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PHYTOCHEMICAL STUDIES ON THE METHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA* LEAVES

[1]Sachin S, [2]Asha Rani, [3]Nagarathna Amresh, [4]Murugan Rajadurai, *[5]Balasubramanian Sathyamurthy

[1, 2] Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054.

[3]Professor, Department of Biotechnology, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054

[4, 5]Professor, Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054

Abstract:

Calotropis gigantea is a plant of herbal importance which belongs to Apocynaceae family because of its large industrial uses and economic values. Different parts of the plant have immense potential to cure various diseases and disorders like asthma, cold, epilepsy, fever, indigestion, leprosy, piles, skin diseases etc. So, in order to understand their pharmacological action, there is a need to scientifically evaluate them at molecular and biochemical level. Our study is formulated to identify the phytochemicals present in the methanolic l extract of Calotropis gigantea leaves through GCMS analysis. The GC-MS chromatogram of methanolic extract of leaves identified nearly 160 compounds and the selected phytochemicals are deteriquantified such as carbohydrates (400 mg/ml), proteins (16 mg/ml), Polyphenols (101.39 mg/ml) and antioxidants (2 mg/ml) are determined using spectrophotometer. By FTIR analysis we can conclude that the caloptropis methanolic l extract is rich in amines and C = O groups.

Key words: *GCMS, free radical, FTIR, polyphenols, amines*

Corresponding author:

Dr. BalasubramanianSathyamurthy,
Professor, Department of Biochemistry,
Ramaiah College of Arts,
Science and Commerce,
Bangalore – 560054
Email: balasramaiah@gmail.com

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INTRODUCTION:

Herbal plants are considered as the most important part of our natural wealth because of its therapeutic diversity and hence it holds their distinct position. From very ancient times till the present day the herbal plants are used for medicinal purposes. Herbal medicines have less side effects and it is easily available in nature. Tropical country like India is blessed with numerous natural resources along with knowledge for its judicious utilization [1]. *Calotropis gigantea* is also a plant of herbal importance. This plant belongs to Apocynaceae family which includes latex bearing plants. The plant *Calotropis gigantea* (Botanical name) is known by different names in English Crown flower, giant Indian milkweed, in Hindi Aak, Arka, Madar, in Sanskrit Ganarupa, Mandara, Vasuka, Svetapushp etc. In India it has other names Ekka (Kannada), Erukku (Tamil and Malayalam) and Jilledi Puvvu (Telugu). It is also known by different vernacular names like, French cotton, Alarka, Rooster tree, Widuri in different parts of world. The genus *Calotropis* R.Br. (Asclepiadaceous) is distributed in tropical and subtropical regions of Asia and Africa [2].

In India *Calotropis* exists in two species viz. *Calotropis* procera and *Calotropis gigantea*. These two species exhibit similar botanical aspects and pharmacological effects. In Ayurvedic medicine the plant *C. gigantea* is known as ―Sweta Arka" and *C. procera* as ―Raktha Arka." The major difference between these two is only in the colour of the flowers; *C. procera* are white whereas *C. gigantea* are pinkish white. The plant is an erect, tall, large, much branched perennial shrub or small tree. It generally grows to a height of 4 meters [3]. Antioxidants are the molecular agents that prevent the oxidation of other molecules either by stopping the transfer of electrons or by donating hydrogen. These agents protect the human body from the effects of free radicals and Reactive Oxygen Species (ROS). Oxidative damages caused by free radicals to living cells causes many chronic diseases such as aging, cancers, cardiovascular, atherosclerosis, cataract, inflammatory, Parkinson's, Alzheimer's, and other degenerative ailments [4]. Medicinal plants contain many antioxidants in the form of vitamins, carotenoids, flavonoids, polyphenols, saponins, enzymes and minerals. These antioxidants possess antiviral, antiinflammatory, anticancer, antimutagenic, anti-tumour, and hepatoprotective properties. Phenols, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extract, the antioxidant activities of which play an important role in the absorption or neutralization of free radicals [5]. The presence of many phytochemicals such as Usharin, gigantin, calcium oxalate, α and β-calotropeol, beta-amyrin, fatty acids (both saturated and unsaturated), hydrocarbons, acetates and the benzoates, a mixture of tetracyclic triterpene compounds and giganteol whereas flavonoids, triterpenoids, alkaloids, steroids, saponins, terpenes, esters of calotropeols, volatile long chain fatty acids, glycosides and proteases have been isolated from different parts of *C. gigantea* especially in the leaves [6].

In India *Calotropis* occupies special importance because of its large industrial uses and economic values. It has various medicinal properties. Different parts of the plant have immense potential to cure various diseases and disorders like asthma, cold, epilepsy, fever, indigestion, leprosy, piles, skin diseases etc., and exhibiting activities that are antiinflammatory, anthelmintic, anticancer and antitumor; as observed in various polyherbal preparations. It is a highly potential plant resource and different parts of this plant are used for multi purposes. The various uses of this plant are biogas production, substitute for petroleum products, cleansing of water, energy plantation, fibers, fodder, latex or rubber, substitute for paper etc. So, in order to understand their pharmacological action, there is a need to scientifically evaluate them at molecular and biochemical level [7]. Therefore in order to understand the role of various phytochemicals present in *C. gigantea*, we carried out this study to identify the possible phytochemical compounds along with its functional groups present in the methanolic extract of *Calotropis gigantea* leaves. Our study is formulated to identify the possible phytochemical compounds along with its functional groups present in the methanolic extract of *Calotropis gigantea* leaves.

MATERIALS AND METHODS:

Preparation of the *Calotropis gigantea* methanolic extract:

10 grams of the dried *Calotropis gigantea* leaf material was powdered and placed in Soxhlet extractor along with 150 ml of methanolic l and refluxed at 60°C for 8hrs. The methanolic extracts were filtered through Whatmann No. 1 filter. The filtrate was evaporated to dryness at 80°C and stored until further analysis. For analysis the dried material was reconstituted in methanolic l in 1 ml methanolic l and µl of the reconstituted material was subjected for GCMS analysis as described below [8].

1. Phytochemical analysis:

A small amount of the methanolic l extract was used for the phytochemical analysis. The phytochemical tests include test for alkaloids, flavonoids, tannins

and phenols, saponins, steroids, terpenoids, carbohydrates, amino acids and proteins.

