

Secondary Metabolites In The Giant Milk Weed *Calotropisprocera* And Their Role In Controlling Plant Diseases

Eman K. Abdul- Karim¹, Aalaa K. Hassan², Rabab A. Naemah³

^{1,2,3}*Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad, Iraq*

E-mail: ²aalaammh@gmail.com

Abstract: *This study aimed to evaluate the efficiency of secondary metabolites extracted from *Calotropisprocera* against plant pathogens in general. The researchers reported that the treatment with those secondary compounds had a positive effect in controlling plant diseases and significantly different from the control treatment (Un treated with those secondary compounds) and the treatments of adding secondary compounds, whether in addition to the soil or seeds, were more efficient in controlling plant pathogens compared to the treatments in which spraying takes place, as the secondary compounds inhibit the growth of pathogenic fungi and reduce the percentage of infection and the intensity of infection, it was reported that the alcoholic extract of leaves and latex of the brocade plant acted to reduce the rates of infection with fungi, especially the fungi that cause seed contamination, and they also found that the alcoholic extracts gave better results than the water extracts against plant diseases in general. As well as the important role in increasing the fresh and dry weight of the plant significantly on the control treatments with the presence or absence of pathogenic fungi.*

Keywords: *Giant milk weed, *Calotropisprocera*, plant diseases*

1. INTRODUCTION

Excessive use of pesticides of chemical and unnatural origin, especially systemic pesticides, to combat plant diseases that affect orchard trees, field crops and vegetable plants has led to harmful environmental pollution on humans, animals, plants, soil, water and air, thus disrupting the ecosystem. The problem of environmental pollution today is considered the problem of the times at the level of the countries of the world as a whole, it exposed an urgent matter with which man coexists and is looking for alternatives to get rid of it as a result of the great harm to human and animal health, whether directly or indirectly, as well as the bad side on the plant component, soil and wildlife and this occurs at a time when the world in general and the third world countries in particular suffer from a shortage of water and food. For this reason, a new trend has emerged in the recent period towards the use of safe alternatives to the environment in general, including the control using natural plant compounds with which the side effect is reduced or absent, for example the use of plant extracts, especially those found in medicinal, aromatic, wild and desert plants, and these plants are naturally found on a large scale in All over Iraq, as it was found that most of these plant sources contain one or more substances in their chemical composition that have the ability to inhibit and prevent the

development of many organisms that cause plant diseases, especially fungi (Mustafa et al., 2010; Hassan and Al-Kuwaiti, 2019).

It is a source of many chemical compounds of great interest, as they represent secondary metabolites and means of defense and protection against other living organisms. They are often called active substances. The plant contains one or more substances. These substances are present in one part or all of its parts, and their concentrations vary. The active substances, depending on the age of the plant and the time of collecting the plant parts (Hamza, 2006) are characterized by having an anti-pathogenic effect (Malik and Chughati, 1979; Masood, 1980) and these compounds include phenols, flavonoids, alkaloids, hydrolyzed proteins, tannins, glycosides, anthocyanins, and terpenes (El-Amirli and El-Kamir, 2010), as well as being safe and non-polluting to the environment (Al-Jalli, 2018). Several studies have proven the great effect of the active substances of plants, including the brocade plant because of its unique properties (Oudhia and Tripathi, 1998), which is called Calotropis and the name is derived from the Greek word meaning the beautiful keel of the boat and It is one of the important plants because all its parts can be used, It possesses many chemical compounds that have a major role in the medicinal and biological activity of the plant against pathogens (Mustafa et al., 2010; Verma et al., 2012) and has the ability to grow in different tropical and non-tropical environments (Singhal et al., 2009) and in all types of soils. It has 140 species and is a sacred plant for the Indians for its association with Martos celebrations, and in the Arab world it is associated with sun worship in ancient times.

