International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Isolation of bioactive components from *Calotropis procera* plant latex-A review

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Abstract

Calotropis procera is a medicinal plant which belongs to family Asclepiadaceae, commonly it is known as "aak" plant. It is widely distributed in subtropical as well as tropical regions of the world. Researchers are interested in latex of *Calotropis procera* because of its medicinal properties, economic value for the cheese and rubber production. Its latex contains many phytochemicals which include cardiac glycosides, phenolic compounds, flavonoids, saponins, terpenes. Different bioactive components from *Calotropis procera* are known to exhibit wound healing properties, for defense mechanism against herbivorous plants, as bio indicator for pollution monitoring, as manure and fertilizers. The white latex of *Calotropis procera* is very rich source of hydrocarbons so it is used as diesel substitutes which contain different nitrates, sulphate and particulate matter. Its latex also has biochemical enzyme purification potential along with photo extraction potential. These phytochemicals are isolated by different methods including fractional distillation, vacuum distillation and High Speed Counter Current Chromatography. Phytochemical analysis is being carried out by using different spectroscopic and chromatic techniques which include GC, GC-MS, HPLC, FTIR and TLC. In this review, by using *Calotropis procera* latex, different biological properties are monitored such as anticancer, anti-inflammatory, anti-glycemic, antibacterial, antimicrobial, antidiarrheal, antimalarial and proteolytic enzyme activities.

Key words: *Calotropis procera*, bioactive components, isolation, biological activities

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1. Introduction

Latex of plants and other secretions are materials which are excreted from the damaged part of plants either by herbivorous insect activity or by external damage. However, these secretions of plants play a vital role in defense mechanism of plants against herbivorous insects. In different studies only the wide range of occurrence of plant latex in plant kingdom have been studied but their different ingredients, proteins and chemicals are not well documented. Now, there has been much progress in this field. Various plant latex proteins including chitin proteins, cysteine protease have been studied for their defense role [1]. All plant secretions contain diverse variety of secondary proteins as well as metabolites, which have a major role in defense mechanism [2]. It is related to species-specific way. There is a wide variety of secondary metabolites for example rubber, alkaloids, furanocoumarins, terpenoids, starch, glucosidase, phosphatase, oxidases, lectins and other secretions in high amount [1]. A wide variety of techniques have been used for the separation of components present in *Calotropis procera* latex which includes (i) fractional distillation (ii) high speed counter current chromatography (iii) fractionation by vacuum distillation (Nadeem *et al*., 2013). The latexes of different plant species have very effective potential against parasitic infections as well as microbes such as many antifungal proteins are present in seeds of herbal plants and these antifungal proteins are used against the protection of embryo from infections [3]. These phytochemicals for example phenolic acid has effective role against the binding of parasites against teeth and urinary bladder lining and in this way it inhibits the risk of (UTI) urinary tract infection and dental cares [4]. GC-MS is very sophisticated and highly recommended technique for the natural compound analysis [5-6]. Molecular mass is determined by GC-MS technique as well as their aging pathway [7]. Chemical composition of the plant latex changes with different seasons [8]. The white latex of *C. procera* is used for cough treatment, loss of appetite, asthama and is purgative [9]. The chemical composition of *C. procera* includes different components in high and low concentration. Saponins and flavonoids are present in minute amount while, resins, tannins and alkaloids are not

recorded in *C. procera* latex. Cardiac glycosides, terpenes and phenolic compounds are recorded in highest concentration [10]. Different investigation concluded that *Calotropis procera* extract has been known to have wound healing capacity, anti-diarrheal activity, anti-inflammatory, insecticidal, antioxidant, anti-carcinogenic, antimicrobial activity, cytotoxic activity and hepatoprotease activity. The investigations will be a footstep for therapeutic as well as pharmacological research [11].

2. Bioactive Components of *Calotropis procera*

Phytochemical analysis of *C. procera* latex extracts confirmed the existance of saponins, alkaloids, tannins, flavonoids and terpenoids. The concentrations of different bioactive compounds were different in various extracts. Highest amount of bioactive compounds was present in aqueous and ethanolic extracts. Lowest amount of bioactive components was present in chloroform extract. It only contains terpenoids and cardiac glycosides. But the results obtained from the species which were collected from different areas of world and analyzed in different laboratories had different chemical compounds. These bioactive components of *Calotropis procera* have antimicrobial activity [12].