2. Gas Chromatography Mass Spectrometry (GCMS) Analysis:

The methanolic extract of the leaves of *Calotropis gigantea* was subjected to GC-MS analysis on a GC-MS Clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC- MS) instrument employing the following conditions: Restek Rtx^R – 5, (30 meter X 0.25 mm) (5% diphenyl / 95% dimethyl polysiloxane), running in electron impact mode at 70 eV; helium (99. 999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1.0 µl was employed(split ratio of 10:1); injector temperature 280 ^{0}C. The oven temperature was programmed from 40°C (isothermal for 5 min.), with an increase of 6 ^{0}C / min to 280 ^{0}C, then ending with a isothermal for 15min at 280°C. Mass spectra were taken at 70 eV; a 0.5 seconds of scan interval and fragments from 40 to 550 Da. Total GC running time was 60 minutes. Interpretation of mass spectrum GC-MS was done using the database of National Institute of Standard and technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library [8].

3. Estimation of Carbohydrates by Ortho-Toluidine Method

Glucose condenses with ortho-toludine in glacial acetic acid when heated to 100^{o}C. The product formed is N-Glycosylamine which was blue green in colour, the absorbance which is measured at 630 nm.

4. Estimation of Protein by Lowry's Method:

Protein content was determined by the method of Lowry et al., (1951). Protein reacts with Folin-Ciocalteau reagent to give a coloured complex. The colour so formed was due to the reaction of alkaline copper protein and the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour depends on the amount of these aromatic amino acids.

5. Estimation of Total Phenols:

Estimation of total phenols were performed by two methods one by F C Method using spectrophotometer and another by HPLC using gallic acid as standard

a. Estimation of Total Phenols by FC Method:

The colorimetric method is the most widely used method for the estimation of total phenolic content. The reagent used for this estimation is the Folin-Ciocalteu reagent, which is a mixture of phosphomolybdate and phosphotungstate. This method consists of calibrating using the standard phenolic compounds. FC reagent reacts with the nitrogen-containing compounds to form a blue coloured complex. The intensity of the color was read at 650 nm.

b. Estimation of total Polyphenols by High Performance Liquid Chromatography (HPLC):

10gms *Calotropis gigantea* powder was extracted with 50ml methanol at 50^{o}C for 4 hours. The methanolic extract of *Calotropis gigantea* were filtered through Whatmann No. 1 filter paper and filtrate was evaporated to dryness. Methanolic extract (10mg/ml) was used for HPLC analysis. The standard gallic acid (100µg/ml) and *Calotropis gigantea* methanolic extract (10mg/ml) were dissolved in mobile phase and 20µL was injected and the elution was monitored at 254 nm.

Concentration of Standard injected : 100µg/ml

Sample concentration : 10mg/ml

6. Estimation of antioxidant property by FRAP assay:

Ferric reducing antioxidant power (FRAP) is a widely used method to determine the antioxidant capacity of the samples. This method uses antioxidants as reductants in a redox-linked colorimetric reaction in which ferric (Fe^{3+}) is reduced to ferrous (Fe^{2+}). The reduction of ferric to ferrous at low pH leads to the formation of a colored ferrous-probe complex from a colorless ferric-probe complex.

7. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis:

Fourier Transform Infrared Spectrophotometer (FTIR) is the most important and powerful tool for identifying the functional groups present in the sample. The wavelength of light observed is the characteristic of the chemical bond. The chemical bonds in a molecule can be determined by interpreting the infrared absorption spectrum.

RESULTS AND DISCUSSION:

1. Phytochemical analysis:

Table – 1: Qualitative analysis for phytochemicals present in methanolic extract of *Calotropis gigantea* leaves

Phytochemicals	Test	Observations	Inference
Alkaloids	Mayer's Test Wagner's Test	Orange coloured precipitate Reddish-brown coloured precipitate	Presence of Alkaloids
Flavonoids	2 M HCl + Aqueous NaOH	Yellow colour is observed	Presence of Flavonoids
Tannins and Phenols	10% Lead Acetate + $FeCl_3$	Brick red colour is observed at top layer of the test tube White colour is observed at the bottom of the test tube	Presence of Tannins and Phenols
Saponins	Foam Test	Appearance of Foam	Presence of Saponins
Steroids	Acetic Anhydride	Violet to Blue or Green	Presence of Steroids
Terpenoids	Salkowski's Test	Absence of Reddish Brown colour	Absence of Terpenoids
Carbohydrate	Benedict's test	Red colour precipitate is observed	Presence of Carbohydrates
Amino Acids	Ninhydrin Test	Purle Colour	Presence of Amino Acids
Proteins	FC Reagent	Green Colour	Presence of Proteins

From the Table – 1 the phytochemical components present in the methanolic extract of *Calotropis gigantea* leaves are found to have alkaloids such as holarrhetine which shows anti-plasmodial activity against one of the most virulent malaria causing protozoa, *Plasmodium falciparum* [9].

Flavonoids referred as a plant secondary metabolite which usually presents in leaves and flower. These give pigmentation as yellow, red or bluish which helps to protect against microbes and insects. The common flavonoids present in the leaves of *Calotropis gigantea* are epicatechin, pterosupin and liquiritigerin has low toxicity and show antioxidant and antihyperglycemic activity [10].

Tannins have a high molecular weight that ranges from 500 KD to 3000 KD. These are generally large polyphenolic compounds having sufficient hydroxyls and carboxyls groups. In *Calotropis gigantea* leaves tannins and phenols are present as a long polymer bound with alkoids, gum, proteins, polysaccharide and other macromolecules. These are astringent, having a bitter taste. They will bind with proteins and get precipitated. Tannins are of two types (hydrolyzed or condensed). The tannins bind with minerals, proteins, homo and hetero polysaccharides [11].

Saponins are glycosides of steroids, steroid alkoids (steroids with nitrogen function) found in plant. Saponins are glycosides with a distinctive foaming characteristic. This provides waxy coating to the plant parts and helps in terms of protection. These are amphiphilic in nature and thus dissolve in water and form froth as soap gives with water. The common saponin present in *Calotropis gigantea* leaves are Sapogenin. Generally the Sapogenin are useful in controlling cholesterol. In general saponins are poisonous to humans and may cause skin rashes if swollen [12].

