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# Phytochemical analysis and GC-MS-MS investigation of methanol extract of *Calotropis procera* aerial parts

**Amjad I. Oraibi**

Al-Manara College for Medical Sciences, Department of Pharmacy

\*Corresponding author email: [amjadibrahim@uomanara.edu.iq](mailto:amjadibrahim@uomanara.edu.iq)

**Mohanad S. Al Jaber**

Al-Manara College for Medical Sciences, Department of Pharmacy

**Oussama Mansour**

Al-Manara College for Medical Sciences, Department of Pharmacy

**Abstract**--The purpose of our study was to examine chemical constituents of *Calotropis Procera* aerial parts using preliminary chemical test and GC-MS-MS analysis since no enough phytochemical investigation had been done regarding the plant in Iraq, the aerial parts leaves stems and fruits of *C. Procera* were extracted in 95% aqueous methanol in soxhlet and fractionated with n-hexane, chloroform respectively. The extract was analyzed using specific chemical reactions to diagnose the chemicals specifically the important secondary metabolites, and then checked further in GC-MSMS to identify the identity of each compound.

**Keywords**---Phytochemical analysis, GC-MS-MS investigation, methanol extract, *Calotropis procera*, aerial parts.

**Introduction**

Plants and more specifically their active metabolites that are referred as secondary metabolite have an extended history of usage in current medicinal system and in systems of traditional medicine, and are the bases of very important and even lifesaving medications such as digoxin, atropine, morphine, quinine, vinblastine and vincristine. Usage of herbal medications and food supplements has extended gradually in the by physician and patients due to the safety profile and believing that is the nature products are safer than chemical materials. Phytochemicals (originating from the Greek word Phyto, that's mean plant) which are naturally active materials, occur in nature as chemical

composites present in plants, providing healthiness benefits for individuals further than those recognized to macronutrients and micronutrient [1]. The phytochemicals role is protection plants from disease and damage and contribute to the plant's color, aroma and flavor. Generally, chemicals that is present in plant known as secondary metabolites and providing protection from environmental hazardous exposure or from insects and pollutions are called as phytochemicals [2]. Phytochemicals also have very significant role in human health. In excess of 4,000 phytochemicals have been categorized [4] and are classified according to their chemical structures, physical, or functional protections in plants [3, 4].

The percentage and the concentration of secondary metabolites vary from part to part of the plant, stems, roots, leaves, flowers, fruits or seeds we found that some part have high concentration of the secondary metabolites while others not contain at all .the outer shell and layer of the plant contain the secondary metabolite in the highest percent of the plant tissue. In addition, the concentration differ even from plant to plant according to the variety growth level of the plant and even the environmental condition [6].the phytochemicals as secondary plant metabolites have many reported and approved biological and therapeutic activity like antioxidant, immune system stimulations, antibacterial, antifungal, reduction in the platelet aggregation and blood clotting modulation of hormone metabolism and antineoplastic property.

The studied plant *calotropis procera* also have extensive use in history for many indications as we see its use in toothache, some respiratory diseases, and elephantiasis, also used topically for some skin diseases, leprosy, and rheumatoid diseases as analgesic [7]. Different parts such as leaves, roots and bark, flower, fruits, stem, and latex of the plant have been reported to have various phytochemicals, which might possess many pharmacological activities. The plant extracts seems to have antimicrobial, insectisidal , acaricidal, schizonticidal , anthelmintic , anti-inflammatory, antidiarrheal, anticancer, and larvicidal activities with lot of other advantageous properties [7, 8]. *Calotropis procera* described as a wonderful gift for humankind since it possessing cardio tonic agents such as calotropin, calotoxin, calotropagenin, uscharin, calactin, amyrrin esters, amyrrin, uscharidin, coroglaucigenin, corotoxigenin, frugoside and calotropagenin [7]. The plant consider important for medical researcher due to its rich nature in many chemicals such as norditerpenic esters, cysteine protease procerain, organic carbonates, alkaloids, sterols, flavonoids, and numerous cardenolides.