2.1. Glycosides

Glycosides are the condensed product of organic hydroxy groups and sugars. The condensation occurs in such a way that the hemiacetal entity remains intact during condensation. Glycosides are present in sap of plant cell and consist of oxygen, carbon and hydrogen which are crystalline in nature, colourless in appearance and water soluble constitute of plant latex. Glycosides consist of one carbohydrate part, which is usually a glucose part, and other part is non-carbohydrate which may be alcohol, glycerol and phenol. Glycosides can be hydrolyzed with the help of ferments as well as mineral acid. The classification of glycosides is based on aglycogen part and glycogen portion or on the basis of pharmacological activities [1]. Cardiac glycosides present in *Calotropis procera* are calotropogenin, calotropin, uscharin, calotoxin and calactin [13].

2.2. Flavonoids

Plant flora widely contains flavonoids, which are polyphenols in nature. Structurally, upto 15 carbon aromatic ring can be attatched in flavonoids. Flavan is the parent compound of flavonoid group. Flavonoids are also pigments in higher plant species. Kaempferol, quercitrin and quercetin are flavonoids which are present in plant species. Catechin, proanthocyanidin, flavans, flavanes, anthocyanidins as well as dihydroflavans are other flavonoid group [4]. *C. procera* reported the 3-O-rutinoside of kaempferol, isorhamnetin-3-O-rutinoside, flavonoid glycoside 5-hydroxy-3, 7-dimethoxyflavone-4'O-b-glucopyranoside and quercetin-3-O-rutinoside in the aerial parts of the plant latex [14].

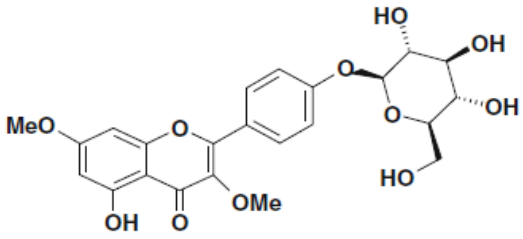

Fig.1. Structure of 5-hydroxy-3, 7-dimethoxy-4'O-beta – D-glucopyranoside

2.3. Phenolics

Phenolic compounds are distributed universaly which are natural pigments and give different colours to the plant fruits. PAL (Phenylalanine ammonia lyase) produces phenolic compounds which are responsible for various functions in plants. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infection. There are three categories of phenolic compounds (1) Non-flavonoid polyphenolics (2) Phenolic acids (3) Flavonoids polyphenolics. Phenolics are used as natural antioxidant and as nutraceuticals, which are found in green tea, apples as well as in red wine for the prevention of cancer and heart ailments. These compounds are also good as anti-inflammatory agents. Chlorogenic, flavones, hesperidin, rutin and neringin are the examples of phenolic compounds present in *Calotropis procera* latex [4].

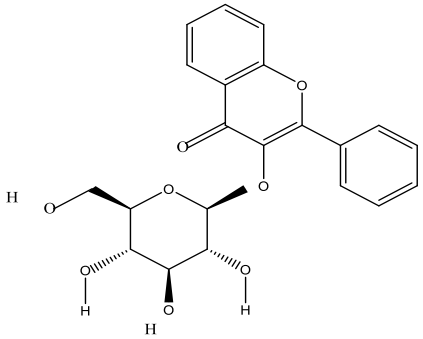

Fig.2. Structure of Flavonol

2.4. Saponins

Plant *Saponaria vaccaria* (*Quillaja saponaria*) is the major plant for the derivation of saponins. It was used as soap in ancient times. Saponins act just like soap in the form of foam when treated with water. When it is hydrolyzed sapogenin is produced, which is aglycogen part. Sapogenin has two types triterpenoidal and steroidal [1]. 3-Epimoretenol is found most commonly in *Calotropis procera* latex [15].

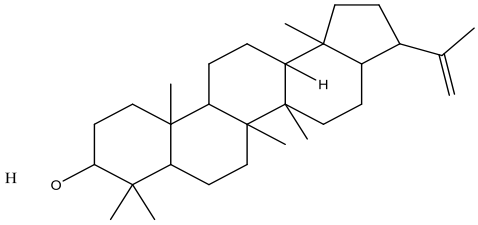

Fig.4.Structure of 3-Epimoretenol

2.5. Terprnes

Terpenes are one of groups of natural products. These are unsaturated hydrocarbons which are flammable and found in latex oleoresins and resins [16]. Terpenoids are classified as monoterpenoids, diterpenoids and triterpenoids. They have general formula (C_5H_8) it depends on number of carbon atoms present. Terpinen-4-ol, eugenol, methanol, camphor and thujone are the examples of monoterpenes [1]. β-Amyrin, α-Amyrin and germanicyl are present in *Calotropis procera* plant latex [17].

