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ANALYSIS OF FATTY ACID, ELEMENTAL AND TOTAL PROTEIN OF *CALOTROPIS PROCERA* MEDICINAL PLANT FROM SINDH, PAKISTAN

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Abstract

*Calotropis procera (*Asclepiadaceae) is a well known medicinal plant with leaves, roots and bark being exported as popular medicine to fight many human and animal diseases. It is locally known as AKK with English name as Milk Weed, grows abundantly in Sindh province of Pakistan. The isolated fatty acid composition in the extract of *C.procera* has 7 saturated fatty acid and 11 unsaturated fatty acid. The essential elements Al, As, Cu, Ca, Cr, Cd, Fe, K, Mn, Na, Pb, and Zn have been analyzed from the medicinal plant in variable range. The total protein in *C. procera* was 27-32%

Introduction

Calotropis procera (Ait). R.Br. of the family Ascelpiadaceace is locally known as Akk.The latex of the plant is used as an antidysenteric, antirheumatic, a diaphoretic, an expectorant and for the treatment of bronchial asthma and skin conditions (Ageel, 1987; Al-Yahya et al., 1990). In African and Asian countries, the latex of *C. procera* is utilized as an arrow poison molluscide, a fungicide, an anti-syphilitic, an anti-inflammatory, a purgative, for the treatment of lepers and bronchial asthma and for milk coagulation in chease making (Watt & Breyer-Brandwijik, 1962; EL`Badwi, 1997; Larhsinil, 1997). *C. procera* is known to contain cardio active glycoside calotropine which has shown an antitumer effect in vitriol on human epidermoid carcinoma cells of the rhinopharynx, it also acts as expectorant and diuretic. *C. procera* is used as expectorant and anthelmintic. Milky juice is reported for the treatment of dropsy and rheumatism to remove Taenia, the treatment of toothache. (Kalita, 2001). However dried leaves are smoked in pipe as cure for cough (Aftab *et al*., 1990; Csurhes *et al*., (1998). *C. procera* is considered for large scale cultivation as an alternative source for producing energy (Hifzul Kabir, 2003); *Calotropis procera* is applied for the treatment of expectorant, analgesic and anti inflammation (Dhiman, 2003) *C. procera* is used for digestion. Oil of leaves is useful in skin eruptions. Latex from *C. procera*, is widely used in folk medicine as a rich source of biologically active compounds capable of promoting diverse benefits such as control of dermal fungal infections, antimicrobial activities and pain relief among other useful properties. The identification and cultivation of plants rich in hydrocarbons as renewable sources of chemicals for use as fuel and chemical feedstock has generated considerable interest (Nielsen *et al*., 1977; Buchanan *et al*., 1978a, b; Calvin, 1978; Saxon, 1980; Wang & Hu.man, 1981; Adams & Machesney, 1982; Campbell, 1983; Jenkins and Ebeling, 1985; Abbott *et al*., 1990; Seiler *et al*., 1991). The

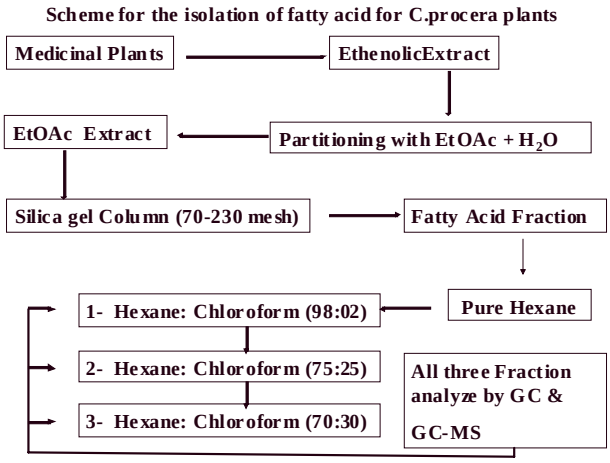
shortage and depletion of worldwide sources of fossil hydrocarbon warrants development of alternative sources of fuels and chemicals. There are thousands of plant species that produce copious amounts of hydrocarbons and these hydrocarbon bearing plants are a special group of shrubs and trees that are being identified and selected to initiate agronomic and genetic improvements (Paul, 1981; Campbell, 1983; Adams *et al*., 1984; Bhatia *et al*., 1984; Margaris & Vokou, 1985) *C. procera* containing hydrocarbons, can be cultivated as fuel crops (Lipinsky, 1981; Isely, 1981; Adams & Machesney, 1982; Roth *et al*., 1984; Emon & Seiber, 1984; Carr, 1985; Carr *et al*., 1986; Carr & Bagby, 1987). Studies have been carried out in India on some Hydrocarbon-bearing plants (Pachauri& Dhawan, 1987; Marimuthu *et al*., 1988; Kalita & Saikia, 2000). However, as the plant resources of India are enormous and unique owing to varied topography and wide climatic conditions, there are a large number of hydrocarbon-bearing plants in the forests, which have not been systematically evaluated as potential sources of hydrocarbon and other valuable phyto chemicals. The response of plants to contamination can be manifested as an increase in the uptake of elements when the effect of pollution is minor. As an alternative sources of energy excessive presence of some essential and trace elements *C. procera* can result in the toxicity of the plant and hence change of leaves colour, inhibition to the germination and growth of seeds or even death of the plant may be evident (Abbasi *et al*., 1992). Other effects of pollution can be described as inhibitory effects, by the fact that the excessive presence of some elements can result in blocking the uptake of other elements and hence depriving the plant from absorbing essential elements from the soil (Altaf 1997). In the present study 7, saturated fatty acid and 11unsaturated fatty acid were isolated. Essential elements Al, As, Cu, Ca, Cr, Cd, Fe, K, Mn, Na, Pb, and Zn have been analyzed from the *C. procera* in variable range. The total protein *C. procera* was 27- 32%.

