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AUTECOLOGY AND METABOLIC PRODUCTS OF *Calotropis procera* (Aiton) W. T. Aiton.

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ABSTRACT

The present study was conducted to evaluate the ecological characteristics and the bioactive metabolic products of *C. procera*. Also, the crude methanol extract of the aerial parts of calotropis was examined for antioxidant and antimicrobial potentialilties. The scrubland dominated by *C. procera* represented an advanced stage of desert vegetation and comprised 33 species related to 14 families. *Tamarix nilotica*, *Alhagi graecorum*, *Launea mucronata*, *Trichodesma africanum* and *Hyoscyamus muticus* were the common associates. The major life-forms were therophytes, geophytes and chamaephytes. The assemblage of this community belongs to eleven floristics categories with predominance of Sahara-Sindian, Sahara-Arabian and Sudano-Zambezian. The flavonoids and polyphenols contents of the aerial parts of *C. procera* were 0.185 and 0.37 g/100g, respectively. Thirteen fatty acids were detected among them undecanoic, palmitic, lauric, myristic and linoleic acids. The *Calotropis* methanolic extract exhibited free radical scavenging activity with IC_{50} value of 0.35 mg ml^{-1}. It is showed antimicrobial spectrum only against *Bacillus subtilis*.
Keyword: *Calotropis procera*, Ecology, Fatty acids, Antioxidant.

INTRODUCTION

Calotropis procera is a Sahara-Sindian species common in Kharga Oasis in Egypt. It ia s soft-wooded, evergreen or semi-deciduous xerophytic perennial shrub, favors open habitat with little competition, grows best in full sun and does not form dense stands, normally occurring as scattered individuals(Plate 1). When cut, all parts of the plant exudes a milky, sticky sap (latex). The bark is corky, furrowed and light gray (Plate 2).

Literature reviews indicated that, traditionally the roots are used to treat diarrheoa, cough, skin diseases, rheumatism, as an expectorant and emetic (Kritika and Basu, 1999). The plant is used in folk medicine to treat renal and rheumatic diseases (Al-Yahya *et al*., 1990). Different parts of the plant have been reported to exhibit anti-inflammatory, analgesic and antioxidant properties (Kumar and Arya, 2006 and Frosi *et al*., 2012). The latex has been proved to have insecticidal activity against different insects (Singhi *et al*., 2004 and Morsy *et al*., 2001). The abundance of latex in the green parts of the plant is a defense strategy against virus, fungi and insects (Rashmi *et al*., 2011). Latex contained 11-23% rubber and can be used as a renewable source of hydrocarbons and intermediate energy resources (Orwa *et al*., 2009). It have reviewed by Al-Yemni *et al*. (2011) and Al-Qahtani, (2012) that *C. procera* has high capacity for taking heavy metals into its tissues due to their abilities to absorb and tolerate heavy metals without serious physiological damage. *C. procera* plays an important role in formation of its natural habitats as it contains allelo-chemical compounds that enable the plant to compete with other species (Steve, 2009). Verma *et al*. (2010) studied the ethno medicinal uses of *C. procera* and its medicinal properties used for treatment of various ailments as fevers, eczema, elephantasis, vomiting,… etc.

Plate (1). *Calotropis* scrub grow on deep silty deposits at Adan (80 km south of Kharga). Note the Scattered Small individuals .

Plate (2). A Copious white sap flows from the cut in *Calotropis* stem. Note the thick furrowed bark.

The oases have special ecological conditions and characteristic vegetation. Several studies have been carried out on the vegetation of the oases of western desert such as Shaded *et al*., 2012; Abd El Ghani and Fawzy, 2006; Abd El Ghani, 1985 and Abu Ziada, 1980.

The present investigation aimed to study the autecolog, flavonoids and polyphenols contents, fatty acids, the antioxidant and antimicrobial properties of *C. procera*.

MATERIALS AND METHODS

Ecological Characters

1. Vegetation Analysis

Twenty stands dominated by *C. procera* were studied in detail including: total coverage, a list of species, families, phenological aspects of growth, life span, life form, chorotype and cover-abundance estimate of each species. Identification and nomenclature of the species were followed Täckholm (1974) and Boulos (2009). Techniques of floristic analysis based on the methods given by Kent and Cocker (1992) and Muller-Dombois & Ellenberg (1974).

