Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(11); 1823-1827

ISSN: 0975-4873

Research Article

Comparative GC-MS Analysis of Bioactive Compounds in Methanolic Extract of *Calotropis gigantea* (L) W.T. Aiton Leaf and Latex

Shalini Sharma, Amita Kumari*, Mamta Sharma

School of Biological and Environmental Sciences, Shoolini University, Solan, 173212, India.

Available Online: 15th November, 2016

ABSTRACT

Calotropis gigantea (family Asclepiadaceae) commonly known as 'Sweta Arka' is a highly medicinal drought resistant and relatively high degree salt tolerant wild plant species of the Indian Himalayan region. Plant contain milky latex in the stem which is used as an antidote for snake poison in dried form. Whereas, dried leaves of the *Calotropis* plant are used as an expectorant and anti- inflammatory for the cure of paralysis and rheumatic pains. The plant has been reported to be effective in more than hundred human diseases. Various bioactive compounds have been extracted from *C. gigantea* leaf, flowers and stem. Present investigation deals with the determination of bioactive constituents from the leaf and latex of *C. gigantea* using GC-MS and their comparative analysis. The GC-MS analysis revealed the presence of total 46 bioactive compounds (24 from leaves and 22 from latex) with valuable activity. Most of the compounds were found to be similar in both leaf and latex, but small variation was also observed in their chemical profile. The chemical compounds observed in only latex were 1-[(T-butyl) dimethyl silyl thin] butane, 1-Hexadecyne, Hexadecane, L-Glutamic acid, Phenol-3-isoproxy-5-methyl, Trocosane and Z-1,6-Tridecadiene. Compounds identified only from the leaves were Azulene, Benalaxyl, Cis-vaccenic acid, Levomenol, Profenofos, β- Tocopherol and β-Sitosterol, whereas the rest of the compounds were similar in both leaf and latex.

Keywords: *Calotropis gigantea*, bioactive compounds, GC-MS, leaf, latex.

INTRODUCTION

Medicinal plants are the gifts of the nature which are widely used to cure a number of diseases. Medicinal properties of these plants are due to the presence of secondary metabolites which are implicated in most plants therapeutic activities[1]. *Calotropis gigantea* belongs to family Asclepiadaceae is one of the drought resistant and salt tolerant medicinal plant[2] which is used in different ways to treat the infectious diseases[3]. Xerophytic adaptation of this plant is due to the presence of latex as well as extensively branched root system and thick leaves with waxy coverage. Hence, this plant is distributed in the tropical and subtropical area of the world and throughout the India[2]. The plant has many medicinal properties as it is used as an antifungal[4] and analgesic agent[5]. The dried leaves of the plant used as an expectorant, anti-inflammatory[6], for the cure of paralysis and rheumatic pains[7], etc. The dried form of latex and roots of this plant may also be used as an antidote for snake poisoning[8]. It is also used as an abortifacient for the cure of piles[9]. The powder form of root and bark used against diarrhea and asthma[10]. Instead of medicinal values, plant also provides a strong fiber from the bark; useful floss from the seeds. Some reports indicated that *C. gigantea* affects germination and seedling vigour of agricultural crops. All parts of *C. gigantea* are toxic due to the presence of several cardiac glycosides (Cardenolides). The medicinal properties in *C. gigantea* is due to the presence of various phytochemical constituents reported from various parts, e.g., plants contain the Cardenolides, root bark contains Benzoylinesolone and Benzoylisolinelone, leaves and stalk of the plant contain Calotropin, and Calotropagenin while the flower and latex contains Uzarigenin, and Terpenol ester[11]. The plant also contains Triterpenoids, Calotropursenyl acetate, Calotropoleanyl Calotropternyl ester, Oleanene triterpenes like ester[12], Cardiac glycosides Calotropogenin, Calotropin, Calotoxin and Calactin[13], etc. *Calotropis* plant has also been investigated for the presence of Cardenolides[14] and Anthocyanins[15]. The present study is based on comparative chemical profiling of *C. gigantea* leaves and latex identified by Gas chromatography and Mass spectrometry (GC-MS). This study indicated the presence of about 22 compounds from latex and 24 compounds from leaves of *C. gigantea.*

MATERIAL AND METHODS

Site selection and sample collection

As the plant has been reported from the lower Himalayan regions which comprise mostly Una, Kangra, Chamba, Solan, Sirmour, Bilaspur, Hamirpur, etc. Therefore, plant sample, i.e., leaves and latex for the present study was collected from district Sirmour (Nahan) of Himachal Pradesh in polyethylene bags and glass bottles containing methanol, respectively. The sampling site is located on 30.5599°N / 77.2935°E, northern India. The average elevation is 932 m above sea level.