Material and Method

Collection of plant material: *Calotropis procera* (Ait). R.Br. (Ascelpiadaceace), were collected from Distt. Nawab Shah during November-December 2005. Reference sample were identified through flora of Pakistan (Nasir & Ali, 1990). The collected plant material was washed with tap water followed by distilled water and dried in shade at room temperature for 20 days.

Extraction: The dried plant were chopped into small pieces and was dipped into 2 litre ethanol (EtOH) for about one month at room temperature. The ethanolic extract was filtered and evaporated under reduced pressure at below 40°C using rotary evaporator, which yielded dark green gummy residue. The extracts were then partitioned with Ethyl acetate (EtOAc) and water which this procedure was repeated 3 times. The (EtOAc) extract was evaporated under pressure and yielded thick greenish residue.

Column chromatography (CC): The residue containing fatty acids fraction was separated on chromatograph over silica gel. (70-230 mesh Merck) column. The column was first eluted with n-hexane and thereafter chloroform was added in order of increasing polarity. First fraction was eluted with pure hexane, fraction “A” was eluted from hexane: chloroform (85:15), fraction “B” from hexane: chloroform (80:20), fraction “C” from hexane: chloroform (75:25), and fraction “D” from hexane: chlorofrom (70:30).



Esterification: All fractions (A-D) were esterified with diazomethane, 0.5 mg of each fraction was dissolved in MeOH and 0.5 ml of diazomethane was added. The reaction mixture was kept overnight at room temperature (28°C) and was then evaporated .The methylated fatty acid fractions were analyzed first by GC and finally by GC-MS.

Gas chromatography-mass spectrometry (GC-MS): The fatty acids analysis was performed on JEOL JMS 600H Agilest 68 g ON, equipped with 30 m×0.32 mmHP-5 column, stationary phase coating 0.50µm. The column temperature was kept at 250˚C for 2 min with increased at the rate of 5˚C per min up to 250˚C. Injector temperature 250˚C, split ratio 1:35, the carrier gas (Helium) flow rate 1.8ml/min.

Spectral data: The GC-mass spectral chromatogram showed the presence of 7 saturated 11 unsaturated fatty acids methyl esters; the significant ions from the mass spectra of these methyl esters are as follows.

n-Heptanoate: GC-MS:: m/z144 (M±,$C_8H_{16}O_2$, 6%) 113 (M±-31, 4%) 101 (.M±-43, 2%) 87 (7%) 73 (100%).

n-Octanoate:158(M±, $C_9H_{18}O_2$. 33%) 143(M±-15, 85%), 127(M±-31, 8%), 115 (M±-43, 38%), 101(75%) 87(82%) 73(59%), (9%).55(100%).

Methyl-nonanotetracnoate: 164(M,$C_{10}H_{12}O_2$,4%), 121(M±-,1%), 105(M±-59, 12%), 104(100%)91(22%), 77(7%), 63(3%), 49(2%).

n-Decenoic acid m/z: 170 (M±, $_{C10}H_{18}O_2$, 25%), 127(M±-43, 7.5%), 113(M±-14,8%), 99(M±-14, 11%), 85(M±-14, 49%), 71(M±-14,69%), 57(M±-14, 100%), 43(M±-14,28%).

n-Nonanoatem/z: 172(M±, $C_{10}H_{20}O_2$,11%), 157(M±-15, 37%), 129 (M±-43, 8%), 111(27%) 97(11%), 83, (37%) 69(28%), .55(100%).