2. Habitat Conditions

Soil samples were collected from each stand at a depth of 25 cm, air dried and sieved through a 2 mm sieve to remove gravel and debris. The procedure followed in estimating their physical and chemical variables were according to the method recommended by Carter and Gregorich (2008), Pansu and Gautheyrou (2006), Margesin and Schinner (2005), Baruah & Barthakur (1997) and Klute (1986).

Phytochemical Analysis

Quantitative estimation of polyphenols and flavonoids

The polyphenols contents was determined using the method described by Sadasivam and Manickam (2008). The flavonoids were extracted and subjected to column chromatograph according to Rolim *et al*., (2005) and Kujala *et al*., (2000).

Investigation of fatty acids

Qualitative and quantitative analysis of the fatty acids of *C. procera* aerial parts were performed using GLC technique, and comparison of their retention data with those of the available reference samples.

Evaluation of antioxidant activity

The antioxidant activity was investigated using free radical scavenging method (DPPH) as described by Kitts *et al*., 2000, with slight modifications (Liyana-Pathirana and Shahidi, 2005).

Screening of antimicrobial activity

The antimicrobial activity of the methanolic extract of *C. procera* aerial parts was examined by the filter paper disc assay (Murray *et al*., 1998) using inoculums for 10^6 bacterial and fungal cells/ ml.

RESULTS AND DISCUSSION

Climate

The climate of Kharga Oasis can be characterized as arid. The practically nil values of rainfall coupled with high evaporation rate and low relative humidity express the extremely dry climatic condition. The hottest months are June, July and August where the maximum temperature is 39.3^{o}C. January is the coldest month where the mean minimum temperature is 5.8^{o}C. The values of relative humidity showed that the atmosphere is dry allover the year with the highest value of 46% in December and lowest value of 12% in June. The highest evaporation rate in June (24.7 mm/ day) and the lowest rate in January (7.8 mm/ day). The wind velocity ranges between (11.1 km/ hr.) in January and (19.8 km/ hr.) in June.

Vegetation

Vegetation of the surveyed stands representing the scrubland community type of *Calotropis procera*, composed of 33 plant species. These species categorized into two groups according to their duration or life-span as follows: 25 perennials (76%) and 8 annuals (24%) Fig (1).

The plant assemblage of this community is related to 14 families. Fabaceae, Poaceae and Asteraceae are representing more than 50% of the recorded species (Fig. 2). Abd El Ghani and Fawzy (2006) and Abd El Ghani (1985) mentioned that these families are the most common in North Africa.

Salama *et al*. (2014) and Abdel-Aleem (2013) reported that environmental stresses reduce species diversity. Stress due to hostile climatic aridity is reflected remarkably on the composition and structure of the plant communities. They generally open and structurally characterized by low number of species. Stress due to the unstable nature of sandy habitat may be added in reducing the species diversity and unequal distribution of species richness (Sheded *et al*., 2012 and Galal & Fahmy, 2011).

Concerning the life forms (Fig. 3), the majority of species are therophytes and geophytes (8 species each), then hemicryptophytes and chamaephytes (7 and 5 species, respectively). This pattern of life forms spectrum displays a strong resemblance to that given by Abu Ziada *et al*., 2015 a & b; Abd El Ghani & Fawzy, 2006; Abd El Ghani, 1981; Salama *et al*., 2013; Al-Sherif *et al*., 2013 and Youcef *et al*., 2012 indicated the predominance of hemicryptophytes followed by therophytes in arid and semi-arid areas.

Dealing with chorotypes, the floristic elements of this community related to eleven floristic categories (Fig.4). Sahara-Sindian, Sahara-Arabian, Sudano-Zambezian, Irano-Turanian and Mediterranean are the main representative chorotypes. Such finding seems to be response to a more hot and dry climate, topographic variation and human influence.