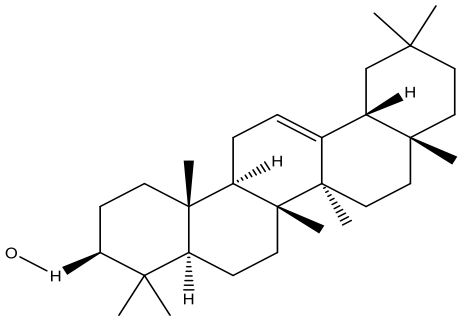

Fig.5.Structure of Beta-Amyrin

3. Methods of Isolation of Bioactive Components

3.1. High speed counter current chromatography

High speed counter current chromatography can be used for the isolation of bioactive components of *Calotropis procera*. It is a recent separation technique in which two immiscible solvents are used in form of two layers for isolation of required components of scented oil where lower layer acts as mobile phase and upper layer works as stationary phase. Initially, stationary phase is filled in helical column and then mobile phase is pumped in the coiled column through inlet that is usually found on chromatographic column. Flow rate of mobile phase is almost 7 milliliter per minutes using head to tail mode. When equilibrium is established, sample of essential oil is introduced which undergo fractionation and continuously detected by detector. Mostly for purification of different bioactive components and functional groups from latex and natural products is carried out. For purification, isolation and separation from high speed counter current chromatography proper selection of extracting solvent is required. Proper selection of extracting solvent following requirements should be fulfilled. (i) Greater than 50 percent of stationary phase should be retained (ii) Solvent should be settled less than 29 seconds (iii) There should be stable target material (iv) Within double layer system required components should be soluble (v) There should be laminated solvent system [18].

3.2. Vacuum distillation

Vacuum distillation apparatus is used for the fractionation of volatile components. In the round bottom flask known amount of sample is introduced. There are three outputs of round bottom flask. First capillary is used for controlling and for enhanced ebullition while second is used for controlling the fractions of latex bioactive components and third is attatched with the packed column. For the protection of column against corrosion titanium alloy is filled within 1.5 meter column. Thermocouple is usually used for the measurement of temperature of upper portion of fractionating column with the precision of ±15 ºC. Thermostatic bath and vacuum pumps are usually used for the maintenance of temperature and pressure respectively with in condenser. The compounds which have very less volatility are fractioned in less time through vacuum distillation and there is also a change in chemical composition which alters its acaricidal activities [19].

Table 1.Phytochemical screening of crude extract and latex of *Calotropis procera* [20]

Chemical constitutes	MeOH	Latex
Alkaloid	-	-
Terpenoid	+	+
Flavonoids	+	+
Anthraquinones	-	-
Tannins	+	-
Phlobatanins	+	-
Saponins	+	+
Glycosides	+	+
Reducing sugars	-	-
Steroids	+	+
Cardiac glycosides	+	+

+ = Present, - = Absent

3.3. Fraction Distillation

Different fractions of latex have different chemical compounds on different temperature ranges so boiling point is such a physical property which plays important role in the fractionation of bioactive components Optimum conditions are usually set for the isolation of bioactive components. Fragrance of every component is different due to different chemical components [19]. For the isolation and separation of bioactive components phytochemical analysis is done with different polarities. Different separation techniques are done for phytochemical analysis such as HPLC, flash chromatography sephadex chromatography and column chromatography. The purified compounds are then used for different biological activities and structure elucidation. Non-chromatographic techniques are also used for the structure identification which are Fourier Transform Infrared spectroscopy and monoclonal antibodies [21].

4. Analysis Methods

TLC (Thin layer chromatography) is usually used for the quick identification method to check how many components are present within mixture. Researchers mostly use this technique for quick response. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound. Sometimes phytochemical sprays are required for visualization of separated components or TLC plates are checked under UV light. This is the procedure to check the identity of compounds [22]. Phytochemical analysis is also

done by HPLC. For HPLC analysis detectors are selected. Detector is set for the optimal detection settings and separation assay must be developed. Analysis by HPLC is done by comparison of peak areas and retention time of peaks. Most commonly UV detectors are used because most classes of compounds show low UV absorbance and this technique is more sensitive to natural compounds. UV sensitivity is of great importance if the required component is present in minute amount. Mass spectrometer and diode array detectors are also used for phytochemical analysis. Sometimes hyphenated techniques LC/MS are used for the analysis of bioactive components. When MS (mass spectrometery) technique is used then much more elaboration of structures takes place. If pure standard compounds are not found then HPLC is used in combination of MS [23].