Decenoate: 184(M±, $C_{11}H_{20}O_2$, 21%), 152(M±32, 2%), 127(M±57, 11%), 113(12%), 99(15%), 85(75%), 71(100%).

Undecadienoate: 196(M±, $C_{12}H_{20}O_2$,10%), 139(M±57, 5%), 125(8%), 111(15%), 97(16%), 83(22%), 69(31%), 52(21%).

9-Dodecenoate: 212(M±, $C_{13}H_{24}O_2$, 43%, 155(M±-57, 12%, 141(14%), 127(5%), 113 (7%), 99(27%), 85 (100%).

Tridecanoate: 222 (M±, $C_{14}H_{22}O_2$. 6%)190 (M±-32, 3%), 177(30%), 140 (100%), 12 (7%) 93 (11%), 65 (15%).

n-Tridecanoate: 228 (M±, $C_{14}H_{28}O_2$.42%) 185 (M±-43 40%), 171 (M±-57, 10%), 157 (7%), 143 (13%), 129 (43%) 115(16%), 101(11%)87 (38%) 73(100%).

Tetradecatrienoate: 236 (M±,$C_{15}H_{24}O_2$ 13.%), 204 (M±-31, 1%), 166(M±-74,7%) 148 (1%), 137 (47 %), 123 (24%) 109(14%), 95(11%) 81 (8%) 67 (100%).

n-Pentadecanoate: 256 (M±, $C_{16}H_{32}O_2$, 46%) 213 (100(M±-43, 16%), 199 (6%), 185 (5%), 171 (6%) 157 (7%), 143 (20%).129 (8%), 155 (2%), 101 (7%) 87 (60%), 74 %).

Hiragonate: 264 (M±, $C_{17}H_{28}O_2$, 100%), 221(M±-43, 35%), 207(8%), 199(5%), 180 (27%)157(8%), 87(20%), 74(100%).

n-Hexadecanoate: 270 (M±, $C_{17}H_{34}O_2$,58%)239 (M±-31, 17%), 227(M±-43,23), 213 (3%), 199(10%), 185(10%) 171(9%), 157 (3%) .143 (23%), 129 (10%), 115 (6%) 101 (8%), 87(64%), 74 (100%).

Heptadecadienoate: 280(M±, $C_{18}H_{22}O_2$60%) 248(M±-32, 25%), 206 (M±-74, 10%), 192(30%) 178(5%) 164 (10%), 150 (20%), 136(10%), 122 (8%), 108 (20%), 95 (25%), 80 (30%), 73 (100%).

Heptadeceonate: 282 (M±, $C_{18}H_{34}O_2$, 20%)225 (M±-57, 5%), 211, (6%), 197 (8%), 183 (7%) 169(8%), 155(10%) .141 (10%), 127 (10%), 113 (12%) 99 (17%), 85(64%), 71 (100%).

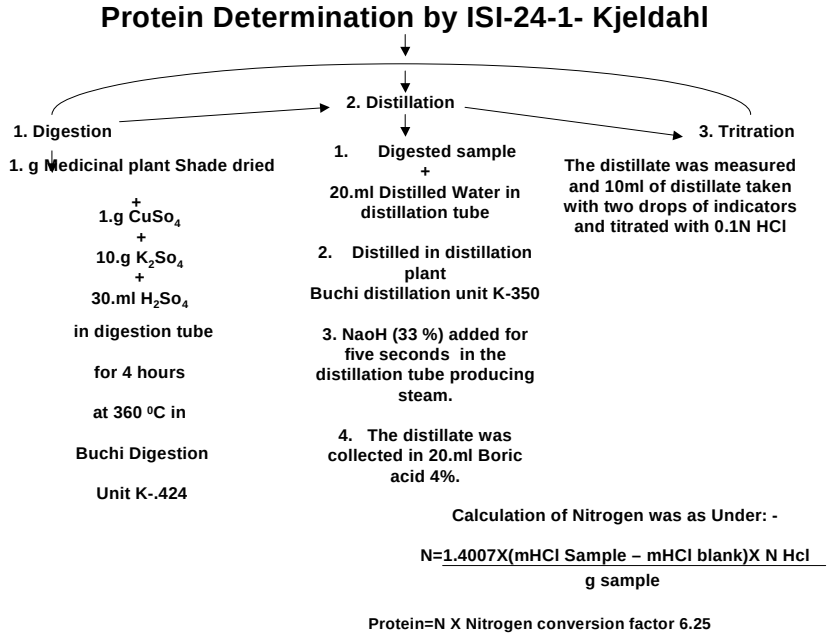
n-Heptadeceonate: 284 (M±, $C_{18}H_{36}O_2$, 19%)241 (M±-43, 8%), 213, (6%), 199 (5%), 185 (5%) 171 (4%), 157 (15%) .143 (9%), 129 (9%), 115 (8%) 101 (58%), 87 (100%).