*Author for Correspondence: amitabot@gmail.com

Table 1: GC-MS analysis of methanolic extracts of *Calotropis gigantea* leaves.

S. No.	Compound name	Retention time	Peak area (%)	Molecular formula	Molecular weight
1.	1-Octanol-3,7-dimethyl	22.78	5.78	$C_{10}H_{22}O$	158.28
2.	2- Methoxy 4-vinyl phenol ethanone	53.21	1.87	$C_9H_{10}O_2$	149.0
3.	4-Methyl-2-phenylindole	4.34	5.26	$C_{15}H_{13}N$	207.0
4.	5-Nonadecen-1-ol	33.40	7.62	$C_{19}H_{38}$	266.9
5.	9,12,15-Octadecatricenoic acid, methyl ester	52.82	2.78	$C_{19}H_{32}O_2$	293.2
6.	Azulene	11.56	3.16	$C_{10}H_8$	125.4
7.	Benalaxyl	22.78	5.78	$C_{20}H_{23}NO_3$	326.9
8.	Beryllium sulfate tetrahydrate	50.19	1.81	BeH_8O_8S	178.1
9.	Biphenyl	3.44	43.28	$C_{12}H_{10}$	154.7
10.	Butane-2,2-dimethyl	6.66	6.00	C_6H_{14}	87.0
11.	Campesterol	49.87	2.65	$C_{28}H_{48}O$	401.0
12.	Cholest-5-en-3 ol, 24, Propylidene(3.beta.)	41.79	3.56	$C_{29}H_{48}O_2$	429.0
13.	Cis-vaccenic acid	22.78	5.78	$C_{18}H_{34}O_2$	281.1
14.	Cyclohexane	3.46	43.28	C_6H_{12}	84.1
15.	Decane	46.98	2.17	$C_{10}H_{22}$	147.0
16.	D-Mannose-1-phosphate sodium salt	6.66	6.00	$C_6H_{13}O_9P$	282.0
17.	Eicosane	46.98	2.17	$C_{20}H_{42}$	281.0
18.	Ethion	53.21	1.87	$C_9H_{22}O_4P_2S_4$	385.0
19.	Guanidine nitriate	51.73	2.43	$CH_6N_4O_3$	121.0
20.	Levomenol	49.87	2.65	$C_{15}H_{26}O$	222.8
21.	Pentacosane	33.40	7.62	$C_{25}H_{52}$	355.1
22.	Profenofos	22.78	5.78	$C_{11}H_{15}BrClO_3PS$	372.2
23.	B-Tocopherol	22.78	5.78	$C_{28}H_{48}O_2$	417.8
24.	β-Sitosterol	46.98	2.17	$C_{29}H_{50}O$	415.1