5. Economic Importance of *Calotropis procera*

Calotropis procera is wild medicinal plant which grows South East Asia to West Africa. The milky white latex is used as purgative in India and its flowers are used as an anti-asthmatic, as stomach tonic and digestive liquid. For the treatment of intestinal worm, enlargement of abdominal viscera and skin diseases, *Calotropis procera* latex is used. For dyspepsia treatment, *Calotropis procera* milky juice is used. The aqueous extract of *Calotropis procera* is also used for the inhibition of cellular infiltration and is for the development of the neoplastic changes within transgenic mouse model of the *hepatocellular carcinoma*. Liver damage induced by CCl_4 is treated with chloroform extract of root of *Calotropis procera* [24-25]. Photoextraction potential is the characteristic feature of *Calotropis procera* which is shown by accumulation ratios under the natural condition. It is well adjusted and multiplies freely through the seed and root [26]. Heavy metals are accumulated in this order Cd > As > Zn > Cu > Pb. Cd as well as As are mobile within soil-plant system while Pb, Cu and Zn are mobile slightly [27]. In urban areas and suburban areas *Calotropis procera* is used as bio-indicator for pollution [28].

6. Applications of *Calotropis procera* Plant Latex

Good quality thread and cloth is prepared by blending and spinning of bast fibres of *Calotropis procera*. For the improvement of the economics of system mechanization is required as manual extraction is slow process. Extraction rate also depends on the stem condition for the separation of fibres. At the beginning of extraction, due to strong bondage of stem fibre extracted fibre quantity is very low. Later on extraction rate is much reduced because fibres decay [29]. *Calotropis procera* which is commonly known as “aak” is used for the thermal insulating material. The aak floss is also used in stuffing the pillows and it becomes powdered because of its brittleness. Aak seeds also have some quantity of oil which are used in varnish, soap and paint industry and can be consumed as manure. It is also used as an insulating thermal material [30].

Procerain enzyme is present in the *Calotropis procera* latex which has some biochemical properties. For enzyme purification *Calotropis procera* latex is used. Precipitation as well as chromatography is used for the extraction of enzymes. *Calotropis procera* latex were used for the purification of procerain by CM-Separose column and Ammonium Sulfate precipitation [31]. *Calotropis procera* secrets irritating volatile components to repel the toxins and desert grazers [32]. Many diseases in India are cured by root bark of *Calotropis procera* such as snakebite, leprosy, malaria and fever [33]. While in Central Africa and Western zone it is used to cure dysentery, dermatitis and elephantiasis [34]. It is also being used for brewing, tanning and curding milk [35]. *Calotropis procera* roots are dried and ground to powder form which is used for the treatment of asthma, spleen enlargement, bronchitis and hepatic diseases [36]. *C. procera* leaves are used for the treatment of burn injuries, rheumatid joints swelling, for the treatment of hair fall tooth aches and paralysis India [37]. *Calotropis procera* leaves are used as soil indicator in the tropical West Africa [38]. In semiarid regions *Calotropis procera* is also used for the production of bioenergy and biofuel [39]. This plant also produces valuable hydrocarbon which are converted into deiseal substitutes. Leave, stem and pods are also used as a source of hydrocarbons. The biodiesel which is produced by *Calotropis procera* latex consists of NO_2, SO_2 and also particulate matter and has high cetane value [40].

7. Biological Activities of *Calotropis procera*

7.1. Anti-hyperglycemic Activity

The *C. procera* latex has been described to show anti hyperglycemic activity which is related to restore liver glycogen and with the normalization process of the oxidative stress markers. The free radical production as antioxidant agent of *Calotropis procera* was found to be comparable with the vitamin C and standard antioxidant agent [41]. *Calotropis procera* latex has toxic effect which has penetrated administration as well as oral ingestion. It is rich in poly-isoprene components which has rubber like component and has pro-inflammatory activity found for its toxicity. Recently, pro-inflammatory and anti-inflammatory effects have been identified by separating the iso-prene fraction from latex. The dried latex aqueous suspension was made which is free from rubber like fractions and has also medicinal values [42].