Plant classification

The plant belongs to the class of Dicotyledons, the order Gentianales, the family Asclepiadaceae, and the genus Calotropis. It is named in Arabic as Ashaar - Asher - Ashkar - Ashoor - Asheer - Asher eggs - Calotropis eggs - camel genus - silky. This plant is called Sodom apple in relation to a city in Palestine and India called (Sanskrit) Arka, Ganarupa, Mandara, Vasuka, Svetapushpa, Sadapushpa, Alarka, Pratapass, (Hindi) Aak, Madar, (Kannada) Ekka, (Tamil and Malayalam) Erukku, (Telugu) Jilledipuvvu Crown flower giant Indian milkweed in English, called in Malaysia Remiga, rembega, kemengu In Thailand Po thuean, paanthuean (northern), rak (central). It is called fauxarbre de soie, mercure vegetal in France and in the Philippines it is called Kapal-kapal (Tagalog) (Kumar et al., 2013).

Plant description

An evergreen plant that may reach a height of 6 meters, many branching and characterized by broad, oval-shaped green opposite leaves covered with cotton bristles. Leaves range between 7.5-15 cm long and 4.5-8.2 cm wide, with a sharp top and a heart-shaped base. The flowers are bisexual and white-green on the outside. The inside of the cup is purplish, and fatty with white color, and consists of 5 sepals, as well as the corolla also consists of 5 petals gathered in apical or axillary inflorescences, and its fruits are green, hollow, spherical to oval, and its seeds are oval and laminated in brown color, its top is covered by silky hairs up to 3 mm long. The bark of the plant is white, yellowish, corky in texture. The plant secretes large amounts of milk. The white plant when cutting any part of the aerial parts, as indicated by Pal and (Sinha 1980) The plant possesses a sticky, white, milky liquid that comes out when part of it is cut, and it is poisonous. It also contains toxic compounds, including Calotropin, Uscharin, and Calotroxin, and it contains a rubber compound, Caoutchouc, and vegetable milk is a white liquid, which is an aqueous emulsion of a mixture of protein, mucus, sugar, alkaloids, acids, tannins, mineral salts, and other substances. (Cauchukia) can be used to make rubber, and plant milk is secreted from cells or special secretory ducts found in the tissues of the

plant. The plant is characterized by its deep and dense roots. The plant multiplies easily and the seeds grow quickly. The plant grows wild and it is a widespread plant where it is found in most environments, especially the sandy environment. But it also grows in heavy clay soils and soft sandy soils. The plant is distinguished by its ability to tolerate salinity and drought (Karschon, 1979).

Its original home is India, China and Malaysia, and it is found in Saudi Arabia, Egypt, Iraq, Jordan, Oman, Yemen, Sudan, some countries of the Maghreb (Libya, Algeria, Morocco), the Horn of Africa, Iran, Vietnam, Afghanistan, Nepal, Syria, UAE, Yemen, Lebanon, Kuwait, Mexico, Nigeria, Senegal, Peru, the United States and many countries of the world.

Secondary compounds contained in the plant

Phenolic compounds

They are secondary compounds consisting of an aromatic ring have one or more hydroxyl groups, and they vary in chemical composition, but they share with each other the presence of a phenolic group (a hexagonal ring have one or more hydroxyl groups) or more in their structural structure. There are more than 30,000 phenolic compounds characterized by their important role in the development of plant defenses as well as their role in protecting plants from pathogens, as well as some of them have potential role in regulating plant growth and as pigments. Phenols are divided into groups according to the number of carbon atoms or the basis of their prevalence or prevalence in the plant kingdom. General properties of phenols. It has the ability to form complex compounds with proteins by hydrogen bonds and is characterized by its sensitivity to oxidative enzymes. It has the ability to form coordination bonds with iron and chromium (Verma et al., 2009; Cardoso, 2021)

:1-Alkaloid

Alkaloid is an organic nitrogen compounds separated from the plant, that is, it is a heterogeneous cyclic compound containing at least one nitrogen atom in the hydrocarbon ring.

-Function of alkaloid in plant:

-Its presence is considered as a regulator of plant growth

-Defense substances possessed by the plant against other organisms because most of them have a bitter taste

- Its importance in the ionic balance in the plant rather than the inorganic bases -They are final products to which the reactions of toxic substances in the plant end, and they are disposed of in the form of alkaloids that are not harmful to the plant, which are preserved in some of its different parts.