### **Habitat**

The plant *c. procera* prefer the growth in open area with few competition with other plants that might be exist. The conditions of the area and environment at which *calotropis procera* grown is usually dry area and the rain is very limited up to 1000 mm beside that the soil that the plant will grow in preferred to be excessive drained soil . It's usually found grown beside the road , beachfront dunes, and commonly emerged in the urban areas. *C. procera* is also found at the elevated areas up to 1,000 m. Because the plant is easy to propagate, manages,

and can grow under the xerophytic condition, sometimes it is also grown as an ornamental plant in dry or coastal areas.[9 ,8]

### **Geographical Distribution**

*C. procera* is innate to Asia in the south part and Indo-China to Malaysia, West Africa, Macaronesia, east Africa North and west Africa, Arabian Peninsula, and Madagascar. The plant is adopted in Australia, North America, central, South America, and West Indies. *Calotropis procera* is now established and culture in numerous countries such as Central and South America, Mexico Pacific islands, Caribbean, and the Australia.[10 ,8]

### **Scientific Classification**

Taxonomy

Kingdom: Plantae

Division: Magnoliophyte

Class : Magnoliopsida

Order: Gentianales

Family: Asclepiadaceae

Genus: *Calotropis*

Species : *Calotropis procera*(Aiton). [11]

### **Other Names or synonyms for *calotropis procera***

*Asclepias procera* Aiton, common vernacular names (Sanskrit) Arka, (Hindi) Aaka. Giant Indian Milkweed. Sodom Apple, Small Crown Flower, Rooster tree, French Cotton in English. Remiga (Malaysia), Dok Hak (Laos), Kapal-kapal (Philippines), Nam t[it] b[at] (Vietnam), Pomme de Sodome (French), Rubik (Indonesia), Mudarpflanzer (German), Algodon Extranjero (Spanish), Oshar (Arabic)Ipekag (Turkish) , Calotropo (Italian), Po Thuean, Paan Thuean (northern [8, 12]

### **Botanical Description**

*Calotropis procera* is perennial plant, shrub, and small tree soft-wooded with a maximum height up to 2.5 m. An abundant amount of latex (white liquid) produces if any part of the plant is cut. Regarding the bark of the plant its look light gray, wrinkled and little corky. *Calotropis pocera* root is simple, in the base, it is woody, and have multiple branch, covered with corky bark. The leaves are simple, sub sessile, opposite-decussate, the leaves are look like slightly leathery and having a fine covering of soft hairs that occasionally sting too. Flowers are shallow bell-shaped, like a campanula, bracteates, complete, bisexual, actinomorphic, pentamerous, hypogynous, pedicellate, multiflowered, umbellate, peduncled cymes with axillary or terminal inflorescence. Five sepals, 5 lobed shortly combined that are 4-5 mm long. Five-lobed petals (Corolla), gamopetalous, twisted aestivation. Androecium has five stamens, gynandrous, anther ditheous, coherent. Gynoecium is bicarpellary, apocarpus, and styles are united at their apex, peltate stigma with five lateral stigmatic surfaces[1]. Anthers are adnate to the stigma forming a gynostegium. Fruit is simple, fleshy, inflated, and subglobose to obliquely ovoid follicle. *Calotropis procera* have large amount of

seeds which looks small, flat, obovate, compressed with silky white pappus at the one end, 3 cm or more long [8, 12, 13]



### **Ethno medical (Traditional) Uses**

The leaves have been used in sun worship since Vedic times, according to legend. Hindu physicians employed the latex from the root bark of *C. procera* to cure skin illnesses, as well as cough, intestinal worms, ascites, and anasarca, as well as enlargements of abdominal viscera, among other things. The white liquid was thought to be a harsh laxative and caustic. Flowers were also employed in medicine since they are thought to help with digestion, catarrh, and hunger. Elephantiasis was also treated with the root's bark. Because latex is administered intact, it was used in the toothache preparations. The blooms of *C. procera* have also been used to treat asthma. The plant was also used to treat a wide range of illnesses. Leprosy, hepatic, and splenic enlargements were all treated with the plant. The leaves were cooked, and the oil obtained was applied topically to cure leprosy. Leaf powder has been used as a substitute for ipecacuanha and has the same wound-healing effects as *Gutta-persica*. Latex has also been used to induce abortion in the early stages of pregnancy [14]. Latex was used by tanners to eliminate hair [8].

