



## Biochemical Profile of *Calotropis procera*

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**Estimates of chemical profiles of whole extract of flowers, leaves and stem of *Calotropis procera* revealed the presence of protein, carbohydrate, amino acid, phenol, peroxidase and phenylalanine ammonialyase. HPLC analysis of *C. procera* revealed the presence of lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid.**

**Key words:** *Calotropis procera*, HPLC, biochemical profile.

Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. Recent awareness of therapeutic potential of several traditionally used plants has opened a dimension for the study and research of medicinal plants (Akhtar *et al.*, 2006). In the Indian systems of medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and research. In western world also, the use of herbal medicines is steadily growing. Public, academic and government interest in traditional medicines is growing exponentially due to the increased reports of the adverse drug reactions and economic burden of the modern system of medicine.

Aqueous extract of leaf, flower and roots of *Calotropis procera* proved most effective in the control of many diseases. *C. procera* contains resin, cardenolids, steroid glucosides, useharin, calotoxin, and calactin. Leaves and stalks contains a bioactive chemical constituents of sterols, resins, cardenolides, calotropin, calotropagenin etc., In flower, the chemical constituents are the high

**Table 1. Biochemical and enzymatic properties of *C. procera***

Parameters	Units	Whole extract of flowers, leaves and stem
Protein	mg/g	10.90
Carbohydrate	mg/g	00.70
Amino acid	mg/g	20.90
Phenol	mg/g	04.99
Peroxidase	unit/L	02.62
Phenylalanine ammonialyase	mg/ml/min	14.00

amount of ash and proteins with varying quantities of alkaloids, and anthocyanins. Root bark and root contain bitter yellow resin but no alkaloid. The whole plant contains flavone glycosides and cardiac glycoside. To understand other chemical profiles of *C. procera*, the following biochemical analysis has been done for total protein, carbohydrate, lipids,

phenol, free amino acid, enzyme analysis such as peroxidase (PO), phenylalanine ammonialyase (PAL).

### Material and Methods

Different parts of *C. procera* like flowers, leaves and stem collected for extraction. Following the dry weight determination the dried parts of leaf, flower and stem were milled and stored in -20°C until extraction.

### Extraction and Biochemical analysis

Separate extractions were conducted for each plant parts. All the plant parts are extracted with water, methanol, ethyl acetate; solvents removed vacuum and stored at -20°C till the bioassay and estimated some of the primary and secondary groups. The method of Schuster (1985) was followed to analyse the free fatty acid methyl esters of *C. procera* (whole plant) sample in HPLC.

### Total protein

The blue colour developed by the reduction of the phosphomolybdic and phosphotungstic components in the folin-ciocalteau reagent by the aminoacids tyrosine and tryptophan present in protein plus colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured using the Shakir *et al.* (1994) method.

### Total carbohydrate

The method of Singh and Sandhu (2005) was followed for estimating the total carbohydrate. Carbohydrates are first hydrolysed in to simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This compound forms green coloured products with anthrone reagent which can be estimated at 630nm.

### Total Aminoacids

Singh and Sandhu (2005) found a method which

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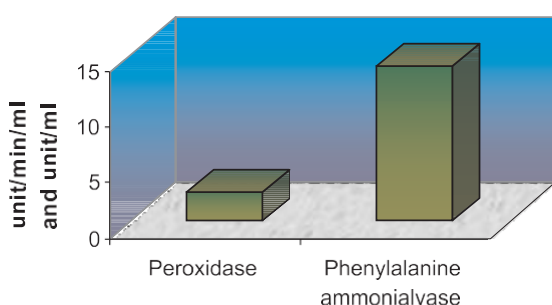
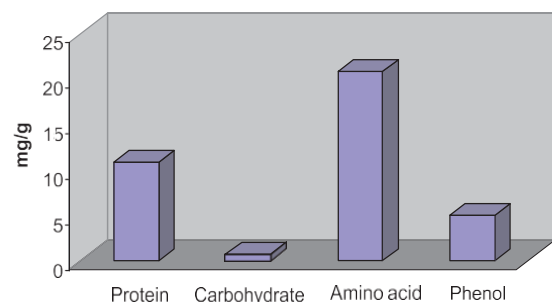
**Table 2. HPLC analysis of free fatty acid methyl esters of *C. procera***

Fatty acid	Area (%)	Retention time (min)
Lauric acid	0.0123	2.141
Myristic acid	0.1905	3.090
Palmitic acid	53.0672	4.761
Stearic acid	3.6439	8.028
Oleic acid	8.3149	8.239
Linoleic acid	14.5222	9.342
Linolenic acid	20.2490	11.306

was followed for estimating total amino acids. Ninhydrin, a powerful oxidizing agent, decarboxylates the alpha-aminoacids and yields an intensely coloured bluish purple product which is colorimetrically measured at 570nm.

#### Total phenol

For estimation of total phenols, the method of Singh and Sandhu (2005) was followed. Phenol reacts with phosphomolybdic acid in folin-Ciocalteu reagent in alkaline medium and produce



**Fig.1 Biochemical profile of whole extract of flowers, leaves and stem of *C. procera***

blue coloured complex (Molybdenum blue).

#### Peroxidase

The method of Singh and Sandhu (2005) was followed for estimating enzyme peroxidase. Guaiacol is used as a substrate for the assay of peroxidase. The resulting oxidized (dehydrogenated) guaiacol is probably more than one compound and depends on the reaction conditions. The rate of formation of guaiacol dehydrogenation product is a measure of the POD activity and can be assayed spectrophotometrically at 436nm.

#### Phenylalanine ammonialyase

The method of Singh and Sandhu (2005) was followed for estimating enzyme peroxidase. Phenylalanine ammonialyase activity is determined spectro photometrically by following the formation of trans cinnamic acid which exhibits an increase in absorbance at 290nm (crude enzyme)/270nm (purified enzyme).

#### Results and Discussion

The biochemical analysis such as total protein, carbohydrates, amino acids, phenol, peroxidase and phenylalanine ammonialyase analyzed during the experiment period and the results are summarized in Tables 1 and 2, and in Fig.1.

From the table 1 and fig. 1 the following chemical profiles viz., protein (10.90 mg/g), carbohydrate (0.07 mg/g), amino acid (20.90 mg/g), phenol (04.99 mg/g), peroxidase (02.62 unit/l )and phenylalanine ammonialyase(14.00 mg/ml/min)were identified. Table 2 shows the results of HPLC analysis in *C. procera*. It revealed the presence of following chemicals such as lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid.

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