- It is considered a stockpile of the elements that the plant needs in its different stages of growth, so that it can benefit from it when needed. The most important of these elements is nitrogen.

They are biosynthesized in some specific cells of different tissues of some medicinal plants and they aggregate or accumulate in the same cells or are transported to be stored in special collection places in the form of solid and crystallizing basic compounds or oily liquid properties as they are found in free and free-moving or frozen non-free forms Combined with organic or mineral acids in the form of salts or derivatives with a bitter taste (Waheed et al., 2016; Ahmed et al., 2016).

Natural benefits of alkaloids:

-It is one of the most important products or final compounds that are left behind in the processes of metabolism, which are called neglected or damaged products or waste products, or they are called Secondary Products, Natural Products, or By Products because the plant does the work or disposes of these materials or waste and its accumulation in the form of crystals or in the form of salt derivatives to be collected and accumulated in order to be stored in some cellular structures of plant cell tissue

-It is one of the reserve organic sources of nitrogen that can be obtained in severe conditions under unsuitable environments for emergency feeding when this mineral element is not provided in the nutritional solution of agricultural soil or a deficiency within plant cells, which works to withdraw nitrogen in its dissolved form after utilizing it and cracking from the complex and self-accumulating alkaloid compound inside the storage cells of these alkaloids in the cells of the same plant.

It is characterized by severe toxicity within the cell juice of the cytoplasm of cells, which in turn leads to the biologically active protection and self-protection of the plant producing these organic compounds, which gives it natural immunity or self-resistance against attacking living organisms such as insects, birds, bacteria, fungi or viruses (Nasrin et al., 2018; Cardoso, 2021).

-It plays an important biological role and an important system during the periods of the plant life cycle that produces these natural products, which are distinguished by biological effects and physiological activities, represented in the biological effectiveness as growth regulators as a result of their activation and raising by increasing plant growth under normal natural conditions.

They are usually found either free or in the form of salts of some vegetable acids, such as citric acid, tartaric acid, or tannic acid, and rarely as a salt form of mineral acids.

General properties of alkaloids:

- Crystallized solids

- It is colorless, odorless, and bitter in taste, and some are colored, such as Berberine, yellow, and Magnoflorine alkaloid, and orange.

- Free alkaloids dissolve in organic solvents such as chloroform and ether and do not dissolve in water, while their salts dissolve in water and do not dissolve in organic solvents.

2-Glycosides

They are important and effective substances in medicinal plants and these organic substances play an important role in the treatment of many diseases. It contains sugar and other non-sugar materials that differ according to plants, which leads to differences in the properties of the glycosides as a result of the difference in the non-sugar part, which is called the aglycon part, which has the therapeutic and chemical properties. It can be decomposed either by using hydrolysis with special enzymes or acids that lead to the separation of the two parts. One of them is a type of sugar called Glycan, such as glucose sugar, rhamnose sugar, or semarose, etc., and the second part is Aglycon, which includes organic compounds such as alcohols, esters, ketones, ... etc. The separation process takes place by removing Molecule of water H₂O (Suraj and Chatterjee, 2015; Al-Luhaiby and Hassan, 2020).

General properties of glycosides

-Most of them are found in liquid form, as they dissolve in water and dilute alcohol, except for resinous glycosides, and some dissolve in organic solvents such as acetone.

-Its solutions taste bitter and shift polarized light to the left

- When it is present in the plant, the cells of that plant also contain special enzymes that act on their decomposition and they reduce Fahlink's solution
- Either it is a crystalline solid or it is in a colorless amorphous form

Glycosides role in plant

- It has a protective role against some insects and living organisms, as it has a disinfectant role

-It is one of the by-products of metabolic processes in plants

- Its role is to get rid of the activity of some toxic and harmful substances to plants
- It is a food storehouse for plants, especially sugars
- Plays a regulatory role in the growth process.