Latex and pear-shaped fruit offer therapeutic benefits. Although latex is commonly thought to be deadly, claims of its toxicity may be exaggerated. Lifting the seeds and pulp from a halved ripe fruit and drinking sheep, goat, or camel milk from the remaining green skin "cup" could provide a safe and effective dose. Rheumatism is treated with poultices produced from the leaves. The Sodom apple is identified by Levey as *Lappdanum asclepiad*, which Al-Kindi employed in a dentifrice, for hair lengthening, and in a mixture for worm removal and air purification during an epidemic[15]. The root was applied to the nostrils after drying and crushing to a powder with goat milk. Latex is used by the Varanasi tribes to remove worms from teeth and in pain remedies. *C. procera* bark has traditionally been used to treat cholera, extract Guinea worms, and aid digestion. The medication has a calming effect on intestinal muscles and is known to increase bile output. Migraine is also treated with the tender leaves.[9]

### Photochemistry of *Calotropis procera* in previous researches

A vast number of articles and researches are published on the phytochemical investigation and chemical properties of *C. procera*. Besides the cardenolides, which took the highest importance, other phytochemicals are also reported from the plant such as sterols, flavonoids, coumarins, alkaloids, triterpenes, saponins, tannins, and hydrocarbons were reported and isolated from *calotropis procera* different parts. *Calotropis procera* are considered toxic in all of its parts, due to the existence of cardenolides (cardiac glycosides). The milky secretion (latex) considered the more toxic one due to the higher concentration of cardiac glycoside. The chief and the most important cardenolides found in the plant are voruscharin, uscharidin, uzarigenin, calotroposide, calactin, calotoxin, uscharin, ascleposide, calotropagenin, coroglaucigenin, calotropin, proceroside, proceragenin, and syriogenin. The concentration of cardenolide are differ in each part of the plant. The most abundant cardenolides in the various parts of *calotropis procera* are uscharin and calotropagenin the highest two cardenolide in the latex; calotropin and calotropagenin in the leaves; uscharidin, calotropin, proceroside, and calactin in the stem; calotoxin and calactin in the root bark; coroglaucigenin and uzarigenin in the fruit pericarp. *Calotropis procera* seeds also contain cardenolides, mainly coroglaucigenin or frugoside[5].

The flavonoids in *calotropis procera* are rutin (quercetin-3-rutinoside): present in the referred plant parts increasingly in concentration Roots, stem, leaves, flowers, and highest one is latex. In addition to the mentioned chemicals *calotropis procera* is also reported to contain resins, fatty acids, proteases, hydrocarbons, amino acids, and many minerals. The polyphenol content varies from 3.3% (leaf) to 4.9% (stem) in different plant parts [16]. *Calotropis procera* flowers mostly contain  $\alpha$ - and  $\beta$ -amyrins, an alkaline phosphate, cycloart-23-en-3 $\beta$ , cyanidin-3-rhamnoglucoside, 25-diol, cyclosadol, procestrol, quercetin-3-rutinoside,  $\beta$ -sitosterol,  $\beta$ -sitost-4-en-3-one, multiflorenol, and stigmasterol. Cyanidin-3-rhamnoglucose and the triterpene calotropenyl acetate are also reported in the flowers[17]. The leaves content of *calotropis procera* was reported as ascorbic acid, calactin, calotoxin, calotropagenin, calotropin, polysaccharide containing D-arabinose, D-glucose, D-glucosamine and L-rhamnose, calotropagenin, and 3-proteinase. The latex contains calotropin,  $\alpha$ -calotropeol, 3-epimoretenol, gigantol, giganteol, isogiganteol,  $\alpha$ -lactuceryl acetate,  $\alpha$ -lactuceryl isovalerate, lupeol, proceroside, proceragenin, syriogenin, taraxast-20 $\alpha$ -(30)-en-(4-methyl-3-pentenoate), 3'-thiazoline cardenolide uscharidin, uzarigenin, voruscharin and  $\beta$ -sitosterol, powerful bacteriolytic enzyme in latex[17].