Table 2: GC-MS analysis of methanolic extracts of *Calotropis gigantea* latex

S. No.	Compound name	Retention time	Peak area (%)	Molecular formula	Molecular weight
1.	D-Mannose-1-phosphate sodium salt	46.98	2.74	$C_6H_{13}O_9P$	282.0
2.	1-[(T-butyl) dimethyl silyl thin] butane	52.82	4.84	$C_7H_{15}F_3O_3SSi$	205.0
3.	1-Hexadecyne	51.92	3.32	$C_{16}H_{30}$	221.0
4.	2- Methoxy 4-vinyl phenol ethanone	6.71	5.86	$C_9H_{10}O_2$	149.0
5.	5-Nonadecen-1-ol	33.40	4.64	$C_{19}H_{38}$	266.9
6.	9,12,15-Octadecatricenoic acid, methyl ester	52.82	4.84	$C_{19}H_{32}O_2$	293.2
7.	Butane-2,2-dimethyl	6.71	5.86	C_6H_{14}	86.24
8.	Campesterol	49.87	3.50	$C_{28}H_{48}O$	401.0
9.	Cholest-5-en-3-ol,24,Propylidene(3.beta.)	41.79	3.69	$C_{29}H_{48}O_2$	429.0
10.	Cyclohexane	3.45	51.52	C_6H_{12}	84.0
11.	Decane	41.79	3.69	$C_{10}H_{22}$	147.0
12.	D-Mannose	49.16	1.87	$C_6H_{12}O_6$	180.1
13.	Eicosane	51.92	3.32	$C_{20}H_{42}$	281.0
14.	Guanidine nitrate	45.09	2.49	$CH_6N_4O_3$	121.0
15.	Hexadecane	52.82	4.84	$C_{16}H_{34}$	227.2
16.	L-Glutamic acid	49.87	3.50	$C_5H_9NO_4$	147.0
17.	Oxadiazon	41.79	3.69	$C_{15}H_{18}$	341.0
18.	Pentacosane	33.40	4.64	$C_{25}H_{52}$	355.1
19.	Phenol,2,5-bis (1,1-dimethylethyl)	49.16	1.87	$C_{14}H_{22}O$	204
20.	Phenol,3-isopropoxy-5-methyl	4.35	3.17	$C_{10}H_{14}O_2$	166.9
21.	Tricosane	46.98	2.74	$C_{23}H_{48}$	326.9
22.	Z-1,6-Tridecadiene	49.57	3.19	$C_{13}H_{24}$	180.3

Extract preparation

Collected plant leaves were air dried and then crushed into a coarse powder using laboratory grinder. The coarse powder was used for methanolic extract preparation. For the preparation of methanolic extract, 5 g dry plant powder was dissolved in 50 ml of methanol and extracted using soxhlet for 24 hours at 50°C. The extract will be then filtered and stored at 4°C in airtight bottles for further use. For latex extract preparation, known quantity of fresh latex (1 ml) was mixed with methanol (1 ml). Then mixtures were placed in shaker for overnight, filtered through the

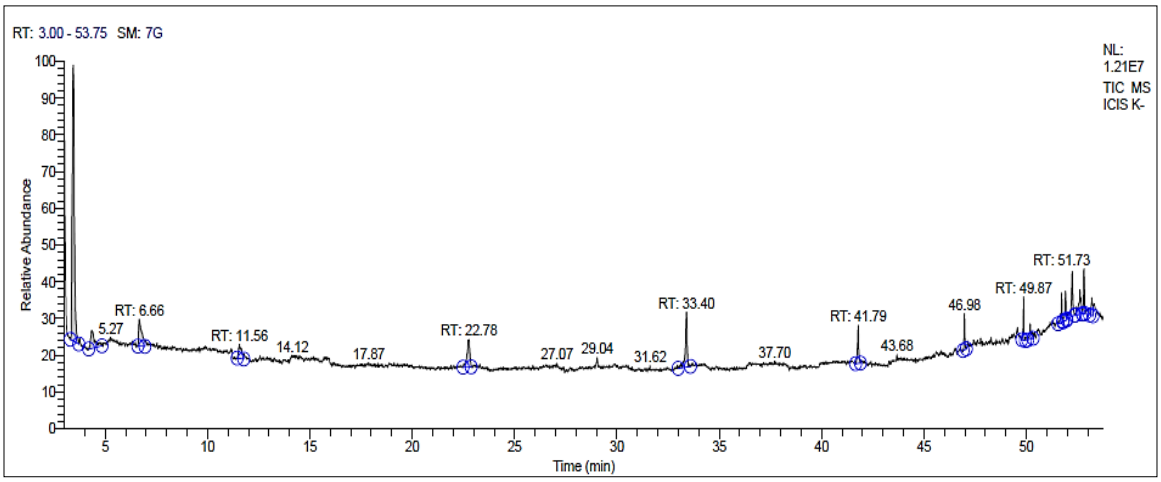

Figure 1: GC-MS chromatogram of methanolic extract of *Calotropis gigantea* leaf.

