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Screening for bioactive compounds in different plant parts of *Calotropis gigantea* L.

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Abstract
The genus *Calotropis* R. Br., belonging to family Asclepiadaceae, is cosmopolitan in distribution. *C. gigantea*, commonly known as safed aak, grows abundantly in Rajasthan. The present study was design to analyze the qualaitative and quantitative in different extracts of *Calotropis gigantea.* Methanolic extracts of root, stem, leaf, flowers and fruit of *C. gigantea* were prepared via soxhlet extraction method. The screening was performed for carbohydrates, lipids, protein, phenols, tannins, flavonids, phytosterols, sennosides, anthraquinones. The color intensity or the precipitate formation was used as analytical responses to these tests. Quantitative analysis of compounds were done by established protocol.

Keywords: *Calotropis gigantea*, secondary metabolites, methanol, chloroform

Introduction
From pre-historic times to the modern era in many parts of the world and India, plants, animals and other natural objects have profound influence on culture and civilization of man. The Indian subcontinent is rich in medicinal plants and is one of the richest countries in terms of genetic diversity of medicinal plants. It exhibits a wide range in topography and climate. In ancient ayurvedic medicine the plant *C. gigantea* is known as "Sweta Arka" Previous studies have been show the presence of various chemical constituents in *C. gigantea* such as toxic glycosides calotropin, uscharin and calotoxin, cardenolides, flavonoids, and saponins [1]. In a continuation in our study, we have reported many primary and secondary metabolites and their qualitative and quantitative analysis have been done.

Materials and methods
Plant materials
Root, stem, leaf, flowers and fruits of *C. gigantea* were collected from local area of Jaipur city, Rajasthan, India.

Extraction: Plant materials were subjected to shade drying (22ºC) for two weeks and then processed at laboratory mill. Air dried coarse powder thus obtained (1 kg) was extracted with methanol in soxhlet extractor by continued successive hot extraction method. Finally the marc was collected and concentrated. Latter, following the established protocols [2] each of the test sample was processed further to used to evaluate the presence of carbohydrates, proteins, tannins and flavonoids. Before doing so, each test sample was reconstituted in methanol and divided into aliquots to perform the qualitative tests mentioned in table 1.

Methodology
Quantification of total soluble sugar [4], starch [5], lipids [5], nucleic acids (RNA/DNA) [6], protein [7], total phenols [8] were done by established protocols, in each test samples. Total presence of Chlorophyll a in test samples were calculated in mg/g of plant material from the equations [9] derived as follows –

$$\text{Chlorophyll a} = \frac{11.3A_{663} - 0.96A_{645}}{\alpha \times 1000 \times w} \times V$$

$$\text{Chlorophyll b} = \frac{18.3A_{645} - 3.9A_{663}}{\alpha \times 1000 \times w}$$

$$\text{Total Chlorophyll} = \frac{A_{652} \times V}{34.5 \times w}$$

(Where, A = Absorbance; V = Volume of each extract; w = weight of the plant material used; α = the length of light path in the cell, which is usually 1 cm).

Similarly, the level of total carotenoids (mg/g) was calculated using the equation [10] given, where E is determined as follows:

$$\Box E\ Car_{480} = [\Box E_{480} + (0.114\ \Box E_{663})] - (0.638 \Box E_{645})$$

(Here, $\Box E\ Car_{480}$ = increase in absorbance at 480 nm due to carotenoids; $\Box E_{480}$ = extinction at 480 nm; $\Box E_{645}$ = extinction at 645 nm; $\Box E_{663}$ = extinction at 663 nm).

Result and discussion

The present study carried out on the methanolic extractions of different plant parts of different of *C. gigantea* revealed the presence of bioactive constituents. Qualitative and quantitative analysis of these extracts are summarized in table 1. And 2. Alkaloids, tannins, phenol, flavonoids, sterols, terpenoids, saponin, anthroquinones, cardiac glycoside, proteins, quinines, carbohydrate, reducing sugar, resin and fats were tested for their presence and absence on the basis of colour intensity or precipitation by using established protocols. Alkaloids were found absent in stem. However, phenols, tannins, flavonoids, tepenoid, cardiac glycosides, protein and quinone were present in all plant parts. Phytosterols, saponin and anthroquinon were found absent in root and stem extracts. No colour reaction was found for anthroquinon in leaves also. Previously, chemical investigation of this plant for the presence of cardiac glycosides, saponins, flavonoids, steroids, terpenoids has been done [11, 12]. The presence of tannins suggests the ability of this plant to play a major role as antidiarrhoec and antihaemorrhagic agent [13], while alkaloids has been implicated for its detoxifying and antihypertensive properties [14, 15]. Furthermore, the presence of phenol observed in the methanolic extract is an indication that the plant might play an important role as dietary antioxidants. Phenolic compounds prevent oxidative damage in living systems [16, 17].

Further, crude plant materials were examined for quantitative analysis of different primary metabolites. Results are shown in table-2. Starch and total soluble sugars were found maximum in stem (4.75 and 10.57 mg/gdw respectively). Ascorbic acid was found maximum in flowers (1.12 mg/gdw). Ascorbic acid (vitamin C), an essential dietary requirement in man, is widely distributed in the plants and due to its universal presence in the actively metabolizing cells. Ascorbic acid plays major role in the plant growth and metabolism [18]. Later, the important role of ascorbic acid was also suggested during the juvenile phase of growth of a plant [19].