Steroids of plant origin are classified into different classes based on their chemical structure, pharmacological activities and source from which they have been isolated. The common steroids present in *Calotropis gigantea* leaves extract are taraxasterol isovalerate. These steroids possess many biological activities like anti-tumor, immunosuppressive, hepatoprotective, antibacterial,

plant growth hormone regulator, sex hormone, antihelminthic, cytotoxic and cardiotonic activity [13].

Terpenoids are also known as Isoprenoids which are derived from 5-carbon isoprene units differing in functional groups having aromatic properties. This property helps in antibacterial, antineoplastic and other medicinal values [11].

Carbohydrates such as mudarine, calactin, calotoxin, calotropagenin, proceroside, syriogenine, uscharidin, uscharin, uzarigenin, and voruscharin are present as cardioglycosides which acts either as stimulant to the heart or inhibits the functions of Na^+ / K^+ ATPase [14].

Amino Acids and Proteins such as trypsin, cysteine proteinase and aspartic proteinase enzymes are found to be present in the leaves. The laticifer fluid which are present in the leaves of *Calotropis*, have strong proteolytic activity which give resistant to phytopathogens, and insects [12].

2. Gas Chromatography Mass Spectrometry (GCMS) Analysis:

From the Figure – 1 and Table – 2 the GC-MS chromatogram of methanolic extract of leaves showed nearly 160 compounds. Most of the compounds which were reported from leaves were found to be rich in Phenol, Biphenyl, Cyclohexane, 4-Methyl-2-phenylindole, D-Mannose-1-phosphate sodium salt, Butane-2,2-dimethyl, DL – Proline, 5 – oxo, methyl ester, 2 – Pyrrolidinecarboxyamide, 5 – oxo, 2 – Pyrrolidinone, 5 – (cyclohexylmethyl), n - Hexadecanoic acid, Azulene, 9, 12, 15 octadecatrienoic acid (z, z, z), Methyl 8, 11, 14 – heptadecatrienoate, Di isooctyl phthalate, Bis (2 – ethylehexyl) phthalate at retention time 3.44, 3.46, 4.34, 6.66, 6.66, 8.436, 8.436, 8.436, 10.655, 11.56, 12.07, 12.07, 15.24, 15.24 with peak area 1.28, 1.28, 1.26, 1.20, 1.20, 2.21, 2.21, 2.21, 11.74, 1.56, 8.14, 8.14, 40.78 and 40.78 respectively. As per literature chloroform was reported as a better solvent system for compound extraction from *C. gigantea*. Our present study shows methanolic l can also be used as a compound for extraction from *C. gigantea* leaves. Methanol solvent could be used along with chloroform as solvent system for various bioactive compound extractions [15].

Biphenyl compounds having the retention time 3.44 and measuring the peak area of 1.28 are used in dye carriers, food preservatives, as fungicide [16]. Cyclohexane having the retention time 3.46 and measuring the peak area of 1.28 are used as solvent and paint remover [17]. 4-Methyl-2-phenylindole having the retention time 4.346 and measuring the peak area of 1.26 is used as chromogenic agent for the determination of estimation of malondialdehyde (MDA) production [18]. D-Mannose-1-phosphate sodium salt having the retention time 6.66 and measuring the peak are of 1.20 are used in a study to assess in vivo targeting of alveolar macrophages and has also been used in a study to investigate genetic engineering of the phosphor carrier protein NPr. [19]. Butane-2,2-dimethyl having the retention time 6.66 and measuring the peak are of 1.20 may be employed as probe to investigate the nature of possible active sites in supported metal catalysts [20]. DL – Proline, 5 – oxo, methyl ester having the retention time 8.43 and measuring the peak are of 2.21 are used as an agonist of glycine receptors, a catalyst for aldol condensation, an essential component of collagen, significant role in the immune system and strengthening of heart muscles and also as an osmoprotectant and therefore is used in many pharmaceutical, biotechnological applications [21]. 2 – Pyrrolidine carboxyamide, 5 – oxo compounds having the retention time 8.43 and measuring the peak area of 2.21 are used for chemotherapy [22]. 2 – Pyrrolidinone, 5 – (cyclohexylmethyl) having the retention time 8.43 and measuring the peak area of 2.21 are used in the electronics industry as a photoresist stripper (usually in combination with other solvents like N-methyl-2-pyrrolidone), and as a chemical polisher of copper in circuit board fabrication. It is also used in the textiles industry as a dye carrier in aramid fabrics [23]. n - Hexadecanoic acid or hexadecanoic acid in IUPAC nomenclature, having the retention time 10.65 and measuring the peak area of 11.74 are used to produce soaps, cosmetics, and industrial mold release agents [24]. Azulene having the retention time 11.56 and measuring the peak area of 1.56 are used in treatment of ulcers, gastritis, athlete's foot, and vein problems [25]. 9, 12, 15 octadecatrienoic acid (z, z, z), having the retention time 12.07 and measuring the peak area of 8.14 are present in cooking oils as linolenic acids. It acts as an antioxidant, anti-inflammatory, acne reductive, skin-lightening and moisture retentive properties when applied topically on the skin [26]. Methyl 8, 11, 14 – heptadecatrienoate, having the retention time 12.07 and measuring the peak area of 8.14 are used as a synthetic insecticide [27]. Di isooctyl phthallate or Bis (2 – ethylehexyl) phthallate having the retention time 15.24 and measuring the peak area of 40.78 are used as plasticizer in medical devices such as intravenous tubing and bags, IV catheters, nasogastric tubes, dialysis bags and tubing,

blood bags and transfusion tubing etc. [28].