3-Coumarins

It belongs to the class of heterocyclic hexagonal compounds containing oxygen as an element in the heterocyclic ring. It arises from the bonding of a benzene ring with a pyron ring to form the class of benzopyron, of which two types can be distinguished depending on the location of the carbonyl group. Coumarins are divided into:

- 1- simple coumarins
- 2- Furanocoumarins
- 3- Pyranocoumarins
- 4- Substituted coumarins in the pyran ring.

Coumarins are found freely or bound in many dicotyledonous or dicotyledonous plants and gather on the surfaces of leaves, fruits or seeds, and their concentration in the plant changes during the day. It is characterized by its role as an inhibitor of free radicals and antioxidant and its important role in protecting plants from pathogens and their tolerance to ultraviolet rays.

4-Tannins

.It is a group of complex chemical compounds resulting from the combination of some uncomplicated phenols with astringent effect. They are amorphous, difficult to separate, soluble in water, alcohol, and glycerin. They do not dissolve in ether or benzene. When dissolved in water it forms an acidic emulsion with an astringent taste and has the ability to Precipitation of proteins and alkaloids from their solutions, and this is the process that takes place when tanning leather. They are found in all parts of the plant such as the bark, leaves, fruits and roots. The tannins are divided into two groups, the first being Hydrolysable tannins, which consist of gallic acid in a polyester form with glucose, and the second Condensed tannins, which consist of Flavonoid, Flavone or Quinones units. It has an important role in protecting the plant parts in which they are located from microbial infections(Mahmood et al ,2019).

The effectiveness of brocade plant in inhibiting the growth of plant pathogens.

The brocade plant was used in the control of many fungal pathogens. Alam et al. (2004) reported that the use of concentrations (1, 1.5, 2, and 2.5%) affected the germination rate of *Fusariumoxysporum* that causes root rot of the Indian pumpkin plant (Piper betle) in a different way. They reached 23, 22, 11 and 9, respectively, after 30 minutes of treatment, while Jabeen et al. (2013) used the extract of leaves and stems of the brocade plant at concentrations 1, 2, 3, 4 and 5% to inhibit the growth of the fungus

