CHAPTER-3

RESULTS AND DISCUSSION

Table-1 : Nature and Colour of each extract of different plant parts

S.No.	Extract/fraction	PLANT PARTS			
		Root	Stem bark	Leaves	Flowers
1.	Petroleum ether	Dark redish (Solid)	Dark redish brown (Semi solid)	Brown (Semi solid)	Light brown (Sold)
2.	Ethanol	Yellowish (Solid)	Light Yellowish (Solid)	Dark brown (Semi solid)	Yellowish brown (Semi solid)
3.	Benzene	Dark brown (Solid)	Yellowish brown (Solid)	Greenish yellow (Solid)	Dark yellowish (Semi solid)
4.	Chloroform	Yellowish brown (Solid)	Blackish brown (Solid)	Brown (Solid)	Dark brown (Solid)
5.	Ethyle acetate	Yellowish red (Solid)	Dark brown (Solid)	Dark blackish brown (Solid)	Brown (Semi solid)
6.	Acetone	Browinsh red (Solid)	Dark red (Semi solid)	Yellowish (Solid)	Dark brown (Semi solid)
7.	Methanol	Light orange (Solid)	Orange (Semi solid)	Yelllowish Orange (Solid)	Dark yellow (Semi solid)

Table-2 : Qualitative Phytochemical Analysis of Different Extracted Plat Part of *Calotropis*.

S.No.	Plant Parts	Carbohydrates	Glycosides	Alkaloids	Flavonoids	Amino acids	Phenols	Tannins	Saponins	Sterols
1.	Root	+	+	+	-	+	+	-	-	-
2.	Stem bark	+	+	+	-	+	+	-	-	-
3.	Leaves	+	+	-	-	+	+	-	-	+
4.	Flowers	+	+	+	-	-	-	-	-	-

Table-3 : T.L.C. of Petroleum Ether Extract of root.

S.No.	Solvent Systems	No. of Spots	Resolution Type	Rf Value in the Best Solution
1.	Benzene	4	a	
2.	Benzene : Ethyle acetate (8:2)	3	a	
3.	Benzene : Ethyle acetate (7:3)	5	b	
4.	Chloroform	4	a	
5.	Benzene : Chloroform (9:1)	8	c	0.12, 0.24, 0.33, 0.36, 0.39, 0.44, 0.55, 0.61
6.	Chloroform : Acetone (9:1)	5	a	

a = Diffused
b = Poor
c= Good

Table-4 : T.L.C. of Petroleum Ether Extract of Stem Bark.

S.No.	Solvent Systems	Resolution Type	No. of Spots	Rf Value in the Best Solution
1.	Benzene	b	4	
2.	Benzene : Ethyle acetate (8:2)	b	3	
3.	Benzene : Ethyle acetate (7:3)	a	5	
4.	Chloroform	b	4	
5.	Benzene : Chloroform (9:1)	c	11	0.09,0.11,0.18,0.21,0.29,0.31,0.37,0.45,0.55,0.62,0.66
6.	Chloroform : Acetone (9:1)	c	9	0.16,0.21,0.29,0.37,0.42,0.48, 0.56,0.61,0.66

a = Diffused
b = Poor
c= Good

Table-5 : T.L.C. of Petroleum Ether Extract of Leaves.

S.No.	Solvent Systems	Resolution Type	No. of Spots	Rf Value in the Best Solution
1.	Benzene	b	5	
2.	Benzene : Ethyle acetate (8:2)	a	4	
3.	Benzene : Ethyle acetate (7:3)	a	6	
4.	Chloroform	a	4	
5.	Benzene : Chloroform (9:1)	c	13	0.23,0.26,0.32,0.36,0.41,0.47,0.53,0.58,0.62,0.66,0.72,0.74,0.75
6.	Chloroform : Acetone (9:1)	c	11	0.11,0.15,0.22,0.26,0.32,0.38, 0.41,0.47,0.56,0.62

a = Diffused
b = Poor
c= Good

Table-6 : T.L.C. of Petroleum Ether Extract of Flowers.

S.No.	Solvent Systems	Resolution Type	No. of Spots	Rf Value in the Best Solution
1.	Benzene	b	4	
2.	Benzene : Ethyle acetate (8:2)	a	5	
3.	Benzene : Ethyle acetate (7:3)	c	8	0.11,0.18,0.32,0.29,0.36,0.44, 0.47,0.53
4.	Chloroform	a	6	
5.	Benzene : Chloroform (9:1)	b	5	
6.	Chloroform : Acetone (9:1)	a	4	

a = Diffused
b = Poor
c= Good

Table-7 : T.L.C. of Ethanolic Extract of Root.