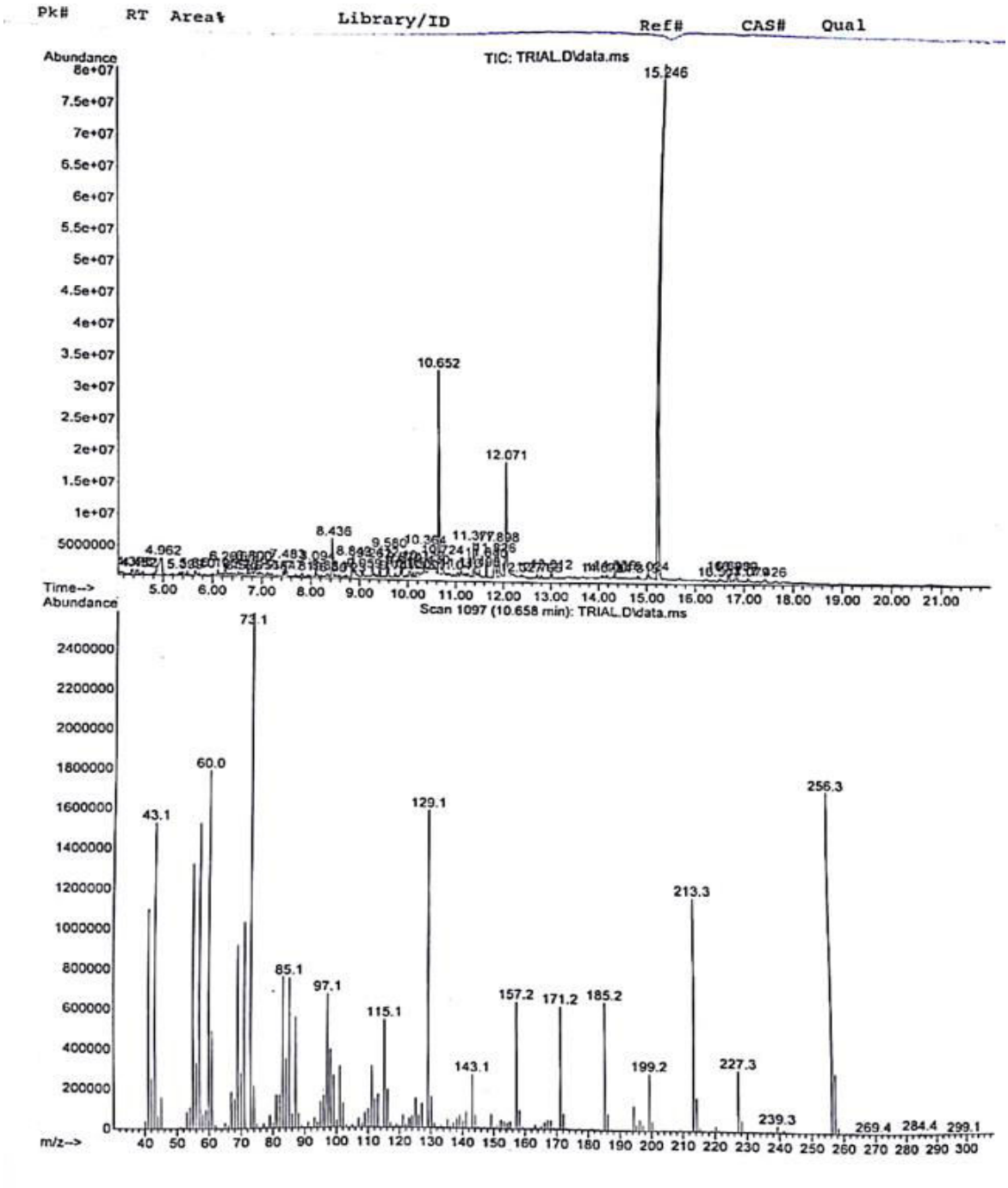

Figure – 1: GC- MS chromatogram of *Calotropis gigantea*

Table – 2: GC- MS chromatogram of *Calotropis gigantea*

Retention Time	Name of the Compounds	Peak area	Activity
3.44	Biphenyl	1.28	As dye carriers, food preservatives, fungicide.
3.46	Cyclohexane	1.28	As solvent and paint remover
4.34	4-Methyl-2-phenylindole	1.26	As chromogenic agent for the determination of estimation of malondialdehyde (MDA) production.
6.66	D-Mannose-1-phosphate sodium salt	1.20	To assess *in vivo* targeting of alveolar macrophages and also used in a study to investigate genetic engineering of the phosphor carrier protein NPr.
6.66	Butane-2,2-dimethyl	1.20	As probe to investigate the nature of possible active sites in metal catalysts
8.43	DL – Proline, 5 – oxo, methyl ester	2.21	As an osmoprotectant , agonist of glycine receptors, catalyst for aldol condensation, essential component of collagen, significant role in the immune system and strengthening of heart muscles.
8.43	2 – Pyrrolidine carboxyamide, 5 – oxo	2.21	Used for chemotherapy
8.43	2 – Pyrrolidinone, 5 – (cyclohexylmethyl)	2.21	It is used in the electronics industry as a photoresist stripper and as a chemical polisher of copper in circuit board fabrication. It is also used in the textiles industry as a dye carrier in aramid fabrics
10.65	n - Hexadecanoic acid	11.74	To produce soaps, cosmetics, and industrial mold release agents.
11.56	Azulene	1.56	In treatment of ulcers, gastritis, athlete's foot, and vein problems.
12.07	9, 12, 15 octadecatrienoic acid (z, z, z)	8.14	Present in cooking oils as linolenic acids. Acts as antioxidant, anti-inflammatory, acne reductive, skin-lightening and moisture retentive properties on the skin.
12.07	Methyl 8, 11, 14 – heptadecatrienoate	8.14	As a synthetic insecticide.
15.24	Di isooctyl phthallate Bis (2 – ethylehexyl) phthallate	40.78	As plasticizer in medical devices such as intravenous tubing and bags, IV catheters etc.