9-Eicosenoate: 324 (M±, $C_{21}H_{40}O_2$15,%), 267 (M±-57, 3%), 253 (3%), 239 (4 %), 225 (4%) 221(5%), 197(5%)183 (5%) 169(5%) 155 (6%), 141(5%), 127 (7%), 113 (8%), 99 (12%), 85 (40%), 71 (62 %), 57 (100%).

Conventional Digestion method (CDM)

Elemental assay: The samples were investigated for elemental analysis by using atomic absorption spectrophotometer (AAS), Hitachi Ltd. 180-50.S.N5721-Appropriate working standard solution was prepared for each element. The calibration curves were obtained for concentration vs. absorbance. The data were statistically analyzed by using fitting of straight line by least square method. All elements were determined in Medicinal plants under this investigation procedure. A blank reading was also taken.

Total protein by kjeldhal: The sample was digested in H_2SO_4. (30 ml) in the presence of catalyst CuSO4, (1g) and K_2SO_4 (10g), after digestion Sodium hydroxide (NaOH, 33%) were added followed by steam distillation, the distillate was collected in 20 ml boric acid (4%). Then nitrogen content was determined by using titration with HCl (0.01 N). A factor of 6.25 was used to evolute total protein



Total protein analysis: Protocol of ISI-24-1-e, was found determination of total nitrogen, which was calculated using a nitrogen conversion factor of 6.25. (Anon., 1999).

Results and Discussion

In the extract of *Calotropis procera* medicinal plant. The isolated saturated (SFAs) and unsaturated fatty acid (UFAs) showed 29.24%Hexadecanoic, moderate % 10.8% n-Heptadecanoate, lowest relative % 1.35%, n-Heptanoate, the highest relative %of (UFAs). 20.35% Tridecadienoic acid, Moderate % 5.37% Tetradecatrienoat and lowest n-Decenoic acid 0.58% The GC mass spectra showed the presences of saturated and unsaturated fatty acids methyl ester. Tables 1-2, showed the presences of 18 different fatty acids (FAs) and these tables also showed the relative retention time (RRT) and relative percentage of occurrence of their methyl ester. The saturated fatty acids were greater quantity (52.92%) than unsaturated fatty acids (47.08%). The comparison with other fatty acid (FA) content found to be the highest in the stem of *C. procera* (65.9%) with 58.7% whole plant extract were (Kalita *et al*., 2004).

Table 1. Seven saturated fatty acids isolated from medicinal plants of *Calotropis procera*.

S. No.	Fatty acids of *Calotropis procera* analyzed as methyl ester.					
	Systematic name	**Common name**	**Molecular Formula**	**Mol. Wt.**	**R.r.t**	**Rel. %**
	Saturated fatty acids methyl ester					
1.	n-Heptanoate	Heptylate	$C_8H_{16}O_2$	144	11.73	1.34
2.	n-Octanoate	Caprylate	$C_9H_{18}O_2$	158	17.1	3.64
3.	n-Nonanoate		$C_{10}H_{20}O2$	172	22.05	1.72
4.	n-Tridecanoate	Tridecylat	$C_{14}H_{28}O_2$	228	25.68	2.62
5	n-Pentadecanoate:	Pantadecylate	$C_{16}H_{32}O_2$	256	30.1	28.84
6.	n-Hexadecanoate	Palmilate	$C_{17}H_{34}O_2$	270	28.72	4.41
7.	n-Heptadecanoate	Margorate	$C_{18}H_{36}O_2$	284	33.48	10.98
	Total					**52.92**

Table 2. Eleven unsaturated fatty acids isolated from *Calotropis procera* medicinal plants.