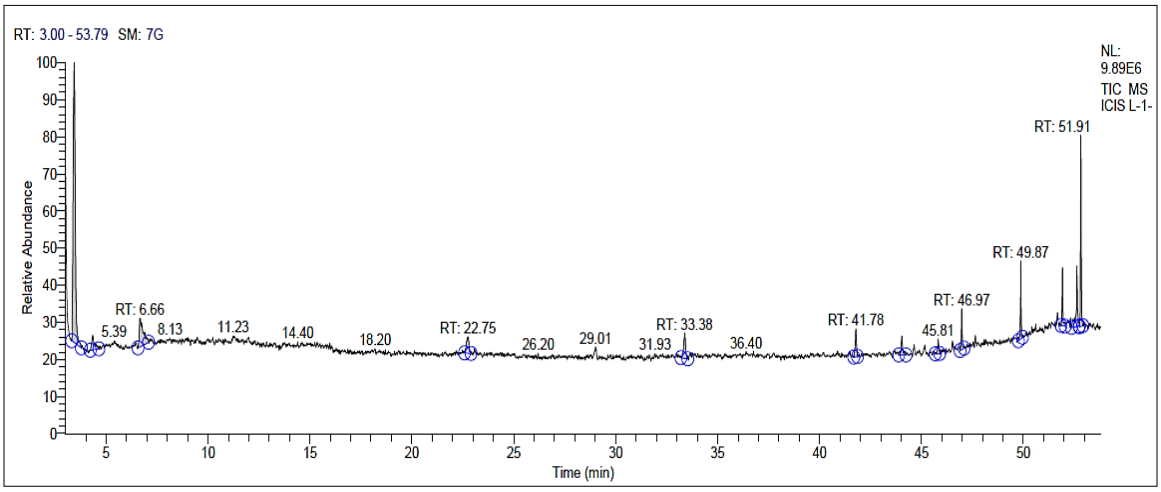

Figure 2: GC-MS chromatogram of methanolic extract of *Calotropis gigantea* latex.

Whatman's filter paper and further used of GC-MS analysis.

GC-MS analysis

GC-MS analysis of the methanol extract of *C. gigantea* leaf and latex was performed using Thermo Scientific Triple Quadrupole GC-MS (Trace 1300 GC, Tsq 8000 triple quadrupole MS) equipped with TG 5MS (30m X 0.25mm, 0.25µm) column. Helium was used as the carrier gas at a flow rate of 1ml/min. using an injection volume of 1.0 µL. Injector temperature was kept at 250ºC and ion source temperature was 230ºC. The oven temperature was maintained at 50ºC isothermal at 280ºC, Mass Spectra transfer line temperature.

RESULTS AND DISCUSSION

Now a day the identification of bioactive compounds from medicinal plants has increased. GC-MS analysis has been found to be an ideal technique for the analysis of volatile and semi-volatile bioactive compounds[16]. In the present study, total 46 compounds were identified from the leaf and latex extract of *C. gigantea* using methanol solvent. Out of 46 compounds, 24 were identified from leaves (Table 1, Fig. 1), whereas 22 were observed from latex (Table 2, Fig. 2). Methanolic extract has also used by Bhagavaty and Mary (2015) for the extraction of bioactive compounds from the leaf and flowers of *C. gigantea*. Various other organic solvents which have been used for the chemical profile of *C. gigantea* plant parts, are Chloroform[17,18], Ethanol[19], Acetone, Hexane and Diethyl ether[20], etc. The identification of compounds in this study is based on the peak area of the compound (which represents the percentage of that compound), its molecular formula and molecular weight. In the present study GC-MS chromatogram of methanolic extract of leaf showed eleven prominent peaks at retention time 3.4, 4.3, 6.6, 11.5, 22.7, 33.4, 41.7, 46.9, 50.1, 51.7 and 53.2. Compound Cyclohexene was observed at retention time 3.46 with peak area 43.28 %. It was the major compound present in leaf of *C. gigantea*. Additionally, Butane-2,2-dimethyl (C_6H_{14}) and D-Mannose-1-phosphate sodium salt ($C_6H_{13}O_9P$) showed their present at Rt 6.66 with peak area 6.0%. Most of the compounds were observed at Rt-22.78 which were Cis-vaccenic acid ($C_{18}H_{34}O_2$), Benalaxyl ($C_{20}H_{23}NO_3$), Profenofos ($C_{11}H_{15}BrClO_3PS$), β-Tocopherol ($C_{28}H_{48}O_2$) and 1-Octanol-3,7-dimethyl ($C_{10}H_{22}O$) having peak area 5.78 %. Cholest-5-en-3-ol,24, Propylidene (3. beta.) ($C_{29}H_{48}O_2$) showed its presence at Rt-41.79 with peak area 3.56 %. Three other compounds Eicosane, Decane and β-Sitosterol were observed at Rt-46.9 with peak area 2.17 %. Remaining identified