Lipid were in high quantity in roots (27.20mg/gdw). Higher levels of lipids investigated in the selected plant species indicate their probable drought tolerance capacity and physiological characteristics under necessarily needed in the ecological adaptation to the semi-arid environment.

Proteins occur throughout the plant cells, both in extrinsic and intrinsic, simple and conjugated forms. In many plant species the exhibited bioactivity, viz. antiviral and others has been attribute to the proteinaceous substances present in their tissues. As a result studies on the quality and quantity of proteins have been undertaken in a large number of plants. In the present investigation, therefore, the selected plant types were assessed for the total protein content, where the higher level of protein was measured in flowers (56.12 mg/gdw).

Phenols exhibit important role in the regulation of plant growth and development. In the present study, phenols were found maximum in leaves (0.019 mg/gdw). Nucleic acids, being the key component of the biochemical processes of the living organism have been estimated in *C. gigantea*. Results indicate that generally the higher concentration of DNA is present in various plant parts than that of RNA. It was also observed that higher level of RNA and DNA were found in the flowers. Chlorophylls are the main photosynthetic pigments in the green plants and hence, their determination is frequently required in most plant analysis. In the present study total level of chlorophylls were found to be higher in leaves (0.03 mg/gdw) while carotene were higher in flowers (0.51mg/gdw).

Table 1: Preliminary phytochemical constituents present in the methanolic extracts different plant parts of *Calotropis gigantea* Linn.

S.N.	Phytochemicals	Colour detection/ precipitate formation	Methanolic extracts of *C. gigantea*				
			Root	Stem	Leaves	Flower	Fruit
1.	Alkaloids (5ml Aliquot+1.27g Iodine+2g KI+100 ml H_2O)	Reddish Brown colour	+	-	+	+	+
2.	Phenols and Tannins (5 ml Aliquot+2ml. Glacial Acetic acid)	Red Colour	+	+	+	+	++
3.	Flavonoids (Shinoda's Test)	Orange colour	+	+	+	+	+
4.	Phytosterols (Salkowaski reaction)	Red colour	-	-	+	+	+
5.	Terpenoid (5ml Aliquot shake with 5ml. Chloroform, add few drops of H_2SO_4)	Dark green colour	++	+	+	+	+
6.	Saponin (Aliquot vigorously shaken with water)	Foam formation	-	-	+	+	+
7.	Anthroquinone (5mlAliquot+1ml conc. H_2SO_4 +1ml. of Ammonia)	Rose pink colour	-	-	-	+	+
8.	Cardiac glycosides (Keller-Killani Test)	Brown ring appear	+	+	-	+	+
9.	Protein (Biuret Test)	Pale purple colour	+	+	+	+	+
10.	Quinone (5ml. Aliquot+5ml. conc. HCL)	Yellow colour ppt.	+	+	+	+	+
11.	Carbohydrate (Molish's test)	Red or purple colour	-	-	+	+	+
11.	Reducing Sugar (Fehling test)	Brick red ppt. at bottom	-	-	+	+	+
12.	Resin (2ml. aliquot+10ml. Acid anhydride+1dror H_2SO_4)	Purple colour	-	-	-	-	-
13.	Test for fats and oils(solubility test)	-	-	+	+	+	+

Table 2: Isolated primary metabolites contents (mg/gdw) from different plant parts of *C. gigantean*

Primary metabolites	Roots	Stem	Leaves	Flower	Fruit
Starch	2.18±5.0	4.75±0.15	2.060±1.14	4.07±0.68	3.48±10.22
Total soluble sugar	2.41±9.10	10.57±6.66	5.42±6.71	1.64±8.05	7.89±1.2
Ascorbic acid	0.22±7.66	0.25±25.11	0.37±0.48	1.12±1.02	0.14±3.45
Lipids	27.20±6.47	8.72±5.13	1.53±4.66	1.05±9.14	1.31±7.16
Proteins	22.98±9.36	29.15±12.04	25.19±6.15	29.18±4.16	56.12±5.13
Phenols	0.019±0.12	0.07±3.65	2.17±4.12	0.064±6.66	1.08±6.12
RNA	1.85±0.66	0.05±4.12	0.12±6.95	5.59±4.87	0.92±6.15
DNA	2.66±0.66	0.10±2.03	1.21±5.12	7.33±6.25	4.29±3.52
Chl.a+b	0.02±0.33	0.20±6.30	0.30±3.71	0.20±1.02	0.01±2.65
Carotein	0.44±6.05	0.10±0.50	0.43±0.66	0.51±2.03	0.24±6.42

Conclusion

Biologically active compounds contain a remarkably diverse assay of organic compounds. All the biochemical compounds are directly/indirectly derived from primary metabolites for the important framework or they also modify the physico-chemical characters of other groups of compounds by combining with them. Phytochemical screening of the crude extracts revealed the presence of saponins, tannins, alkaloids, other phyto constituents which were reported during present investigation were cardiac glycosides, flavonoids, glycosides, steroids, terpens and tannins. The obtained results provide a platform for further advance investigations.

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Conflict of Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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