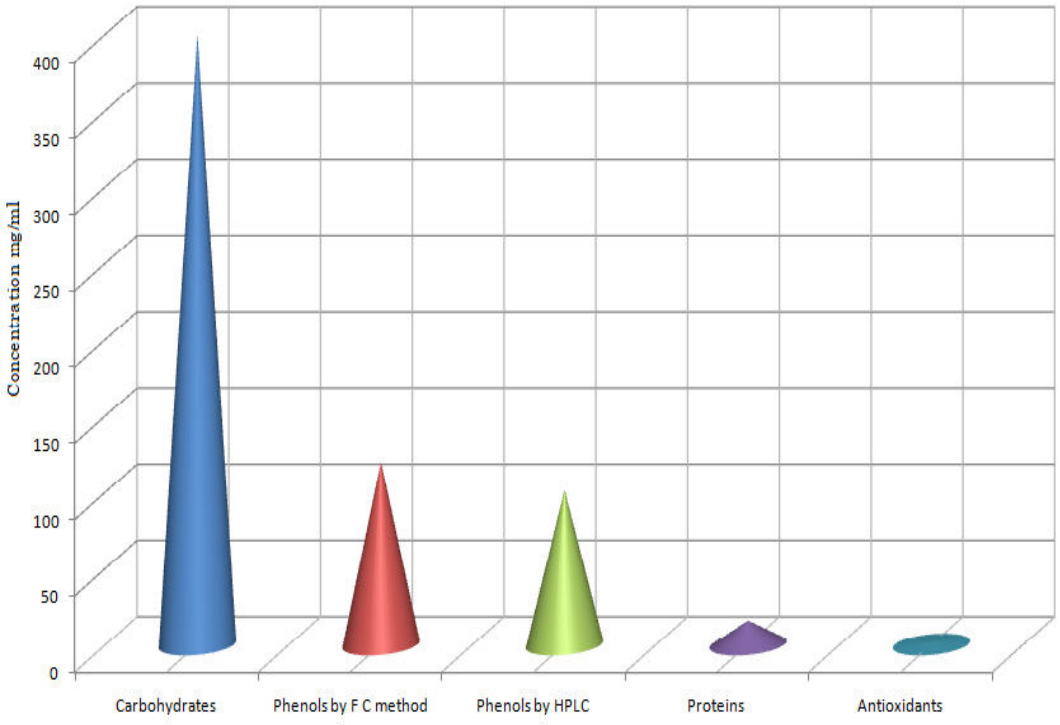

Figure – 2: Comparison of conc. of Carbohydrates, proteins, phenols and antioxidants present in methanolic l extract of *Calotropis gigantea* leaves

3. Estimation of Carbohydrates by Ortho-Toluidine Method:

From the Figure – 2, the total amount of carbohydrates present in the methanolic extract of *Calotropis gigantea* leaves was found to be 400 mg/ml. The Carbohydrates present in the *Calotropis gigantea* leaves as cardioglycosides. It includes mudarine, calactin, calotoxin, calotropagenin, proceroside, syriogenine, uscharidin, uscharin, uzarigenin, and voruscharin which acts either as stimulant to the heart or inhibits the functions of Na^+ / K^+ ATPase [29].

4. Estimation of Protein by Lowry's Method:

From the Figure – 2, the amino Acids and Proteins such as trypsin, cysteine proteinase and aspartic proteinase enzymes are found to be present in the leaves. The laticifer fluid which are present in the leaves of *Calotropis*, have strong proteolytic activity which give resistant to phytopathogens, and insects [30].

The total amount of proteins present in the methanolic extract of *Calotropis gigantea* leaves was found to be 16 mg / ml. The milky latex from the leaves has been used in traditional medicine to cure skin infections, poison, ulcer, enlargement of spleen, liver, abdominal glands, colic piles, worms and different inflammatory diseases. Latex consists of protein like laticifer and osmotin has potent anti-fungal, antimycoplasmal, anti-inflammatory, insecticidal, larvicidal, antioxidant and radical scavenging activities, and anticancer properties. These protein exhibits mild toxic effects on heart, liver and kidneys; that included multi-focal coagulation necrosis of cardiac fibers and vacuolized hepatocytes [31].

5. Estimation of total phenols:

a. By F C Method using spectrophotometer:

The total amount of phenols present in the methanolic extract of *Calotropis gigantea* leaves was found to be 120 mg /ml.

b. Estimation of total phenols by HPLC using gallic acid as standard:

The chromatogram of gallic acid standard and Poly Phenol Overlay present in *Calotropis gigantea* extract are shown in the Figure – 3 and Figure – 4. Using the area and height compared with standard the Polyphenol concentration present in *Calotropis gigantea* leaves extract can be calculated. The amount of Polyphenols present in *Calotropis gigantea* extract is 101.39 mg/ml.

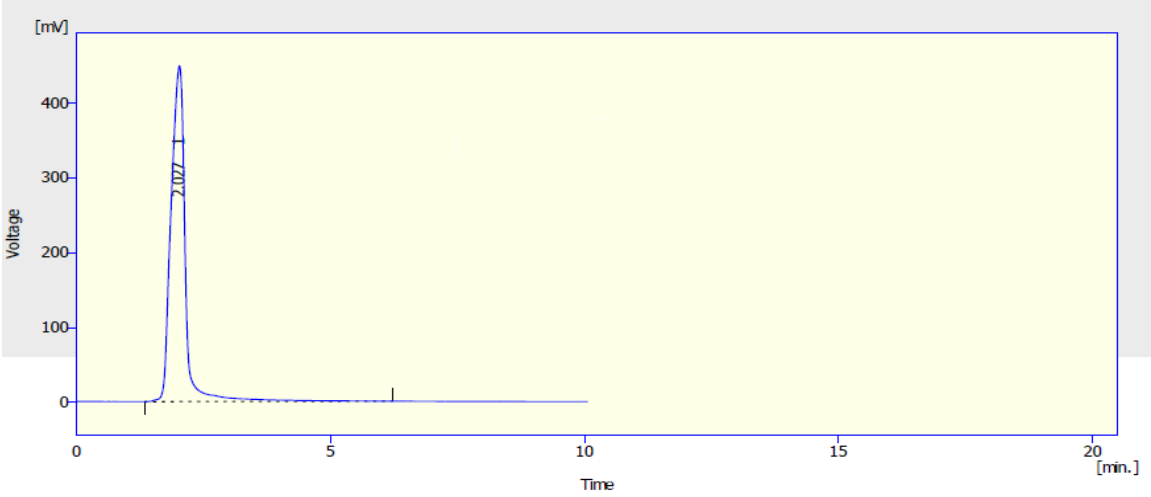

Figure – 3: HPLC chromatogram of Standard Gallic Acid

Table – 3: HPLC chromatogram of Standard Gallic Acid

	Retention Time (Min)	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.027	8663.530	448.434	100.0	100.0	0.29
Total		8663.530	448.434	100.0	100.0	0.29

