desmodium elegans Dc* · Fatty acid composition · Brine shrimp · Antibacterial and insecticidal activities

INTRODUCTION

Desmodium elegans DC syn: *Desmodium tiliaefolium* (Family: Fabaceae) is a perennial deciduous shrub 1.5-2.5 meters tall and papilionaceous shaped white flowers. The flowering period is from June to September. *Desmodium* is a genus of 300-350 species distributed in tropical and temperate areas of the world except Europe and New Zealand [1]. The genus *Desmodium* is distributed in Pakistan, India, Nepal and Bhutan. In Pakistan, it is widely distributed and represented by ten species. The roots of *Desmodium elegans* are used in folk medicine as carminative, diuretic and tonic and powder leaves for healing wounds [2-4]. Juice of the bark is often employed in the treatment of peptic ulcers and sometimes combined with the bark juice of *Bauhinia malabarica* for treatment of cholera [2].

Extensive literature study revealed that fatty acid with unsaturation irrespective of manosaturated or polysaturated have been used in lowering the risks of heart disease, against inflammation and in enhancing immune system [5-9].

Previous Studies: A review of literature revealed that some alkaloids having different residual bases have been reported from the roots of *D. elegans* [10]. However, there is no report on fatty acid composition from the aerial parts of this plant. Therefore, the aim of current investigation was to study the fatty acid composition and antimicrobial besides insecticidal properties of the aerial parts of *D. elegans* against certain strains as highlighted in the abstract and the text.

Present Studies

Extraction of Oil: About 100 g of chopped plant material was extracted with n-hexane (300 ml) for five hours through Soxhlet extraction apparatus. The n-hexane extract was concentrated *in vacuo* to afford yellowish oil which was subsequently subjected to esterification under standard experimental conditions [11].

Preparation of FAMEs: The above mentioned oil was subsequently transformed into corresponding methyl esters following the reported procedure [11]. A known amount of sample (equivalent to 25mg fat) was added to

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0.1ml of internal standard (1.37 mg) and 1.5 mg of sodium hydroxide in methanol (0.5N), sealed and heated in boiling water bath for 5 minutes. The hydrolyzed sample was cooled in ice-bath and 10% BF_3 (2.5 ml) solution in methanol was added slowly over a period of 10 minutes. The solution was then sealed and heated in boiling water bath for 30 minutes and cooled. The cooled contents of reaction tube were diluted with n-hexane (2 ml), neutralized with saturated $NaHCO_3$ aqueous solution followed by treatment brine. The hexane extract was filtered through 0.45µm membrane filter and the filtrate (1µl) was injected to GCMS using auto injector system.

Chemical Reagents: Boron trifluoride solution in methanol (10%) was purchased from Fluka Chemie (Buchs, Switzerland), Sodium hydroxide solution (methnolic; 0.5 N) and Sodium chloride (analytical grade) were obtained from Merck (Damstadt, Germany) while methanol (HPLC grade), n-hexane (HPLC grade) were obtained from Fisher Scientific (Leicestershire, UK). Helium gas (99.9999%) from Pak gas (United Arab Emirates) was procured. Methyl ester of tridecanoic acid and fatty acids methyl esters (FAMEs) 37 components standard mixture were obtained from AccuStandard (Newhaven, Connecticut USA).

Preparation of Standard: Internal standard was prepared by dissolving 13.7 mg of standard tridecanoic acid methyl ester in n-hexane (1 ml). External standard was prepared by diluting 10 mg of 37 components FAMEs mix standard in dichloromethane (10 ml). From this solution further working standard solutions were prepared.

GC-MS Conditions: A gas chromatograph from Shimadzu, hyphenated to a mass spectrometer QP 2010 plus (Tokyo, Japan) equipped with an auto-sampler (AOC-20S) and auto- injector (AOC-20i) was used. Helium was used as a carrier gas. All chromatographic separation was performed on capillary column (TRB-FFAP; Technokroma) having specifications: length; 30m, i.d; 0.35 mm, thickness; 0.250µm, treated with polyethylene glycol. Other GC-MS conditions are: ion source temperature (EI); 250 °C, interface temperature: 240°C, pressure, 100 KPa, solvent cut time; 1.8min. 1µL injector was operated in split mode with split ratio 1:50 with injection temperature of 240°C. The column temperature started at 50°C for 1 min and changed to 150°C at the rate of 15°C /min. The temperature was raised to 175°C at the rate of 2.5°C /min and held for 5 minutes. Then the temperature was increased to 220°C at the rate of 2.5°C /min and kept constant for 3 minutes. Total elution time was 43 minutes. MS scanning was performed from m/z 85 to m/z 380.GC-MS solutions software provided by supplier was used to control the system and to acquire the data identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from NIST library (NIST 05).