Table 3: Activity of bioactive compounds identified in methanolic extract of *Calotropis gigantea*

S. No.	Compound name	Activity
1.	Biphenyl	Used in dye carriers, food preservatives, as fungicide.
2.	Cyclohexane	Used as solvent and paint remover.
3.	D-Mannose-1-phosphate sodium salt	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.
4.	Azulene	Have anti-inflammatory, analgesic, antipyretic, and platelet-inhibitory actions.
5.	Cis-vaccenic acid	Shows anti-carcinogenic properties, Inhibition of telomerase enzyme.
6.	Profenofos	Used as pesticide, toxic compound to human.
7.	β –Tocopherol	Act as antioxidant and vitamin.
8.	β-Sitosterol	Used for heart diseases and high colestrerol. Used for boosting the immune system and for preventing the common cold and flu (influenza), colon cancer, cervical cancer, HIV/AIDS, rheumatoid arthritis, tuberculosis, allergies, psoriasis, as well as for gallstones, fibromyalgia, migraine headache, systemic lupus erythematosus (SLE), asthma, bronchitis, hair loss and chronic fatigue syndrome.
9.	Decane	Used for industrial purpose or as a type of hydrocarbon solvent.
10.	Levomenol	Antimicrobial activity and wound healing
11.	Campesterol	Check the level of cholesterol in body
12.	Ethion	Act as insecticide-affect a neural enzyme called acetylcholinesterase and prevent it from working
13.	Azulene	It is used in treatment of ulcers, gastritis, athlete's foot, and vein problems
14.	2- Methoxy 4-vinyl phenol ethanone	Used as flavoring agent
15.	L- Glutamic acid	Act as amino acid
16.	D-Mannose	Used for preventing urinary tract infections (UTIs) and treating carbohydrate-deficient glycoprotein syndrome, an inherited metabolic disorder
17.	Oxadiazon	Used as herbicide
18.	5-Nonadecen-1-ol	Used to make surfactants, lubricating oils, pharmaceuticals
19.	2- Methoxy 4-vinylphenol ethanone	Used as flavoring agent

compounds are shown in Table 1. The GC-MS chromatogram of methanolic extract of latex showed total 22 compounds. Most of the compounds which were reported from leaves were also present in latex. Additionally, latex was also found to be rich in Phenol,2,5-bis(1,1-dimethylethyl) $C_{14}H_{22}O$, Z-1,6-Tridecadiene ($C_{13}H_{24}$), L-Glutamic acid ($C_5H_9NO_4$), Hexadecane ($C_{16}H_{34}$), 1-[(T-butyl) dimethyl silyl thin] butane ($C_7H_{15}F_3O_3SSi$) at retention time 49.16, 49. 57, 49.87, 52.89, respectively. Literature reported chloroform as a better solvent system for compound extraction from *C. gigantea*[21]. But the present study confirms methanol as a good for compound extraction from *C. gigantea.* This solvent (methanol) could be used with chloroform as solvent system in pharmaceutical companies for compound extraction having various biological activities. Instead of these, table 3 listed the medicinal importance of the compounds, identified from leaf and latex of *C. gigantea* which supports that the plant has much and different pharmaceutical value. Although, additional research is necessary to purify those compounds which are responsible for therapeutic activities.

CONCLUSION

The present study proved *C. gigantea* as medicinal important plant because of the presence of various active compounds. The study confirms the variation between the chemical constituent of leaf and latex, which shows their different potential of therapeutic activities. The present preliminary research concludes that these chemical constituents can be used for the development of various traditional medicines. Further investigations required to separate the novel active compounds from the leaf and latex methanolic extract which may create a new way to treat incurable diseases.

ACKNOWLEDGEMENT

Authors are thankful to Dr. Kalpana Chauhan, Associate professor, School of Chemistry, Shoolini University, Solan for her unreserved help during research work and result interpretation.