S. No.	Systematic name	Common name	Molecular formula	Mol. Wt.	R.R.T	Rel. %Ag
1	Methyl nonanotetracnoate		$C_{10}H_{12}O_2$	164	19.28	1.34
2.	n-Decenoic acid		$C_{10}H_{18}O_2$	170	18.28	0.58
3.	9-Decenoate		$C_{11}H_{20}O_2$	184	23.37	2.49
4.	Undecadienoate		$C_{12}H_{20}O_2$	196	21.6	0.96
5.	9-Dodecenoate:		$C_{13}H_{24}O_2$	212	23.63	1.15
6	Tridecatrienoate	---	$C_{14}H_{22}O_2$	222	19.72	1.15
7.	2,4,5-Tetradecatrienoat	Tetradecatrienoat	$C_{15}H_{24}O_2$	236	18.82	5.37
8.	Hiragonate		$C_{17}H_{28}O_2$	264	28.63	2.30
9.	Heptadecadienoate		$C_{18}H_{22}O_2$	280	33.18	20.35
10.	Heptadecenoate	Heptadecenoate	$C_{18}H_{38}O_2$	282	39.47	6.40
11.	9-Eicosenoate	Gadoleate	$C_{21}H_{40}O_2$	324	36.38	4.99
	Total					**47.08**

7 Saturated, 11 Unsaturated, Total compounds =18

Total % age of Saturated ± Unsaturated fatty acid = 99.99

(Mol.wt = Molecular weight, R.R.T. = Relative retention time, Rel % age = Relative percentage)

Elemental analysis: *Calotropis procera* (Ait). R.Br. *(Ascelpiadaceace)* from, the different locations of Sindh was analyzed for the composition of As, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Pb and Zn elements (Table 3). The amount of Ca was the highest among them. Ca varied according to the collection point. Maximum amount 1481.2 mg/kg of Ca was present in the samples of collected from Daulatpur Saffan and minimum amount 9.0 mg/kg, was present in the samples from Jamshoro. The concentration of Mg, K, Fe, Zn was higher than other elements and the amount of Cd, As, Pb and Cr was minimum 0.12 to 0.97 mg/kg, whereas Cu and Mn was11.9 to 12.33 mg/kg and Zn 5.15 to 2.022 ppm As 40.2 µg to 30.11 µg in *C. procera* from Sindh. Variations of elemental concentrations varied from high in Ca (1481.2 ppm) and low in K (387.8ppm) where the K, Mg, Ca. was reported in maximum values, wile Na, Mn, Zn, Cr are present in minimum range. If we compare with others work K 10.190, Mg 8630, Mn 40.Fe 65.4, Na 4450, Zinc 40.2, Cr 0.74 (Altaf,1997), Mg, 9600, Mn 155, Fe 66.4, Na 2700, Zinc 38.89, Cr 0.514, K 34200, Ca, 13700,(Altaf, 2006). These differences could be ecological, collection time or as increasing pollution or environmental, factors.

Table 3. Elemental analysis of *Calotropis procera* from different areas of Sindh (ppm ± SE).

Elements	Daulatpur Saffan	Nawab Shah	Hyderabad	Jamshoro
Cd	2.69 ± 1.73	1.18 ± 1.10	0.97820 ± 0.07	1.68 ± 0.3
Pb	0.27 ± 0.23	0.13 ± 0.97	0.6 ± 0.10	0.1 ± 0.6
Cr	0.121 ± -0.33	0.18 ± 0.10	0.21 ± 0.7	0.9 ± 0.4
Cu	1.5 ± 0.988	11.9 ± 0.122	9.77 ± 1.36	10.74 ± 1.2
Mn	15.27 ± 12.33	11.11 ± 10.30	10.25 ± 2.6	9.80 ± 1.65
Zn	5.394 ± 0.66	5.15 ± 3.01	3.11.0 ± 1.12	2.02 ± 1.9.3
Fe	29.55 ± 8.9	5.55.90 ± 0.01	6.022 ± 5.183	0.44 ± 2.01
Ca	1481.2 ± 1331.4	10.330 ± 1 259.	10.0 ± 9.03	9.0 ± 10.10
K	387.8 ± 3490.1	330.0 ± 11.0	285 ± 211.0	123.0 ± 145.0
Mg	12.33 ± 15.27	1122.0 ± 1044	1023 ± 12.12	934.0 ± 12.0
As	37.3 ± 56. 0µg/kg	40.2 ± 35.21	30.11 ± 20.1	29.20 ± 22.14
Na	46.4 µ ± 4449.4	401.2 µ ± 4040	39.2 ± 39.3	30.21 ± 30.11

Total protein analysis: In *Calotropis procera* of Sindh, the highest value of total protein recorded was 50.80% of dry weight (Daulatpur), 32.11% (NawabShah), 25% (Hyderabad) and (Jamshoro), 29.45% from different sites. If compared, whole plant portion *Calotropis procera* protein (7.87-14.63% the highest total protein was reported in *Calotropis procera* in leaf extracts 23.94, stem 8.94, bark 12.69 (Kalita *et al*., 2004).

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