Brine Shrimp Cytotoxicity: *Artemia salina* (brine-shrimp eggs) was used to determine cytotoxicity of fatty acid according to the protocol [12]. Ten shrimp and 5ml seawater and different concentrations of (10, 100, 1000 µg/ml) were added to separate vials. Etoposide (LD_{50}= 7.465µg/ml) was used as a reference cytotoxic drug. All the vials were incubated at 26 °C for 24 hrs and the survived brine shrimps were counted. The data was analyzed with a finny computer program to determine the LD_{50} values with 95% confidence interval.

Antibacterial Activity: The fatty acids were screened against various human pathogens employing agar well diffusion method [13]. Nutrient agar plates were swabbed with a 2-8 h broth culture of the respective bacteria. Samples (3 mg/ml) were added into the wells drug in the medium of these plates. The plates were incubated at 37°C for 14-19 hrs and the activity was determined by measuring diameter of zones of inhibition (mm).

Insecticidal Activity: The insecticidal activity was determined by direct contact application method on filter paper [14]. 3 ml of the fatty acid (1 mg/ml) was applied to filter paper of 90 mm diameter and was placed in separate Petri dish along with 10 adults insects of each *Tribolium castaneum, Rhyzopertha dominica* and *Callosbruchus analis* respectively, *Permethrin* (235.71 µg/cm^2) was used as reference insecticide. All these were kept without food for 24 hrs after which mortality count was performed.

RESULTS AND DISCUSSION

The individual peaks in Fig.1 were properly identified along with relative concentration in the mixture. A total of 19 components were identified consisting of both saturated and unsaturated fatty acids (Table 1). The important components in the highest concentration are palmitic acid (59.01%), stearic acid (12.33%), behenic acid (7.48%), arachidic acid (4.20%), tetracosanoic acid (3.84%), tricosanoic acid (2.37 %) and margaric acid (2.36%) while other components are in trace amount.

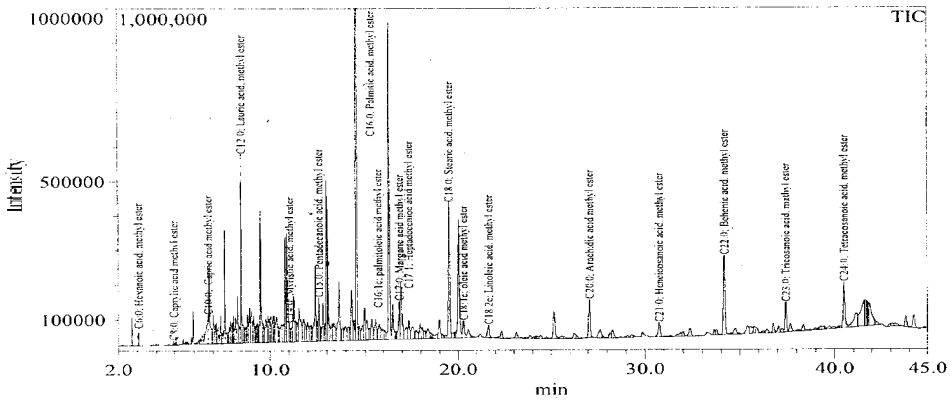

Fig. 1: GC-MS chromatograph of fatty acid methyl ester of *Desmodium elegans*

Table 1: Brine shrimp assay of fatty acid of *Desmodium elegans*

Dose (µg/ml)	No of shrimps	Mortality %	LD 50µg/ml 3382.49	Std. Drug (Etoposide)
LD50µg/ml				
10	30	2	-	7.4625
100	30	7	-	7.4625
1000	30	11	-	7.4625

Table 2: Quantitative result of fatty acid methyl esters by GC-MS

Peak#	Methyl Ester of	Retention Time (min)	Conc (%)	Area	Std. Dev
1.	C6:0; Hexanoic acid	33137	0.71	3.056	0.003
2.	C8:0; Caprylic acid	9726	0.21	4.937	0.001
3.	C10:0; Capric acid	15960	0.34	6.763	0.002
4.	C11:0; Undecanoic	5740	0.12	7.618	0.005
5.	C12:0; Lauric acid	97192	2.08	8.505	0.003
7.	C14:0; Myristic acid	5410	0.12	11.040	0.002
9.	C15:0; Pentadecanoic acid	94418	2.02	12.575	0.001
11.	C16:0; Palmitic acid	2753292	59.01	14.581	0.005
12.	C16:1c; Palmitolic acid	15289	0.33	14.985	0.002
13.	C17:0; Margaric acid	110179	2.36	16.849	0.003
14.	C17:1; Heptadecanoic acid	10786	0.23	17.344	0.001
15.	C18:0; Stearic acid	575299	12.33	19.526	0.002
16.	C18:1c; Oleic acid	15583	0.33	20.304	0.002
19.	C18:2c; Linoleic acid	25612	0.55	21.633	0.004
23.	C20:0; Arachidic acid	196058	4.20	27.068	0.005
27.	C21:0; Heneicosanoic acid	67485	1.45	30.746	0.001
31.	C22:0; Behenic acid	349201	7.48	34.192	0.001
34.	C23:0; Tricosanoic acid	110415	2.37	37.433	0.002
35.	C24:0; Tetracosanoic acid	179267	3.84	40.514	0.002

* Standard deviation values for three measurement results; ^{O} Average of three measurement results

The results obtained from the GC-MS analysis showing the relative concentration of individual esterified fatty acids (Table 2) based on external standard method and standard deviation values among the three results in each case. Analyses were performed in triplicates and values of area and concentration are the average of the triplicate.