Beside what's mentioned The latex also contains 11-23% rubber, the triterpenoids  $\alpha$ - and  $\beta$ -amyrin, lupeol, taraxasteryl acetate,  $\alpha$ - and  $\beta$ -calotropeol, 3-epimoretenol, multiflorenol, cyclosadol, several triterpene esters, the sterols  $\beta$ -sitosterol and stigmasterol, the non-toxic cysteine proteases calotropin, procerain and procerain-B and the alkaloid choline[17]. The root-bark contains benzolisoloneolone, benzolloneolone, long-chain fatty acids, and C (18) isoursane. *Calotropis procera* also reported to contain calactinic acid, choline and O-pyrocatechuic acid, taraxasterol, its  $\phi$ -isomer: taraxasteryl isovalerate and taraxasteryl acetate [17]. The Presence of four new ursane-type triterpenes: Vrsa-13(18), 19(29)-diene-3 $\alpha$ -yl-acetate, 18 $\alpha$ H-urs-19(29)-en-3-one, 18 $\alpha$ H-ursa-

12, 20(30)-diene-3  $\alpha$  -yl-acetate and 18 $\alpha$ H-urs-12- en-3 $\alpha$ -ol, were reported from the root bark [17]. Mudarine as principal cardioactive constituent present in the leaves is reported.

### **Material and Method**

All the chemicals and reagents used for the research were of analytical grade. Regarding the GC-MSMS was Agilent Gas chromatography GC. GC/MS analysis was carried out in Environmental Water Research and Technology Directorate/Ministry of Sciences and Technology.

### **Plant collection and identifications**

Aerial parts of calotropis procera were collected from Wassit in middle of Iraq in July 2020. The plant were identified and authenticated by Mr. mohanad Salim Pharmacognosy Department, of Almanara collage of medical sciences.

### **Preparation of plant material**

Plant parts were air-dried at room temperature and milled into powder for extraction. The powder (200 g) was extracted in 95% methanol using soxhlet apparatus. The resultant extract was concentrated using a rotary evaporator to get rid of methanol and to get a greenish black semi-solid final extract. Solvent partitioning of the crude methanol extract was done using different solvent according to the increased polarity starting with hexane and chloroform. Fractionation was carried out using n-hexane and chloroform, with 250 ml each solvent and repeated three times against water . With using the separating funnel, we get the portioning completed. Each fraction was separated in a different beaker and then dried using rotary evaporator. The aqueous methanol fraction was used for further studies after subjecting the different fractions to a preliminary study.

### **Preliminary phytochemical screening**

Phytochemical profiling of crude extract and aqueous methanol fraction of calotropis procera and different chemical reactions were carried out to determine the class of secondary constituents that is present in the plant.

### **Gas chromatography-mass spectrometry (GC-MS) analysis**

GC-MS analysis were carried out as shown below

Agilent 190915-433UI

Hp-5ms Ultra Inert

In Front SSZ Inlet He

Out MSD

Initial 60 c

Pressure 7.037 psi

Flow 0.9ml/min

Average Velocity 34772 cm/sec

Holdup time 1.4379 min

## Result

Different qualitative phytochemical screening tests were done to establish the chemical composition of each extract; these tests provide important information regarding the type of secondary metabolites present in the plant to establish a suitable procedure for isolation of these metabolites from the different extracts. The most important phytochemicals or secondary metabolites which were screened are flavonoids, alkaloids, coumarin, terpenoids, phenols, and saponins. Preliminary phytochemical examination of crude plant extracts for detection the presence of flavonoids, coumarin, phenols, and terpenoids and cardiac glycoside. The results of these tests are summarized in table 1.1.