Macrophominaphaseolina that causes charcoal rot disease in many crops, They reported that the leaf extract is more effective in inhibiting the growth of pathogenic fungi. As for Pathak and Zaidi (2013) they demonstrated the effectiveness of the latex of brocade plant in inhibiting the fungi associated with the seeds of the wheat plant *Alternaria alternata*, *A. clamydophora*, *Aspergillus niger*, *A. flavus*, *Rhizopusorysappe* and *Mucor. Fusarium spp.*, *Drechslera australiensis*, *Penicillium spp.*, *Curvularialunata* and *Cladosporium* compared with chemical pesticides and thus reduced the toxicity effect of pesticides. As reported by Viji et al. (2013) the effectiveness of the acetone extract of the brocade plant leaves in inhibiting the fungi pathogenic on the rice crop where the inhibition percentage reached 7.1, 7.4 and 7 mm, respectively, for each of *Penicillium sp.*, *Aspergillus sp.*, *Aspergillus niger*, while it reached 10.5, 10.8 and 10.7 mm, respectively, for the aqueous extract of brocade. Suraj and Chatterjee (2015) reported the high effectiveness of ethanolic brocade leaf extract by 50% in inhibiting the fungi *Botrytis cinerea*, *Rhizopusoryzae* and *Fusariumoxysporum*. Also, Manoorkar et al. (2015) confirmed the efficacy of brocade leaf and latex extract in inhibiting the growth of seed-borne fungi in soybean, sunflower, mustard and groundnut. *Curvularialunata*, *Alternariaalternata*, *Rhizoctoniasolani*, *Fusariumsolani*, *Penicilliumchrysogenum*, *Aspergillusniger*, *A. flavus*, *A.terrus*, *A. fumigatus*, *Rhizopus sp.* It showed that the ethanolic extract of latex was the best in inhibiting fungi compared with the ethanolic extract of leaves and aqueous extract of latex and leaves. Waheed et al. (2016) reported that 7% concentration of methanolic brocade leaf extract inhibited the growth of *Macrophominaphaseolina* by 37%, while it reached 31, 23, 20 and 15 at concentrations of 5.5, 4, 2.5 and 1%. The concentration of 20% of the extract of the daibag plant achieved a high effectiveness in reducing the fungi transmitted by barley seeds, reducing its recurrence and increasing the percentage of seed germination (Ahmad et al., 2016). Nasrin et al. (2018) Effectiveness of brocade extract at a concentration of 25% in inhibiting the growth of the fungus *Fusariumoxysporum f. sp. Lycopersici* that causes fusarium wilt disease on tomato by 87%. Mahmood et al. (2019) found that treating wheat seeds with brocade extract at concentrations of 0.5, 1, and 1.5 mg/ml achieved an increase in the germination rate and reduced the growth of fungi, as it was noted that the concentration 1.5 was the best, as the germination rate reached 93.3% compared to the control treatment, which amounted to 73.3% and inhibited the growth of the fungi *Penicilliumnotatum* and *Aspergillusflavus*, which amounted to 81.4 and 59.2% compared to the control treatment, which had a 0% inhibition rate. Bader (2019) explained that the laboratory results of the second instar larvae of *Meloidogyne incognita* were affected when they were treated with concentrations (50, 75, 100)% of Ashar plant extract for 3 days at laboratory temperature and this led to their cessation of movement where the highest level of lethal nematodes reached at the high concentration 100% with average of 25% The results showed that the high concentration of the plant extract was positive compared with the control treatment in terms of the number of root-knot nematodes (250 g of soil), While the results of the plastic house showed that the use of the extract of Al-Ashar plant with a high concentration of 100% recorded a clear improvement in the vegetative growth of tomato plants, which amounted to 20.08 grams, compared to the control treatment, which recorded 10.20 grams. Santana and Cardoso (2021) showed the effectiveness of the aqueous extract of the brocade plant in inhibiting the growth of the fungi *Phomopsissojajae* and *Sclerotiniasclerotiorum* that caused the disease of dry stem and pods and white rot on soybeans at concentrations 5, 10, 15 and 20% and the effectiveness increases with increasing concentration.

2. CONCLUSION

We conclude from this article that the use of the Giant milk weed with aqueous and alcoholic extract as well as latex, led to the inhibition of the growth of fungi, in general, the alcoholic extract and latex showed high effectiveness in resisting fungal pathogens from the aqueous extract.