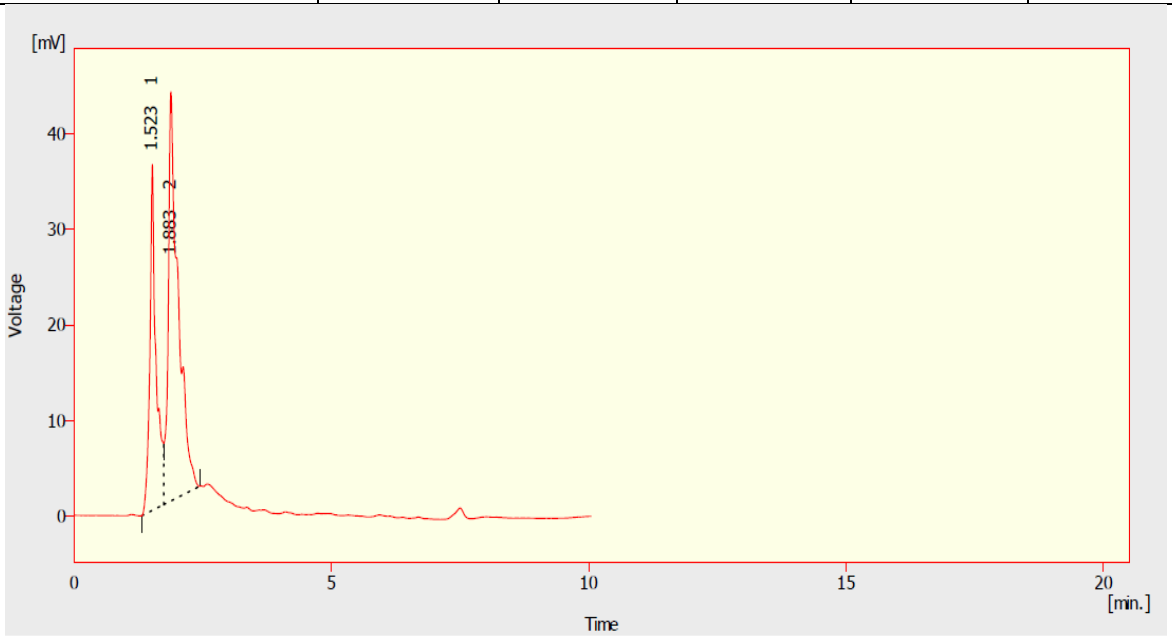

Figure – 4: HPLC chromatogram of Poly Phenol Overlay present in *Calotropis gigantea* extract.

Table – 4: HPLC chromatogram of Poly Phenol Overlay present in *Calotropis gigantea* extract

	Retention Time (Min)	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.523	302.719	36.183	34.5	45.8	0.10
2	1.883	575.682	42.786	65.5	54.2	0.20
Total		878.401	78.968	100.0	100.0	

From the Figure – 2, 3, 4, and Table – 3, 4 the total phenolic content determined by the Folin-Ciocalteau reagent will not exhibit the accurate quantity of the phenolic composition in the extract and hence HPLC determinations of polyphenols were performed [32]. The amount of polyphenols present in *Calotropis gigantea* extract is 101.39 mg/ml. there are about 17 different compounds contributes total polyphenol composition. They are Ellagic acid, Tannic acid, Gallic acid, Chlorogenic acid, Coumaric acid, Myrecitin, Ferulic acid, Quercetin, Coumarin, Cinnamic acid, Kaempferol, Benzoic acid, Catechin, Luteolin, Rutin, Acacetin and Caffeic acid. The major polyphenolic compounds present in the leaves extract are Ellagic acid which has good antioxidant and antimicrobial properties.

6. Estimation of antioxidant property by FRAP assay:

From the Figure – 2, FRAP assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Electron donating antioxidants can be described as redox reaction. Total antioxidant power may be referred analogously to total reducing power. The total amount of antioxidants present in the methanolic extract of *Calotropis gigantea* leaves was found to be 2 mg / ml.The radical scavenging and inhibition of lipid peroxidation by the extract was due to the quenching free radicals or reduction of Fe^{3+} to Fe^{2+}, which can be attributed to the presence of number of polyphenolics such as flavonoids, anthocyanins etc. [33]. The polyphenols present in *Calotropis gigantea* extract are about 17 different compounds contributes total polyphenol composition. They are Ellagic acid, Tannic acid, Gallic acid, Chlorogenic acid, Coumaric acid, Myrecitin, Ferulic acid, Quercetin, Coumarin, Cinnamic acid, Kaempferol, Benzoic acid, Catechin, Luteolin, Rutin, Acacetin and Caffeic acid. The major polyphenolic compounds present in the leaves extract are Ellagic acid which has good antioxidant properties [34].

7. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis:

FT-IR spectroscopy is used to identify some qualitative aspects regarding the organic compounds in a plant *Calotropis gigantea* Linn. Several indicator bands that are pertained to functional groups represent chemical components or metabolic products. The FT-IR spectrum exhibits the characteristic finger print band features. The infrared spectrum is able to identify not only the major components in organic materials, but also to find some differences among them. From the Figure – 5 and Table – 5, the very strong absorption bands at 3416.46 cm^{-1} which is representative for N-H stretching vibrations, characteristic of the presence of amino acids. The bands at 2927.37 cm^{-1} is due to the stretching vibration of $–CH_3$ and $–CH_2$ groups indicative of the chlorophyll groups [35]. The 1642.08 cm^{-1} band is due to stretching vibration of carbonyl group characteristic of the secondary amides and other compounds containing C=0 group [36]. The strong bands at 1413.64 cm^{-1} represent the bending vibrations of CH indicative of the lignins. The 1245.54 cm^{-1} band in all samples predict the presence of ester carbonyl. The C-O-C groups exhibit strong bands at 1061.65cm^{-1}. The band at 553–633 cm^{-1} represents C-O-O and P-O-C bending of aromatic compounds (phosphates) [12].