Table 1.1  
Qualitative profile of the phytochemicals found in the plant

Extract	Flavonoids	Alkaloid	Cardiac glycoside	Coumarins	Terpenoid	Tannins	saponin
result	+	-	+	+	+	+	+

## Gas chromatography-mass spectrometry (GC-MS) analysis GC-MS-MS result for n-hexane

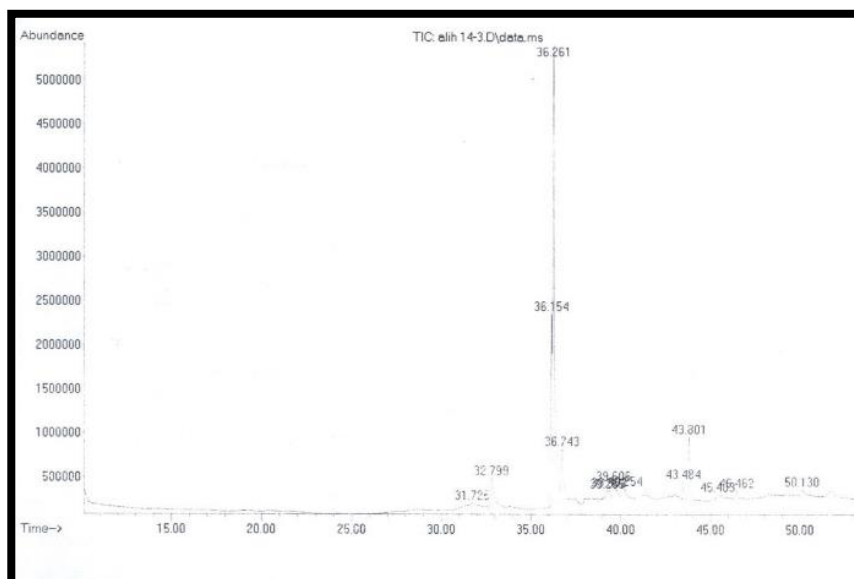
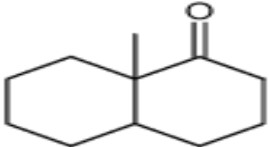
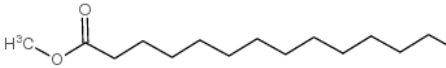
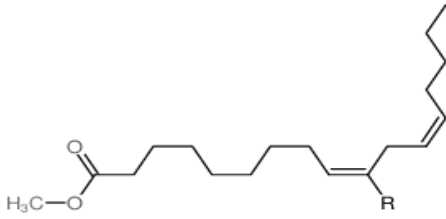
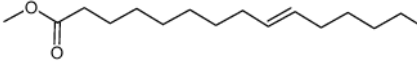
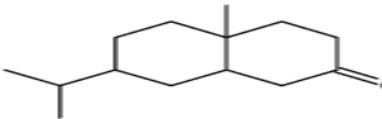
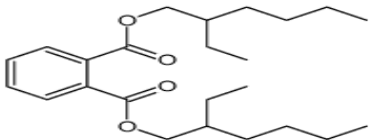
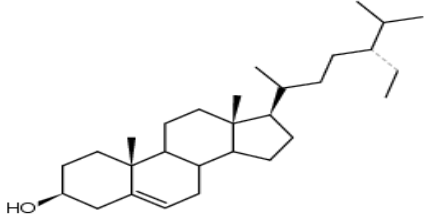


Table 1.2  
Compound identified by GC-MS-MS in n-hexane

No	RT	AREA %	LIBRARY ID	Cas no.	quality
.					

1	31.7 2	2.03	1(2H) -Naphthalenone, octahydro-8a-methyl	91280-39-6	
2-	32.7 9	7.17	Hexadecanoic acid methyl ester	112-39-0	
3-	36.1 5	4.08	9,12-Octadecanoic acid( z,z )-,methyl ester	112-63-0	
4	36.2 6	50.51	9-Octadecenoic acid, methyl ester , (E)	1937-62-8	
5	39.2 5	1.27	2 ( 1H)-Naphthlaenone, octahydro-4a-methyl-7- )1-methylethyl)-,, (4a.alpha.,7.beta.,8a.bet a.)	54594-42-2	
6	43.0	7.04	Bis(2-ethylhexyl) phthalate	117-81-7	
7	45.4 0	0.27	Beta-sitosterol	31793-83-6	