S.No.	Solvent Systems	Resolution Type	No. of Spots	Rf Value in the Best Solution
1.	Chloroform	a	4	
2.	Chloroform : Methanol (8:2)	a	5	
3.	Chloroform : Methanol (7:3)	b	7	
4.	Chloroform : Methanol (6:4)	c	10	0.13,0.15,0.18,0.21,0.31,0.36, 0.38,0.41,0.55,0.61
5.	Chloroform : Methanol (1:1)	d	6	
6.	Butanol : Acetic acid : Water (4:1:1)	a	5	

a = Diffused
b = Poor
c= Good

Table-8 : T.L.C. of Ethanolic Extract of Steam Bark.

S.No.	Solvent Systems	Resolution Type	No. of Spots	Rf Value in the Best Solution
1.	Chloroform	a	7	
2.	Chloroform : Methanol (8:2)	a	5	
3.	Chloroform : Methanol (7:3)	c	12	0.17,0.19,0.21,0.24,0.29,0.33,0.37,0.41,0.44,0.58,0.67,0.72
4.	Chloroform : Methanol (6:4)	b	4	
5.	Chloroform : Methanol (1:1)	a	6	
6.	Butanol : Acetic acid : Water (4:1:1)	c	13	0.06,0.13,0.18,0.21,0.27,0.31, 0.38,0.42,0.56,0.58,0.59,0.64, 0.70

a = Diffused
b = Poor
c= Good

Table-9 : T.L.C. of Ethanolic Extract of Leaves.

S.No.	Solvent Systems	Resolution Type	No. of Spots	Rf Value in the Best Solution
1.	Chloroform	b	6	
2.	Chloroform : Methanol (8:2)	b	5	
3.	Chloroform : Methanol (7:3)	a	8	
4.	Chloroform : Methanol (6:4)	c	11	0.04,0.08,0.09,0.11,0.14,0.21, 0.27,0.31,0.36,0.42,0.55,0.66
5.	Chloroform : Methanol (1:1)	b	6	
6.	Butanol : Acetic acid : Water (4:1:1)	a	7	

a = Diffused
b = Poor
c= Good

Table-10 : T.L.C. of Ethanolic Extract of Flowers.

S.No.	Solvent Systems	Resolution Type	No. of Spots	Rf Value in the Best Solution
1.	Chloroform	b	7	
2.	Chloroform : Methanol (8:2)	b	6	
3.	Chloroform : Methanol (7:3)	a	4	
4.	Chloroform : Methanol (6:4)	b	5	
5.	Chloroform : Methanol (1:1)	a	8	
6.	Butanol : Acetic acid : Water (4:1:1)	c	12	0.06,0.12,0.14,0.02,0.24,0.32, 0.38,0.42,0.48,0.55,0.63,0.68, 0.74

a = Diffused
b = Poor
c= Good

Table-11 : Analysis of Carbohydrates in different Plant parts

S.No.	Sugars	Root	Stem bark	Leaves	Flowers
1.	Glucose	+	+	+	+
2.	Maltose	+	-	-	+
3.	Xylose	-	+	-	-
4.	Arabinose	-	-	+	+
5.	Galactose	+	-	-	-
6.	Fructose	-	-	-	-
7.	Ribose	-	+	-	-
8.	Mannose	+	-	-	-
9.	Saccharose	-	-	-	-

+= Present, - =Absent

Table-12 : Analysis of Amino acids different plant parts.

S.No.	Amino acids	Root	Stem bark	Leaves	Flowers
1.	Alanine	+	+	+	+
2.	Histidine	-	-	-	-
3.	Lysine	+	-	-	-
4.	Proline	-	+	-	-
5.	Threonine	+	+	-	-
6.	Tyrosine	-	-	-	-
7.	Valine	+	-	-	+
8.	Seriene	-	-	-	-
9.	Leucine	-	+	-	-
10.	Isoleucine	+	-	+	-
11.	Glycine	+	+	+	-
12.	Glutamic acid	-	-	+	-
13.	Cystine	+	-	-	-
14.	Arginine	+	-	-	-
15.	Aspartic acid	+	+	-	-

+= Present, - =Absent

Table-13 : Analysis of Phenols in different plant parts.

S.No.	Phenols	Root	Stem bark	Leaves	Flowers
1.	Syrinic acid	+	+	-	-
2.	Nanilic acid	-	-	+	-
3.	p-hydroxy benzoic acid	-	-	-	-
4.	Resorcinol	-	-	-	-
5.	Catecol	+	-	-	-
6.	Protocatechuic acid	+	+	+	-
7.	Orcinol	+	+	+	+

+= Present, - =Absent

Table-14 : Analysis of Alkaloides in different plant parts.