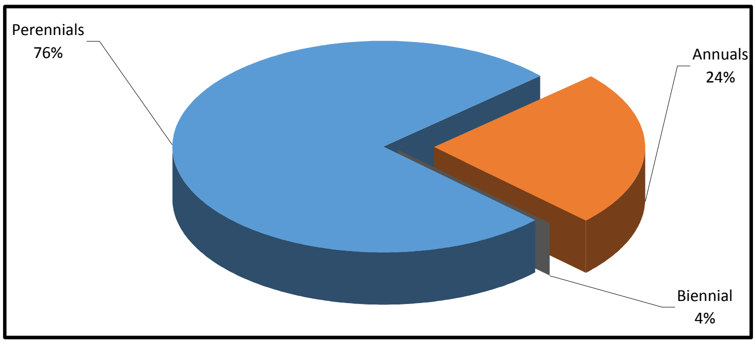

Fig. (1): Life Span of the recorded species in *Calotropis procera* community type .

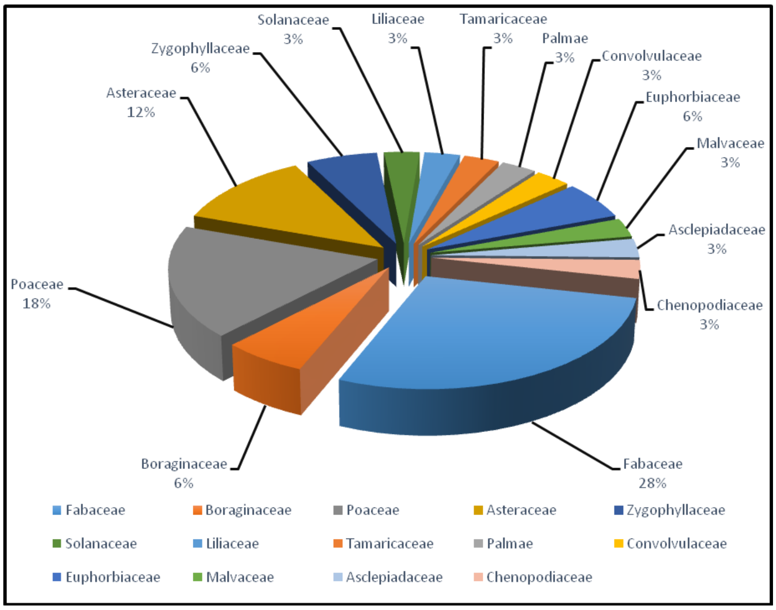

Fig. (2): Plant families of the recorded species in *Calptropis procera community* type.

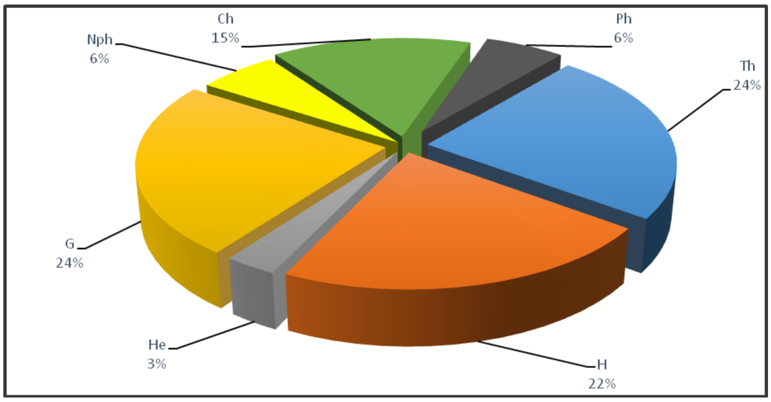

Fig. (3) Life form spectra of the recorded species in *Calotropis procera*. ■Th = Therophytes, ■H = Hemicryptophytes, ■He = Helophytes, ■G = Geophytes, ■Nph = Nanophanerophytes, ■Ch=Chamaephytes and ■Ph=Phanerophytes.

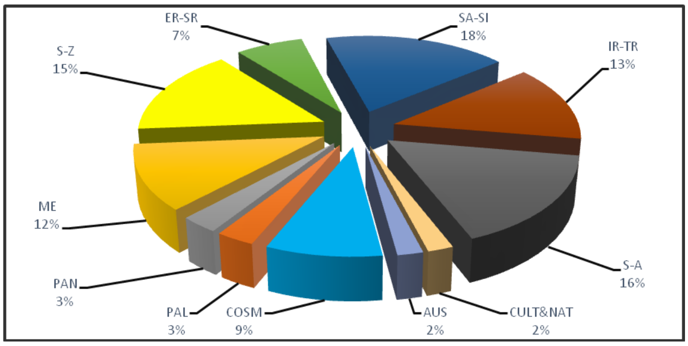

Fig. (4) Chorotypes of the recorded species in *Calotropis procera* community type. ■COSM = Cosmopolitan, ■PAL = Palaeotropical, ■PAN = Pantropical, ■ME = Mediterranean, ■S-Z = Sudano-Zambezian, ■ER-SR = Euro-Siberian, ■SA-SI = Saharo-Sindian, ■IR-TR = Irano-Turanian, ■S-A = Saharo-Arabian, ■CULT and NAT = Cultivated and Naturalized, and ■AUS=Australian.