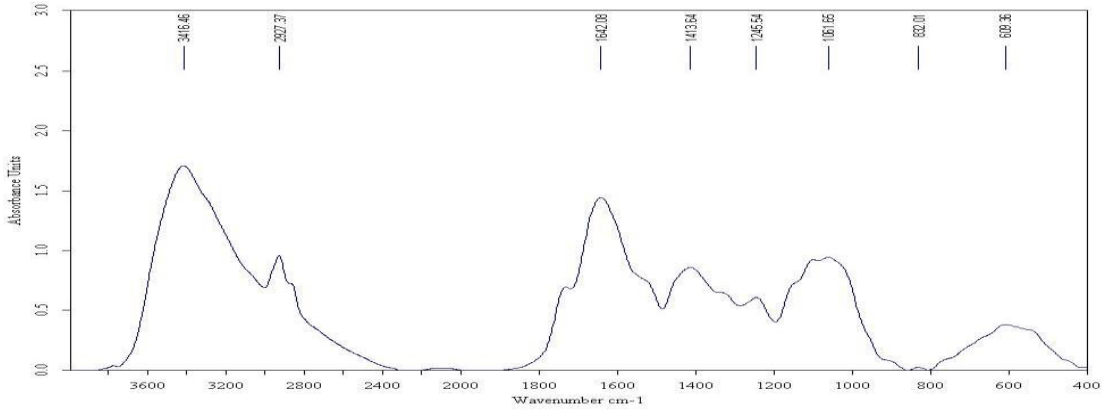

Figure – 5: Infrared spectroscopy spectrum for *Calotropis gigantea* methanolic l extract

Table – 5: Infrared spectroscopy table for *Calotropis gigantea* methanolic l extract

S.No	Frequency	Group	Intensity
1	3416.46	Amines(N-H)	Medium
2	2927.37	Alkyl(C-H [stretching])	Medium-Small
3	1642.08	Ketones (C=O [stretching])	Broad, small
4	1413.64	Aromatic(C=C[stretching])	Medium, variable
5	1245.54	Ethers and alcohols(C-O-C[stretching])	Small
6	1061.65	Primary alcohol (C-O [stretching])	Medium
7	609.36	Alkyl halide (C-Cl)	Small, Variable

CONCLUSION:

The phytochemicals present in the methanolic extract of *Calotropis gigantea* leaves contains Alkaloids, Flavonoids, Tannins / Phenols, Saponins, Steroids, Terpenoids, Carbohydrates, Amino Acids and Proteins. Overall these phytochemicals possess Anti-molluscicidal activity, Anti-implantation activity, Anti-fungal activity, Anti-inflammatory activity, Insecticidal activity and Hypotensive. The GC-MS chromatogram of methanolic extract of leaves showed nearly 160 compounds. Most of the compounds which were reported from leaves were found to be rich in Phenol, Biphenyl, Cyclohexane, 4-Methyl-2-phenylindole, D-Mannose-1-phosphate sodium salt, Butane-2,2-dimethyl, DL – Proline, 5 – oxo, methyl ester, 2 – Pyrrolidinecarboxyamide, 5 – oxo, 2 – Pyrrolidinone, 5 – (cyclohexylmethyl), n - Hexadecanoic acid, Azulene, 9, 12, 15 octadecatrienoic acid (z, z, z), Methyl 8, 11, 14 – heptadecatrienoate, Di isooctyl phthalate, Bis (2 – ethylehexyl) phthalate at retention time 3.44, 3.46, 4.34, 6.66, 6.66, 8.436, 8.436, 8.436, 10.655, 11.56, 12.07, 12.07, 15.24, 15.24 with peak area 1.28, 1.28, 1.26, 1.20, 1.20, 2.21, 2.21, 2.21, 11.74, 1.56, 8.14, 8.14, 40.78 and 40.78 respectively. Our present study shows methanol can also be used as a compound for extraction from *C. gigantea* leaves for various bioactive compound extractions. The bioactive compounds identified in our study have various medicinal importances. The total amount of carbohydrates present in the methanolic extract of *Calotropis gigantea* leaves was found to be 400 mg/ml mostly in the form of cardioglycosides. The total amount of proteins present in the methanolic extract of *Calotropis gigantea* leaves was found to be 16 mg / ml due to the presence of trypsin, cysteine proteinase and aspartic proteinase enzymes in the laticifer fluid which are present in the leaves of *Calotropis.* The amount of Polyphenols present in *Calotropis gigantea* leaves extract is 101.39 mg/ml in which Ellagic acid contributes 25%. Ellagic acid is a natural antioxidant as well as antimicrobial agents and hence *Calotropis gigantea* leaves are having high medicinal values. The total amount of antioxidants present in the methanolic extract of *Calotropis gigantea* leaves was found to be 2 mg / ml which may be due to the presence of number of polyphenolics such as flavonoids, anthocyanins etc. As per the data analysis of FTIR from using infrared spectroscopy correlation table for *Calotropis gigantea* methanolic extract, it was found that amines groups at the frequency of 3416.46 and ketones at frequency of 1642.08 gave maximum peaks. Hence, we can conclude that the caloptropis methanolic extract is rich in amines and C = O groups.

Suggestion for further research:

The present methanobotanical study on *Calotropis gigantea* leaves proved *C. gigantea* leaves confirm the existence of various biological active molecules with its possible functional groups. Moreover, these leaves are already in use for a wide range of treatments traditionally such as fever, indigestion, cough, cold, asthma, nausea, vomiting, diarrhea etc. The present study may be an initiative for further phytochemical and pharmacological investigations required to separate the novel active compounds from the leaves to formulate new drug in order to treat incurable diseases.

REFERENCES:

1. Vaidya A; Pharm. Res. India (Pharma pulse-supplement), 1998, 44–45.
2. The Wealth of India; Raw Materials. Council of Scientific and Industrial Research, New Delhi. 1959, 2;20–
3. Endress M E and Bruyns P V; A revised classification of the Apocynaceae s.l. The Botanical Review. 2000, 66; 1:1–56.
4. Chaudiere J and Ferrari RI; Intracellular antioxidants: From chemical to biochemical