S.No.	Alkaloides	Root	Stem bark	Leaves	Flowers
1.	Atroscin	+	+	-	-
2.	Berberine	-	-	-	-
3.	Brucine	+	-	+	-
4.	Coranine	-	-	-	-
5.	Thebaine	+	-	-	-
6.	Strychine	+	+	-	-
7.	Aconitine	-	-	-	-
8.	Tropine	+	+	+	-
9.	Procaine	-	-	-	-
10.	Naceine	-	+	-	+

+= Present, - =Absent

Table-15 : Analysis of Glycosides of different plant parts.

S.No.	Glycosides	Root	Stem bark	Leaves	Flowers
1.	Methyl 1α-D-lyxopyranoside	+	+	-	-
2.	Methyl 1β-D-xylopyranoside	-	-	-	-
3.	Methyl 1-β-D-arabinopyranoside	-	-	-	-
4.	Methyl 1β-D galactofuranoside	-	-	+	-
5.	Methyl 1α-D-galactopyranoside	+	-	-	+
6.	Methyl 1β-D galactopyranoside	+	-	-	-
7.	Methyl 1β-cellobioside	-	+	-	-
8.	Methyl 1α-D-glucanopyranoside	-	-	-	-

+= Present, - =Absent

Results and Discussion

India is rich with around 80,000 plant species. Only 5-10% have been phytochemically analysed and only about 150 species have been commercially explored. Ancient people of our country were in better state in the past, as far as importance and utilization of plants is concerned. As people throughout the world are diverting their attention towards the plant wealth for various purposes, detailed studies of every plant species offer a great research oppertunity and vigorous search for drugs from plants which are important from medicinal point of view during last century has resulted in the discovery of many active chemical constituents from inumerable plant species. A good number of these have been found to be of great use in the treatment of various ailments and improvement of health. Many compounds have been adopted by modern pharmacopoeia, after a thorough chemical and therapeutic investigations.

Calotropis is an important medicinal plant with many therapeutic values. This plant was observed to be used against many chronic and dangerous diseases. For example asthma, brochitis, cataract, dysentery, epilepsy, elephantiasis, syphilis, leukorrhea, leucoderma, cuts, wounds and swellings etc. by rural and local inhabitants of considered sites as it is clearly indicated by from ethnobotanical surveys carried out during this study. On account of above ethnomedicinal importance, this plant was selected for phytochemical studies.

Qualitative analysis was carried on different plant parts to know the presence of some active priciple constituents of different chemical nature. All the plant parts

were extracted separately in soxhlet extractor assembly with the series of solvents. (Fig.1, 2, 3, 4, 5.....). The colour of different plant parts extracted fractions obtained after the preliminary phytochemical extraction given in Table (1). Persual of Table (2) indicate that different plant parts indicate the presence of different active chemical principles. Carbohydrates, glycosides, amino acids etc. are some chemical constituents which were found to be present in almost all plant parts; their quantity may be less or more. Tables (3, 4, 5 & 6) indicate that the petroleum ether extract of different plant parts analysed by thin layer chromatography with 6 different solvent systems for detecting the presence of various active chemical constituents. The resolution has been categorised as (a) poor, (b) diffused and (c) good. In case of root as shown in table (3) maximum number of spots i.e. 8 were observed with solvent system of Benzene and chloroform (9:1). In case of stem bark (Table-4) maximum number of spots were 9 and 11 with solvent system of chloroform : Acetone (9:1) and Benzene : Chloroform (9:1). In case of leaves (Table-5) maximum number of spots were 11 and 13 with the solvent of chloroform : Acetone (9:1) respectively. As observed in the flowers (Table-6) maximum number of spots were 8 with the solvent system, Benzene ethyl acetate (9:1). Tables (7, 8, 9 & 10) indicate the thin layer chromatography of ethanolic extract of different plant parts i.e. root, stem bark, leaves and flowers by using 6 different solvent systems. The ethanolic extract of root (Table-7) showed maximum number of spots i.e. 10 with the solvent system of Chloroform : Methanol (6:4) and Butanol : Acetic acid : Water (4:1:1) respectively. The resolution of stem bark (Table-8) was

observed in solvent systems of Chloroform : Methanol (6:4) and Butanol : Acetic acid : Water (4:1:1) the extract resolved in 12 and 13 spots respectively.

The maximum number of spots were 11 in leaves extract (Table-9) using solvent systems of Chloroform and Methanol (6:4) and the extract of flowers (Table-10) resolved in 14 components in the solvent system of Butanol : Acetic acid : Water (4:1:1). It was concluded better system for phytochemical analysis.