Habitat conditions

The scrubland type dominated by *C. procera* is common in Kharge Oasis. Table (1) gives the analyses of twenty five soil samples representing the range of soil conditions associated with this community. The soil is sandy textured with predominance of fine sand (particle size lies within class 0.5 – 0.211 mm). the percentages of silt and clay are generally low and ranged from 2.80 – 19.22% and 1.02 – 4.23%, respectively. Moisture content is low and varies from 0.10 to 4.8%. porosity and water holding capacity varied within a wide range (38.9 – 50.5% and 29.3 – 54.8%, respectively).

The organic carbon content is low (0.1 – 1.5%). $CaCO_3$ content fluctuated within a narrow range varying between 2.5 and 12.0%.

T1

Soil salinity is low in the different localities. All soil samples have salinity level ranged from 0.05 to 0.412%. The electric conductivity varied between 128 to 252 μ mohs/ cm. The obtained results indicate that *C. procera* is intolerant to salinity. Sulphates constitute the main bulk of the soluble salts while chloride and bicarbonates attained low concentrations in all samples. The soil reaction is slightly alkaline to alkaline with pH values vary from 7.2 to 9.2.

Phytochemical analysis

Quantitative estimation of polyphenols and flavonoids.

The mean values of flavonoids and polyphenols contents of *Calotropis procera* aerial parts were 0.185 ± 0.004 g/ 100g and 0.370 ± 0.005 g/ 100g, respectively.

Fatty acids constituents of the lipoidal matter of *C. procera*

The results of GLC analysis of the fatty acids methyl esters are presented in Table (2). Thirteen fatty acids were detected in *Calotropis* shoot. The main of these acids were undecanoic, palmitic, lauric, myristic, linoleic, oleic and lignocertic. The other six fatty acids were present with low concentrations.

Table (2): GLC analysis of fatty acids of *Calotropis procera* shoot.

Fatty acid	Retention time (min).	Relative Area %	Conc. (g /100g)
Undecanoic acid	9.075	11.83536	0.843
Palmitic acid	15.725	10.24244	0.729
Lauric acid	10.173	8.46415	0.603
Myristic acid	11.466	8.02019	0.571
Linoleic acid	20.200	7.87618	0.561
Oleic acid	19.240	6.92161	0.493
Lignocertic acid	27.837	6.71920	0.478
Linolenic acid	21.471	4.19141	0.298
Tridecanoic acid	10.950	3.33575	0.238
Pentadecanoic acid	13.576	3.14584	0.224
Stearic acid	18.850	1.32928	0.095
Arachidonic acid	24.034	1.25761	0.089
Margaric acid	17.405	0.91715	0.065
Unknown	12.435	3.08203	0.219
Unknown	9.678	2.75946	0.196
Unknown	15.253	1.39564	0.099
Unknown	24.695	1.22051	0.087

Antioxidant activity

According to the IC_{50} (the concentration of the extract required to inhibit the initial DPPH free radicals) *C. procera* methanolic extract exhibit high free radical scavenging activity (IC_{50} value equal 0.35 mg ml^{-1}. The extract of the natural antioxidant catechol attained the IC_{50} value of 0.03 mg ml^{-1}. The result of the present study is in coherence with that reported by Cai *et al*., 2004 and Miser-Salihoglu *et al*., 2013. This could be attributed to the hydroxyl groups in phenolic compounds which have a significant role in antioxidant activity.

Antimicrobial activity

Calotropis shoot was found to possesses antimicrobial spectrum only against *Bacillus subtilis* with inhibition zone of 7 mm. This result showed that the methanolic extract of *C. procera* shoot has little antibacterial activity and devoid of any antifungal activity. This finding clearly contradicts of the results of Hassan *et al*., 2006 and Malik & Chughtai, 1979 and Mascolo, 1988.