mechanisms. Food and Chemical Toxicology, 1999, 37:949–962.
5. Prabha M R and Vasantha K; Antioxidant, Cytotoxicity and Polyphenolic Content of *Calotropis procera* (Ait.) R. Br. Flowers. Journal of Applied Pharmaceutical Science. 2011, 1; 7:136–140.
6. Kumar P S, Suresh E and Kalavathy S; Review on a potential herb *Calotropis gigantea (L.)* R. Br. Scholars Academic Journal of Pharmacy. 2013, 2; 2:135–143.
7. Tenpe C R, Upaganlawar AB, Dongre PA and Yeole PG; Screening of methanolic extract of *Calotropis gigantea* leaves for hepatoprotective activity, Indian drugs. 2007, 44; 11:874–875.
8. Balasubramanian S, Ganesh D, Kiran K S, Prakash K J M, Surya Narayana VVS; "GC-MS Analysis of Phytocomponents in the Methanolic Extract of Mentha arvensis (Corn Mint)". International Journal of Chemistry and Pharmaceutical Sciences. 2014; Vol. 2 (6):878–881.
9. Misra M K, Mohanty MK, Das PK; Studies on the method – ethnobotany of *Calotropis gigantea* and *C. procera*. *Ancient science of life.* 1993; XIII (1&2):40–56.
10. Ali G, and Neda G; Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research. 2011, 5(31):6697–6703.
11. Sharma N, Shankar R, Gupta N, Prakash P; A preliminary phyto-pharmacognostical evaluation of *Calotropis gigantea* (L.) R. Br. (Alarka or Mandara) Root. International Journal of Ayurvedic Medicine. 2016; 7(1):44–48.
12. Suresh A, Yasuhiro. T, Seikichi. S, Kazuhiko. T, Shigetoshi. K; *Tetrahedron Letters.* 2002; 43,1473.
13. Francisco A M, Nuria C, Rosa M.V, Jose M.G; Bioactive steroids from Oryza sativa L. Steroids. 2006; 71:603–608.
14. Begum N, Sharma B, Pandey RS; *Calotropis procera* and *Annona squamosa*: Potential Alternatives to Chemical Pesticides. British J Appl Sci Technol. 2013; 3(2):254–67.
15. Singh M, Javed K; Comparative study of chemical composition of *Calotropis gigantea* flower, leaf and fruit essential oil. European Chemical Bulletin. 2015; 4(10):577–480.
16. Adams N G, Richardson D M; "Isolation and Identification of Biphenyls from West Edmond Crude Oil". Analytical Chemistry. 1953; Vol 25 (7), 1073–1074.
17. Warnhoff E W; "The Curiously Intertwined Histories of Benzene and Cyclohexane". J. Chem. Educ. 1996; 73(6):494.
18. Yasir Hasan Siddique, Gulshan Ara, Mohammad Afzal; "Estimation of lipid peroxidation induced by hydrogen peroxide in cultured human lymphocytes", Dose-response: a publication of International Hormesis Society. 2012; 10(1):3–17.
19. Jaeken J and Carchon H; The carbohydrate-deficient glycoprotein syndromes: an overview, J. Inher. Metab. Dis. 1993; 16:813–820.
20. Burch R and Paal Z; The use of 2,2-dimethylbutane (neohexane) as a probe molecule of metal catalysts. Applied Catalysis A: General. 1994; 114(1):9–33.
21. Verbruggen N, Hermans C; "Proline accumulation in plants: a review". AminoAcids. 2008; 35 (4):753–759.
22. Najera C, Yus, M; Pyroglutamic acid: A versatile block in asymmetric synthesis. Tetrahedron: Asymmetry. 1999; 10:2245–2303.
23. Albrecht Ludwig Harreus, Backes R, Eichler J O, Feuerhake R, Jäkel C, Mahn U, Pinkos R, Vogelsang R; "2-Pyrrolidone" in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH. 2011; Weinheim.
24. Kingsbury K J, Paul S, Crossley A, Morgan D M; "The fatty acid composition of human depot fat". Biochemical Journal. 1961; 78:541–550.
25. Yamamura, Kimiaki, Kawabata, Shizuka, Kimura, Takatomo, Eda, Kazuo, Hashimoto, Masao; "Novel Synthesis of Benzalacetone Analogues of Naphth[a]azulenes by Intramolecular Tropylium Ion-Mediated Furan Ring-Opening Reaction and X-ray Investigation of a Naphth[1,2-a]azulene Derivative". The Journal of Organic Chemistry. 2005; 70 (22):8902.
26. Darmstadt G L, Mao-Qiang M, Chi E, Saha SK, Ziboh VA, Black RE, Santosham M, Elias PM; "Impact of tropical oils on the skin barrier: possible implications for neonatal health in developing countries". Acta Paediatrica. 2002; 91(5):546–554.
27. Amer A, Mehlhorn H; Larvicidal effects of various essential oils against Aedes, Anopheles, and Culex larvae (Diptera, Culicidae). 2006; 99(4):466–472.
28. Sampson J, de Korte D; "DEHP-plasticised PVC: relevance to blood services". Transfusion Medicine. 2011; 21 (2):73–83.
29. Begum N, Sharma B, Pandey RS; *Calotropis procera* and *Annona squamosa*: Potential Alternatives to Chemical Pesticides. British J Appl Sci Technol. 2013; 3(2):254 – 267.

30. Suresh Kumar P, Suresh E, Kalavathy S; Review on a potential herb *Calotropis gigantea* (L.) R. Br. Scholars Acad J Pharm. 2013; 2 (2):135–143.
31. Mossa J S, Tariq M, Mohsin A, Ageel A M, Al-Yahya MA; Pharmacological studies on aerial parts of *Calotropis procera*. Am J Chin Med. 1991; 19:223–231.
32. WuX, BeecherGR, Holden JM, Haytowitz DB, Gebhardt SE, Prior R L; Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J. of the Agricultural and Food Chemistry. 2004; 52:4026–4037.
33. Alluri, Tayl V K S, Dodda V N R, Mulabagal. S, Hsin sheng V, Gottumukkala T; Assessment of bioactivity of Indian plants using brain shrimp (*Artemia salina*) lethality assay. *Intl.J.Applied sci.Engineering*. 2005; 3(2):125–134.
34. Mohamed A A, Ali S I, Sameeh MY, Abd El-Razik T M; Effect of solvents extraction on HPLC profile of phenolic compounds, antioxidant and anticoagulant properties of Origanum vulgare, Research J.Pharm. and Tech. 2016; 9(11):2009–2016.
35. Bellamy L J; *Infrared spectra of complex molecules*, Chapman Hall, London, 1975.
36. Mueen ahmed K K, Rana A C, Dixit V K; *Calotropis* species (Asclepediaceae). A comprehensive review, *Pharmacognosy Magazine*. 2005; 1(2):48– 52.