Study of Table (1) revealed that qualitative analysis of different plant parts by applying various chemical tests. For example, for carbohydrates; Molish test, Fehling test and Tallen test were being applied to test presence of active chemical constituents in different plant parts extracts. Like this, various other tests were applied for detecting the presence of other chemical constituents, such as amino acids, glycosides, alkaloids etc. Different plant parts showed variation in their chemical constituents but carbohydrates, amino acids, glycosides, alkaloids, phenols etc. were found to be present more or less in all the considered plant parts. Root stands first among all the four different plant parts due to the presence of almost all components except sterols saponins. Thin layer chromatography technique was applied to know the presence of number of carbohydrates, amino acids, alkaloids, phenols, glycosides etc. in different plant parts. For example in case of carbohydrates, the system was Ethyl acetate : Acetic acid : Water : Methanol : Water (70:10:10:5) and sprayer Benedict solution. In case of amino acids, system was N-Butanol : Acetic acid : Water (50 : 30 : 20) and sprayer was Ninhydrin. Rf

value of each spot, was measured and was compared with Rf value of standards which run simultaneously (Harbron, 1982).

Tentative identification of carbohydrates was done and result reveals (table-11) reveals that maximum number of sugars were found to be present in root and stem bark. Four sugars are present in the root and stem bark i.e. Maltose, Sacchorose, Fructose, Galactose. In leaves ; two in sugars were found i.e. Maltose and Galactose. Above results clearly indicate that flowers possess minimum nutrient value as it has minium number of sugars. Sucrose and glucose were found to be present in all four different plant parts. Karawya et al. (1977) concluded that root, leaves, flowers of Moghut species contain sucrose and glucose.

Table (12) reveals that maximum number of amino acids were found in root and stem bark. Glycine, Alanine and Tryptophan were the amino acids of common occurence. Arginine and valine was found to be present only in root. Glutamic acid was found in leaves. Shaw et al (1981) reported the presence of 2, 4-Diamino-3-methyl butanolic acid, a novel amino acid in root nodule of *Lotus tenuis.*

Table (13) indicates that only two or 3 phenolic compounds were observed to be present in root, stem bark and leaves. In root syrinigic acid, catecol and orcinol was detected and in stem bark only orcinol was found to be present. In leaves, nanilic acid was found to be present. Phenolic compounds play major key role in the development and internal resistance of plant and possess high nutritive and medicinal values. Gaind and Gupta (1973) reported various phenolic compounds in

the leaves of *Kalanchoe pinnata.* Shibuya (1984) reported ferulic acid, p-coumaric acid and diferulic acid phenols in the alkaline extract of rice endo-sperm cell wall. Newby et al. (1980) reported p-hydroxybenzoic, vanillic, p-coumaric and ferulic acid were present both in the free and bound forms in the fractions. Kamal et al. (1983) reported the isolation of plumbagin, droserone, isoshinonolone and new napthalenon etc. from petrol extract of roots of *Plumbago ze ylanic* L.

Results in the (table -14) indicates maximum alkaloides were found only in root and stem bark. Flowers and leaves gave negative test in qualitative analysis. Maximum number of alkaloides were present in the root and stam bark in comparison to leaves. Root showed the presence of Strychnine, Tryopine, Arecoline, Thebaine. Stem bark showed the presence of Atroscin procaine and strychnine. Siddiqui et al. (1979) investigated alkaloid in *Crotolaria candicans.* Akinloye and Court (1980) identified twenty one alkaloides from *Rauwolfia oreogiton.*

Result in given (table-15) clearly indicats that glycosides were present in almost all the parts of the plant. Root gave positive result for methyl 1-α-D-lyxopyranoide, α-D-glactopynoside and Methyl 1-α-D-galactopynoside. Stem bark gave positive result for methyl 1-α-D-lyxopyranoside and methyl 1-β-Cellobioside, leaves for methyl 1-β-D-galactofuranoside and flower 1-α-D-galactopyranoside.

The result given in (Table-16) reveals that phenols were detected only in root, stem bark and leaves only in the ethnolic extract. The so were found to be absent in flowers. 2-3 Dimethyl phenol and 2-5 dimethyl phenol were observed in

root and stem bark. p-hydroxybenzoic and catecol were found to present in leaves. Phenolic compounds play major role in the development of internal resistance of plants and they also possesses high medicinal nutritive values.

Above results obtained after the qualitative analysis clearly show that this plant is rich in various active chemical constituents and therefore it has highest ethnomedicinal and nutritional values.