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البيئة الذاتية والنواتج الأيضية لنبات العشار

محمد السيد على أبو زيادة , مها محمد عبد المنعم الشامي و نور كاظم حامد الكريشى
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تشمل تلك الدراسة التعرف على الخصائص البيئية لعشيرة نبات العشار المزدهر بالواحة الخارجة بالصحراء الغربية ، بقياس نسب التواجد (المدى الإجتماعى) والوفرة والطرز المظهرية وصور الحياتية والمجموعات الفلورية ، أيضا دراسة خصائص التربة بقياس القوام والرطوبة والمسامية والسعة المائية والكربون العضوى وكربونات الكالسيوم والأملاح الكلية الذائبة والأنيونات والأس الأديدروجينى . وخصص جزء للدراسة الفيتوكيميائية لنبات العشار وتتضمن تقدير الفلافونيدات والفينولات والأحماض الدهنية والكشف عن فاعلية المستخلص الميثانولى كمضاد للاكسدة ومضاد ميكروبي .

أوضحت النتائج أن الكساء الخضرى لعشيرة نبات العشار النامى بالواحة الخارجة متناثر وغير كثيف ويعزى معظم الغطاء النباتى للنوع السائد . تضم العشيرة 33 نبات تتبع 14 فصيلة ومن أهم الأنواع المرافقة نباتات العبل والعاقول والمرار والغدوان والسكران وكان النوع الأول والثانى لهما مدى بيئى وإجتماعى واسع ، وكانت عناصر الفصائل البقولية والنجيلية والمركبة والبوراجينية هى الأوفر ، أيضا أمكن تمييز سبعة صور حياة أهمها النباتات قصيرة الأجل والأرضيات وشبه المختبئات . وأظهر التحليل الفلورى للعشيرة وجود عناصر تابعة لأحد عشر إقليما مناخيا منها إقليم صحارى – سينديان وإقليم صحارى – أربيان وإقليم سودانو – زامبياذيان وايرانو – تورانيان . وأظهر تحليل التربة أن القوام والمسامية والرطوبة والأملاح الكلية الذائبة وتفاعل التربة أهم العوامل المحددة لتوزيع ووفرة نبات العشار .

كان محتوى المجموع الخضرى من الفلافونيدات والفينولات 0,185 و 0,37 جم / 100 جم . كما تم فصل وتقدير قيم ثلاثة عشرة حمضا دهنيا ، وكان للمستخلص الميثانولى للمجموع الخضرى لنبات العشار فاعليه كمضاد للأكسدة وكمضاد ميكروبى

Table (1): Analysis of soil samples collected from twenty five representative stands of *Calotropis procera*. M.C.=moisture content, Por.=porosity, W.H.C.=water–holding capacity, Org. C=organic carbon, T.S.S=total soluble salts, and E.C.=electric conductivity.