7.2. Antimalarial activity

C. procera leaves are very effective for preventing the entrance of parasites into red blood cells. Liver secretes merozoites, recognize it then attach and enter the red blood cells by ligand receptor interaction within 60 seconds. Proteins of *P. falciparum* have different red blood cells receptors than duffy binding protein (DBP). It was observed

by some researcher that there was a sharp decrease in the protein content after 48 hrs in the treated iRBCs (infected red blood cells) which could probably be due to the effect of certain phytochemicals in blocking the synthesis of this protein (RESA). *Calotropis procera* shows antimalarial activity which is also used in traditional medicine.

7.3. Anticandidial Activity

Calotropis procera dried latex show anticandidial activity. *Cystein proteases* and enzymes are responsible for anticandidial activity. Fungal strains are also treated with *C. procera* latex [43].

7.4. Anti-inflammatory Activity

Calotropis procera dried latex is used as a potent antimalarial and anti-inflammatory agent. PBZ (phenyl butazone) oral dose of DL (dried latex) (500 mg/kg) as well as atropine which was produced by *C. procera* dried latex gives relief against diarrhea when used with castor oil. For the confirmation of *Calotropis procera* anti diarrhoel activity dried latex effect was investigated for induced intestinal accumulation of fluid as well as intestinal transit. Intestinal tansit is decreased by dry latex. Only dry latex is enough for inhibition of castor oil induced enterlooping [44]. Studies have revealed the use of toxic substances in converting milk into chees.

7.5. Antimicrobial Activity

Against some pathogenic fungi and bacteria, different plant extracts can be used [45]. Extracts of dried latex of *Calotropis Procera* in different solvents were made and antibacterial activities were monitored by some researchers. Dry latex of *Calotropis procera* exhibited inhibition mechanism against all bacteria. Chloroform extract of *Calotropis procera* dried latex exhibited only antibacterial activity while ethanolic and aqueous extracts were beneficial against microbes. The investigation was also concerned with the synthesis of latex silver nanoparticles (LAg-NPs) using *Calotropis procera* latex as reducing as well as capping agent, the synthesized LAg-NPs were characterized using UV-Visible spectrophotometer analysis, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD) and Transmission Electron Microscopy (TEM). The synthesized nanoparticles exhibited a variable growth inhibition against the tested strains. A significant increase in the effect of nanoparticles compared with crude latex and latex serum [46].

7.6. Immunomodulatory Activity

Murine macrophage cell line RAW2647 was evaluated by some researcher to produce nitric oxide (NO) and inducible nitric oxide (iNOS) by the action of *Calotropis procera* immunomodulatory activity. In it,cultured cells *were directly in concern with plant extract which gave* results. So, it was concluded that *Calotropis procera* latex extract activated immune system and effector cells and enhanced the NO production and iNOs gene expression. It was one of the most important observations because it showed the contribution of *C. procera* towards pharmacological actions [47].

7.7. Proteolytic Enzyme activity

Enzyme extracted from the *Calotropis procera* plant latex is used for the tenderization of chicken, beef and pork muscle. Chunks of muscles from chicken, beef and pork were marinated in distillated water and crude enzyme extracts of 0.5%, 0.3%, 0.2%, 0.1% and 0.05% on 4 °C for 60 minutes. Different physical and chemical activities were checked from marinated samples. The results exhibited decrease in the moisture content; firmness and toughness of sample muscle with the addition of crude enzyme extract. Cooking yield and water holding capacity was same as without using crude enzyme extract. pH was increased in case of beef and chicken while no change in pH was observed in pork muscles. Proteolytic activity was enhanced with the addition of crude enzyme extract of *C. procera* dried latex extract. So for the effective tenderization of the meat *Calotropis procera* dried latex can be used alternatively [48].

8. Conclusion

A critical analysis of literatures have shown that the *Calotropis procera* plant latex is the good source of various important bioactive compounds and therefore has major applications in the medicinal as well as in agricultural field for pest control. *Calotropis procera* latex has wide applications in industrial sector, cosmetics and textile industry but there is need for extensive research in this regards. It has diverse pharmacological activities and is also used as bio indicator for pollution monitoring. Latex of *Calotropis procera* is also a rich source of hydrocarbons so it is used as diesel substitutes. It might be more useful for successful control of insect pests. *Calotropis procera* has been reported for various biological activities such as immunomodulatory, anti-inflammatory, antimalarial, anti-glycemic, anticancer, anticandidial and proteolytic activity.

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