REFERENCES

1. Ogunleye D, Ibiyoye S. Studies of antimicrobial activity and chemical constituents of *Ximema americana*. Tropical Journal of Pharmaceutical Research. 2003; 2(2): 239-241.
2. Sharma AP, Tripathi BD. Assessment of atmospheric PAHs profile through *Calotropis gigantea* R.Br. leaves

in the vicinity of an Indian coal-fired power plant. Environmental Monitoring and Assessment. 2009; 149(1): 477-482.
3. Hemalatha M, Arirudran B, Thenmozhi A, Rao USM. Antimicrobial effect of separate extract of acetone, ethyl acetate, methanol and aqueous from leaf of Milkweed (*Calotropis gigantea* L.). Asian Journal of Pharmaceutical Research. 2011; 1(4): 102-107.
4. Larhsini M, Bousaid M, Lazrek HB, Jana M. Evaluation of antifungal and molluscicidal properties of extracts of *Calotropis procera*. Fitotrapia. 1997; 68(4): 371-373.
5. Mohsin A, Shah AH, Alaha MA, Tariqi MO, Ageel AM. Analytic anti-pyretic activity and phytochemical screening of some plants used in traditional Arab systems of medicine. Fitoterapai. 1989; 60(3): 174-177.
6. Kapur SK, Sarin YK. Medico-botanical survey of medicinal and aromatic plants of Katra valley (J.K. State), India. Indian Drugs. 1984; 22(1): 4-10.
7. Sebastian MK, Bhandari MM. Medico-ethno botany of mount Abu, Rajasthan, India. Journal of Ethno Pharmacology. 1984; 12(2): 223-230.
8. Basu A, Sen T, Ray RN, Chaudhari A. Hepatoprotective effects of *Calotropis procera* root extract on experimental liver damage in animals. Fitoterapai. 1992; 63(6): 507-514.
9. Gupta DK, Bhutani KK. Triterpenoids from *Calotropis procera* root and bark. Indian Journal of Chemistry. 1996; 35(10): 1079-1084.
10. Singh VP, Sharma SK, Khare VS. Medicinal plants from Ujjain district, Madhya Pradesh Part II. Indian Drugs and Pharmaceutical Index. 1980; 15(5): 7-12.
11. Yoganarasimhan SN. Medicinal plants of India. Regional research institute (Ay.) Bangalore, Tamil Ayurvedic uses and pharmacological activities of *Calotropis procera* Linn. Asian Journal of Traditional Medicines. 2011; 6(2): 97.
12. Ansari SH, Ali M. Norditerpenic ester and pentacyclic triterpenoids from root bark of *Calotropis procera* (Ait) R. Br. Pharmazie. 2001; 56(2): 175-177.
13. Ahmed KKM, Rana AC, Dixit VK. *Calotropis* species (Ascelpediaceae): A comprehensive review. Pharmacognosy, Magazine. 2005; 1(2): 48-52.
14. Seiber JN, Nelson CJ, Lee SM. Cardenolides in the latex and leaves of seven *Asclepias* species and *Calotropis procera*. Phytochemistry. 1982; 21(1): 2343-2348.
15. Ahmed UAM, Zuhua S, Bashier NHH, Muafi K, Zhongping H, Yuling G. Evaluation of insecticidal potentialities of aqueous extracts from *Calotropis procera* against H enosepilachna elaterii Rossi. Journal of Applied Science. 2006; 6(1): 2466-2470.
16. Grover N, Patni V. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticose* (L) Kurz. leaves. International Journal of Pharmacy and Pharmaceutical Science. 2013; 5(4): 291-295.
17. Shirsat MK, Mahatma OP, Umesh SP, Bais SK, Dwivedi J. GC-MS analysis of *Calotropis gigantea* whole plant chloroform extract. Journal of Pharmaceutical and Biosciences. 2013; 1: 26-29.
18. Sureshkumar P. Phytochemical assessment on various extract of *Calotropis gigantea* (L) R. BR. Through GC-MS. Internal Journal of Pharma Bio Sciences. 2013; 4(2): 802-810.
19. Divya R, Manimegalai K. Preliminary phytochemical screening and GC-MS profiling of ethanolic flower extract of Calotropis gigantea Linn. (Apocynaceae). Journal of Pharmacognocy and Phytochemistry. 2013; 2 (3): 28-32.
20. Bhagvathy S, Mary J. Antioxidant and antidiabetic potential of *Calotropis gigantea* in RIN-5F pancreatic cell lines. Human Journal. 2015; 5(1): 176-199.
21. 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.