Samples No.	Physical characteristics										Chemical characteristics								
	Mechanical Analysis							M.C. %	Por. %	W.H.C. %	Org.C %	$CaCO_3$ %	Analysis of 1 : 5 water extract						pH
	Particles Size mm (%)																		
	>2.057	2.057-1.003	1.003-0.500	0.500-0.211	0.211-0.104	0.104-0.053	<0.053						T.S.S %	E.C. µ mohs /Cm	Cl^- %	SO_4^{--} %	CO_3^{--} %	HCO_3^- %	
1	0.09	4.31	13.62	40.16	31.40	9.08	1.39	2.4	45.2	38.7	1.2	4.0	0.110	237	0.03	0.20	0.0	0.09	7.6
2	0.07	8.21	19.67	34.70	27.51	6.52	3.09	1.8	50.5	47.1	0.7	9.0	0.200	197	0.06	0.08	0.0	0.09	7.4
3	0.08	12.71	19.96	33.20	25.26	6.20	2.60	3.1	49.5	42.2	1.5	8.5	0.200	165	0.03	0.12	0.0	0.09	7.6
4	1.02	10.61	18.63	32.85	30.17	4.02	3.15	2.0	40.4	47.2	0.3	2.5	0.300	170	0.09	0.24	0.0	0.06	7.8
5	0.02	1.05	11.53	47.70	29.40	8.33	1.97	2.3	46.6	35.4	0.6	9.5	0.280	252	0.08	0.37	0.0	0.03	7.5
6	0.01	1.25	10.86	43.75	30.23	11.46	2.44	0.4	44.4	36.2	0.3	8.5	0.340	160	0.09	0.32	0.0	0.09	7.3
7	0.01	1.22	2.44	36.11	47.33	8.84	4.05	2.8	45.0	42.2	1.5	5.5	0.300	180	0.06	0.28	0.0	0.03	7.3
8	0.01	0.27	0.94	34.26	50.75	9.60	4.17	4.8	46.3	39.7	1.2	4.5	0.300	170	0.03	0.28	0.0	0.06	7.2
9	0.03	0.21	9.15	46.13	36.21	7.02	1.25	2.7	44.7	31.3	0.9	6.0	0.200	145	0.04	0.21	0.0	0.06	7.5
10	0.02	0.05	2.53	36.57	49.23	9.45	2.15	2.7	47.1	35.8	0.9	7.5	0.260	194	0.01	0.12	0.0	0.03	7.5
11	0.08	10.26	8.64	35.93	26.95	7.80	2.24	2.1	47.8	42.9	1.0	6.5	0.200	217	0.04	0.14	0.0	0.09	7.5
12	0.55	12.66	24.79	29.52	24.17	5.11	2.87	2.6	44.9	44.7	0.9	5.5	0.260	168	0.06	0.18	0.0	0.08	7.7
13	0.02	1.15	11.19	45.72	29.81	9.89	2.20	1.4	45.5	35.8	0.5	9.0	0.300	206	0.08	0.34	0.0	0.06	7.4
14	0.01	0.74	1.69	35.18	39.04	19.22	4.11	3.8	45.6	41.0	1.4	5.0	0.300	175	0.04	0.28	0.0	0.05	7.2
15	0.03	0.13	5.84	41.35	42.72	8.28	1.70	2.7	45.9	33.6	0.9	6.7	0.270	170	0.02	0.26	0.0	0.05	7.5
16	0.00	0.06	1.08	49.48	41.80	6.25	1.61	0.1	39.2	29.3	0.2	8.0	0.412	239	0.10	0.16	0.0	0.06	8.5
17	0.00	0.00	0.60	51.80	43.51	3.60	1.02	0.1	41.6	29.5	0.1	6.6	0.085	128	0.01	0.05	0.0	0.06	8.2
18	0.00	0.00	0.20	49.00	46.61	2.80	1.40	0.3	44.8	29.6	0.2	6.1	0.050	177	0.02	0.02	0.0	0.06	8.7
19	1.30	1.90	4.10	36.00	46.60	7.50	2.10	0.1	38.9	45.6	0.1	6.0	0.068	183	0.01	0.04	0.0	0.03	8.8
20	7.23	0.82	4.59	24.32	42.21	15.74	4.23	0.7	41.2	35.8	0.2	11.0	0.139	171	0.01	0.05	0.0	0.06	8.8
21	1.49	0.59	8.64	38.51	33.92	12.65	3.34	0.8	41.2	31.5	0.2	12.0	0.245	163	0.01	0.08	0.0	0.06	9.1
22	0.19	0.55	13.03	50.45	26.54	6.94	1.21	0.7	46.8	29.8	0.1	10.7	0.090	182	0.01	0.11	0.0	0.03	9.0
23	0.30	0.49	6.55	40.20	45.70	5.00	1.20	0.4	42.0	51.7	0.1	6.2	0.060	168	0.02	0.11	0.0	0.03	9.2
24	0.50	0.30	5.10	36.75	48.60	6.50	1.49	0.7	49.6	54.8	0.1	7.1	0.073	167	0.03	0.07	0.0	0.03	9.1
25	0.14	4.91	12.10	33.52	34.61	12.05	2.63	2.5	45.8	39.6	0.9	6.6	0.211	187	0.05	0.24	0.0	0.06	7.5
Mean	0.53	2.98	8.70	39.33	37.21	8.39	2.38	1.8	44.82	38.84	0.6	7.1	0.212	183	0.04	0.17	0.0	0.06	8.0
S.E	1.43	4.19	6.71	6.94	8.53	3.66	1.00	1.26	3.06	6.97	0.48	2.21	0.10	28.30	0.03	0.10	0.0	0.02	0.69