3. REFERENCE

- [1] Abhilash PC and N Singh.2008. Distribution of hexachlorocyclohexane isomers in soil samples from a small scale industrial area of Lucknow, North India, associated with lindane production, *Chemosphere*, , 73: 1011 – 5.
- [2] Ahamed M,AC Rana, and VK Dixit.2005. Plant Review *Calotropis* species (Asclepiadaceae): A comprehensive review, *Pharmacognosy Magazine*; (2): 48 – 52.
- [3] Ahmad,L. ; N. Pathak and R. K. Zaidi. 2016. Antifungal Potential of Plant Extracts against Seed-borne Fungi Isolated from Barley Seeds (*Hordeumvulgare* L.). *Journal of J Plant Pathology &Microbiology*.7(5):1-4.
- [4] Al-LuhaibyAK.,and AK.Hassan.2020. Evolution the ability of some organic compounds is protecting bean seedling against infection with *Rhizoctoniasolani* .*Plant ARCHIVES*,20(1):86-90.
- [5] Bakry FA.2009. Use of some plant extract to control *Biomphalariaalexandrina* snails with emphasis on some biological effects: world *Applied Sciences Journal*, 6(10): 1335 - 1345.
- [6] El-Kamali, H.H. and M.Y. El-Amir. 2010. Antibacterial activity and phytochemical screening of ethanolic extracts obtained from selected Sudanese medicinal plants. *Current Research Journal of Biological Sciences*, 2: 143-146.
- [7] Hassan AK.,and NA.Al-Kuwaiti.2019.Saponin:Anew promising plant compound to control plant pathogens ,*Ecology , Environment and Conservation Paper*,25(2):485-487.
- [8] Jabeen, K.; N.Waheed,.;S. Iqbal.2013. Antifungal potential of *Calotropisprocera* against *Macrophominaphaseolina*. *Life Sci. J.*, 10, 572-576.
- [9] Jain SK., BK.Sinha,andA Saklani.1989. Medicinal plants known among tribal societies of India, *Ethnobotany*,2: 1 – 92.
- [10] Karschon, R.M.1979. Contributions to the arboreal flora of Israel: *Calotropisprocera* (Willd.) R.Br La-Yaaron, 20,(1-6) , 41-48. (Hebrew and English).
- [11] Khairnar, A.K. ;S.R.Bhamare and H.P. Bhamare .2012. *Calotropisprocera*: anethnopharmacologicalup date. *Advance Research in Pharmaceuticals and Biologicals*. 2(11):142-156.
- [12] Kumar, P.S. ; E. Suresh and S.Kalavathy.2013. Review on a potential herb *Calotropis gigantean* (L.) R.BR. *Scholars Academic Journal of Pharmacy* .2(2):135-143.
- [13] Mahmood, R.K. ; M. H. Ibrahim and S. A.Al shukri.2019. Effect of *Calotropisprocera* extract on fungi accompanied to wheat grains in the storages of Babylon province. *Plant Archives* . 19(2): 1275-1278.
- [14] Malik NN,MID Chughati.1979. Antimicrobial activity of *Calotropisprocera* — a preliminary study, *Pak. J. Sci*, 31: 127–129.
- [15] Manoorkar V. B. ; S.V. Mandge and B. D. Gachande.2015. Antifungal Activity of Leaf and Latex Extracts of *Calotropisprocera* (Ait.) against Dominant Seed-Borne Storage Fungi of Some Oil Seeds. *Bioscience Discovery*. 6(1):22-26.
- [16] Masood A,S.Haq, S.H.Anjum, M.Saxena.1980. Further studies on the effect of some plants extracts on the mortality of *Maloidogyiniincognite*, *J. Sci. Res. Plants Med*, 1: 18–22.