The brine shrimp cytotoxicity assay showed that the fatty acids are sufficiently toxic at higher concentration i.e., 1000 ppm. Fatty acids were tested against five bacterial strains, which show that fatty acids displayed moderate antibacterial activity against Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis and Bacillus subtilis.

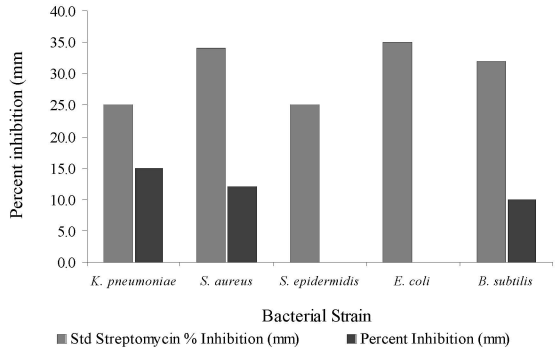

Fig. 2: Antibacterial activity of the fatty acid of *Desmodium elegans.*

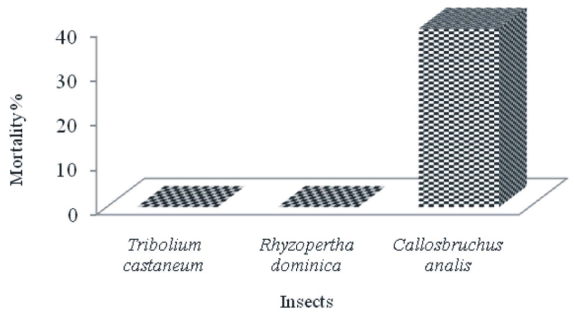

Fig. 3: Insecticidal activity of the fatty acid of *Desmodium elegans.*

The results of insecticidal activity of fatty acids are displayed in Fig. 3 showing that fatty acid exhibit moderate insecticidal activity against *Callosbruchus analis.*

CONCLUSION

The GCMS analysis of the fatty acid obtained from *Desmodium elegans* and their biological activities was the first ever attemt on this plant and this study was performed for the first time.

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REFERENCES

1. Nasir, E. and S.I. Ali, 1977. Flora of West Pakistan, Ferozsons printing press Karachi, pp: 100.
2. Chopra, R.N., S.L. Nayar and I.C. Chopra, 1986. Glossary of Indian medicinal plants(Including the Supplement), Council of Scientific and Industrial Research, New Delhi.
3. Manandhar, N.P., 2002. Plants and People of Nepal, Timber Press Oregon.
4. Kumar, M., Y. Paul and V.K. Anand, 2009. Ethnobotanical Leaflets, pp: 5.
5. Calder, P.C., 1999. Lipids, 34: 137-140.
6. Hamberg, Mats and Hamberg, Gunvor, 1996. Phytochemistry, 42: 729-732.
7. Hargrove, R.L., T.D. Etherton, T.A. Pearson, E.H. Harrison and P.M. Kris-Etherton, 2001. The Journal of nutrition, 131: 1758.
8. Villa, Barbara, Calabresi, Laura, Chiesa, Giulia, Risè, Patrizia, Galli, Claudio and Sirtori and R. Cesare, 2002. Pharmacol. Res., 45: 475-478.
9. Siscovick, D.S., T.E. Raghunathan, I. King, S. Weinmann, K.G. Wicklund, J. Albright, V. Bovbjerg, P. Arbogast, H. Smith and L.H. Kushi, 1973. JAMA, the journal of the American Medical Association, 274: 1363.
10. Ghosal, S. and R.S. Srivastava, 1973. Phytochemistry, 12: 193-197.
11. Shantha, N.S. and Napolitano, 1992. E. Guillermo, Journal of Chromatogrpy. A, 624: 37-51.
12. Atta-ur-Rahman, M.I. Choudhary and J.W. Thomsen, 1999. Manual of bioassay techniques for natural product research, Academic Press, Amsterdam.
13. Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: a convenient bioassay for active plant constituents, Planta Medica, 45: 31-34.
14. Ahn, Y.J., G.H. Kim and K.Y. Cho, 1995. Bioassay system for insecticidal compounds, in Third symposium on Biochemical Methodology for the research and development of bioactive substances Proceedings, Seoul, Korea, pp: 495-506.