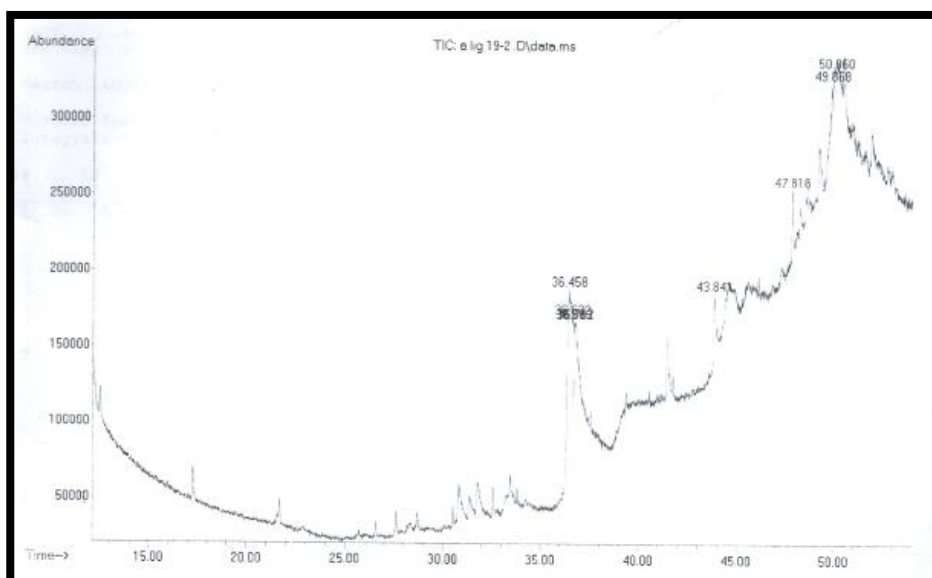
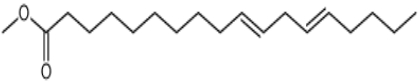
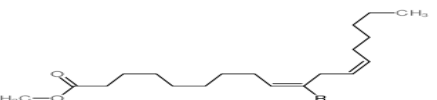
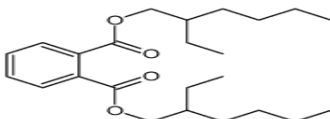
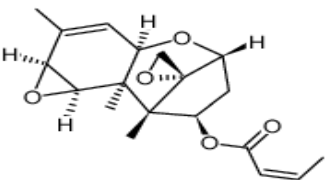
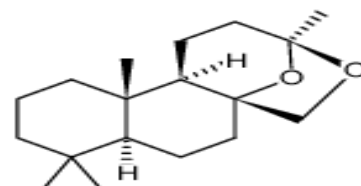
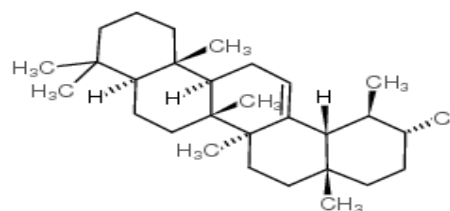


Figure 1.2. GC-MS-MS chromatogram of Chloroform fraction

Table 1.3  
Compound identified by GC-MS-MS in chloroform fraction

No.	RT	ARE A %	LIBRARY ID	Cass number	quality
1	36.46	43.5 9	10,13-Octadecadienoic acid, methyl ester	56554-62-2	
2-	36.63	8.35	9,12-octadecadienoic acid (z,z)-,methyl ester	Methyl lineolate	
3-	43.83	8.89	Bis(2-ethylhexyl) phthalate	117-81-7	
4	47.81	8.84	Trichothec-9-en-4- ol,7,8:12,13-diepoxy-,2- butenoate, [4. beta. (z),7. beta.,8. beta]	212284-11-7	

5	49.872	4.59	Urs-12-ene	464-97-1
6	50.05	7.35	5H-3,5a-Epoxy-naphth[2,1-c] oxepin, dodecahydro-3,8,8,11-tetramethyl, [3s-(3.alpha.,5.alpha.,7.alpha.,11a.beta.)-11b.alpha]-Urs-12-ene	1153-34-0



## Conclusion

The preliminary analysis and the GC-MSMS revealed the existence of many chemicals that is potentially of medicinal values as shown in the previous tables.

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