- [17] Meena, A.K.; A.K. Yadav; U.S. Niranjana; B. Singh; A.K. Nagariya ; K. Sharma; A. Gaurav ; S. Sharma and M.M. Rao. 2010. A review on *Calotropis procera* Linn and its Ethnobotany, Phytochemical, Pharmacological profile. *Drug Invention Today*. 2(2):185-190.
- [18] Moustafa, A. M., Ahmed, S. H., Nabil, Z. I., Hussein, A. A. and Omran, M. A. 2010. Extraction and phytochemical investigation of *Calotropis procera*: effect of plant extracts on the activity of diverse muscles. *Pharma. Biol.* 48: 1080-1090.
- [19] Nart T, S. Poonsab and T. Gritsanaruck. 1984. The environmental implications of the use of *Calotropis gigantea* as a textile fabric. *Agriculture, Ecosystems & Environment*, 11(3): 203 – 212.
- [20] Nasrin, L. ; S. Podder and M.R. Mahmud. 2018. Investigation of Potential Biological Control of *Fusarium Oxysporum* sp. *Lycopersici* by Plant Extracts, Antagonistic sp. and Chemical Elicitors In Vitro. *Fungal Genomics and Biology*. 8(1):1-4.
- [21] Orwa C, Mutua A, Kindt R, Jamnadass R and Simons A; Agroforestry Database: a tree reference and selection guide version 4.0 (<http://www.worldagroforestry.org/af/treedb/>).
- [22] Oudhia P, S.S. Kolhe, R.S. Tripathi . 1998 . Allelopathic effect of *Blumeolacera* L. on rice and common Kharif weeds. *Oryza* 1998a: 35 (2): 175-177. 10. Oudhia P, Kolhe SS, Tripathi RS. Germination and seedling vigour of mustard as affected by allelopathy of *Blumeolacera* L. *Agriculture Science Dig* 1998b: 18 (3): 183-186.
- [23] Pal, G. & Sinha, N. K.. 1980: Isolation, Crystallization and properties of Calotropins DI & DII from *Calotropis gigantea*. *Archives of Biochemistry and Biophysics* 202(2): 321-329.
- [24] Pathak, N. and R.K. Zaidi. 2013. Comparative study of seed dressing fungicides and *Calotropis procera* latex for the control of seed-borne mycoflora of wheat. *Annals of Biological Research*. 4(4):1-6.
- [25] Pereira W and J. Seabrook. 1996. Asking the Earth the Spread of Unsustainable Development, Second impression The Other India Press, Goa, India Mapusa, 1996; 403 - 507.
- [26] Rathod VM; Control mashi in paddy, Honey bee, 1998; 9(4): 13.
- [27] Samantaray S, GR. Rout and P. Das. 1999. Studies on the uptake of heavy metals by various plant species on chromite mine soils in sub-tropical regions of India, *Environmental Monitoring and Assessment*, 55: 389 – 399.
- [28] Santana, S.S. and P.G. Cardoso. 2021. Antagonism of Plant Pathogens by *Calotropis procera*. *Journal of Agricultural Science*. 13(4):120-127.
- [29] Shahidul, A.; M.R. Islam; M.A. Sarkar; A.N. Chowdhury; M.S. Alam and M.W. Lee. 2004. In vitro effect of fungicides, plant extracts and smoke on conidial germination of *Fusarium oxysporum* root rot pathogen of *piper betle*. *Mycobiology*. 32(1):42-46.
- [30] Sharma AP and BD. Tripathi. 2009. Assessment of atmospheric PAHs profile through *Calotropis gigantea* R.Br. leaves in the vicinity of an Indian coal-fired power plant, *Environ Monit Assess.*, 149: 477 – 482.
- [31] Shilpkar P, M. Shah and DR. Chaudhary. 2007. An alternate use of *Calotropis gigantea*: Biomethanation, *Current science*, 92: 425 – 436
- [32] Singhal A, S.N. Shah and V.L. Kumar. 2009. Effect of aqueous suspension of dried latex of *Calotropis procera* on hepatorenal function in rat. *J. Ethnopharmacol.*, 122:172-174.
- [33] Suraj S. and P. Chatterjee. 2015. Antifungal Activity of 50% Aqueous-Ethanol Extract of Leaves of *Calotropis procera* R.Br. *International Journal of Scientific and Research Publications*. 5(11):230-235.
- [34] Sureshkumar P. 2013. Phytochemical assessment on various extracts of *calotropis gigantea* (L.) R. Br. through gc-ms, *Int J Pharm Bio Sci*, 4(2 B): 803 – 810.

- [35] Usha K, Singh B, Praseetha P, Deepa, DK.Agarwal, R.Agarwal and A.Nagaraja.2009. Antifungal activity of *Daturastramonium*, *Calotropisgigantea* and *Azadirachtaindica* against *Fusariummangiferae* and floral malformation in mango, Eur. J. Plant Pathol., 124: 637 – 657.
- [36] Verma, R.,G.P.Satsangi, and J.N. Shrivastava, 2012. Analysis of phytochemical constituents of the ethanolic and chloroform extracts of *Calotropisprocera* using gas chromatography-mass spectroscopy (GC-MS) technique. J. Med. Plant Res. 7: 2986-2991.
- [37] Viji R.; P. Alaguraja ; P. Mani and S. Velavan . 2013. Biological control of *Calotropisgigantea* leaf extracts against pathogenic fungus, infecting *Oryzasativa*. International Journal of Research in Pure and Applied Microbiology. 3(4): 107-112.
- [38] Waheed,N. ; K. Jabeen ; S. Iqbal and A. Javaid. 2016. Biopesticidal activity of *Calotropisprocera* L. against *Macrophominaphaseolina*. Afr J Tradit Complement Altern Med. 13(6):163-167.