



Phytochemical and Antibacterial Screening: A Review on Leaves and Latex of *Calotropis Procera*

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ABSTRACT

Various investigations exposed antibacterial activity and phytochemical screening on leaves and latex extract of *Calotropisprocera*. Over all methodused for study was first to isolate the extracts of petroleum ether, methanol, ethanol, ethanol-170%, ethyl acetate, chloroform and water and all the above extract were than evaluated for the phytochemical test and the antibacterial activity. The results shows that the extract of leaves and latex of *Calotropisprocera* shows remarkable antibacterial activity specially with five bacteria's namely *Salmonella typhii*, *Escheria coli*, *S. aureus*, *S. flexerni*, *E. faecalis* along with this the results also revealed that the best extraction solvent for antibacterial activity of leaf and latex extract is alcohol followed by chloroform and water.

Keywords: Antibacterial, Phytochemical, Screening, Evaluated

INTRODUCTION

India has been known for its use on herbal drugs since the ancient past. Looking back in times we could find the people are very much dependent on plant sources as they are beneficial. The home remedies and DIY has been the most common and favourite way of dealing with diseases for us Indian as we've practiced this since ages and now it has been incorporated in our genes to search for these ingredients in our kitchen or in the nature. Research has explored the nature of the secondary metabolites that in various medicinal plants. Medicinal plants shows a precious, renewable source for new drugs. The world health organisation has claimed that 80% of the world's population count on the herbal method of treatment of various diseases Around 500,000 plant spices were estimated but only a small amount has been investigated phytochemically. More than 130 drugs in the world's markets comes from higher plants either directly or synthetically [1], [2], [3], [4], [5]. Although hundreds of plants were tested for antifungal and antibacterial properties, the majority of them have not been adequately evaluated and processed well. [6]. The whole Himalayan belt of gods world is the home to several medicinal plants, as in India not only the mythological facts says so but always the science has given approval to this very fact. Himalayan Region with background information on family, habit and nativity. In India, out of 17000 species of the higher plants, 7500 are known for their medicinal uses. Currently 25% of the drugs are obtained from plants, many others are synthetic analogous built on the prototype substances obtained from the plant. [7]

Calotropis procera is a plant of Asclepiadaceae family and it is a large, broad leaf, evergreen plant with a strong odour, abundant in the tropical regions of Asia and Africa, which is commonly known as Milkweed, Apple of Seldom and Madar. *Calotropis procera* is used as a folk medicine and is not a new name in Indian household as it is used as ornamental plant due to beautiful white flower. It has been reported that the plant possess potential antimicrobial, anthelmintic, ant inflammatory, anticancer, purgative, anticoagulant, analgesic, and antipyretic characteristics and is also used in the treatment of leucoderma, leprosy, liver and abdomen diseases [8]. The latex of *Calotropisprocera* has been known for important indigenous medicinal uses due to its laxative, antisyphilitic and analgesic action [9]. *Calotropisprocera* flowers causes temporary paralysis of red stomach worm in sheep and notably reduces egg count percent of gastrointestinal nematodes in naturally infected sheep [10]. Dry latex of *Calotropis procera* has potential anti-cancer properties due to its differentiable targets and non-interference with regular pathway of apoptosis [11]. The pharmacological properties of *Calotropisprocera* is a versatile plant for the pharmaceutical industry to develop new drugs [12]. Medicinal plants have no doubt remained the major sources of traditional medicine worldwide [13]. The main objective of this review is to study the effect of various solvent extracts obtained from the leaf and latex of *Calotropisprocera*, also to compare their qualitative and phytochemical screening by using standard tests because successful extraction, determination and isolation of biologically active components from plant material are largely dependent on the type of solvent used [14].

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PHYTOCHEMICAL DISCUSSION OF PLANT

The whole plant contains is incorporated with loads of phyto constituent which possess different remedial effects, it is said to be used in digestion, diarrhoea, jaundice, boils etc. The plant was also recommended by some to treat leprosy, splenic enlargement, dropsy, hepatic and worms [15]

LATEX

The latex and fresh leaves are recommended to be applied on painful sour joints. The latex of *Calotropis procera* is acidic with the density of 1.02g/ml the composition of the latex depends on the environment season [15]. The latex possesses a rigid composition of with compounds namely calactin (1), cholin (2), calotropin (3), calotoxin (4), uscharine (5), syriogenin (6) uzarigenine (7), voruscharin (8), proceragenine (9), L-lactuciferol, proceroid, tetraxasterol, β -amyrin, calatropeol 3-epimoretenol lupeol trypsin [16]. 3, 7, 11-Trimethyl 2, 6, 10, 12-pentadecatrien-1-ol, 2, 6 dimethyl tetra-1, 5-decaene and were also isolated from the latex [17].

The latex contains two distinct cysteine peptidase, procerain and procerain B. However, a new cysteine peptidase were purified from *Calotropis procera* latex. The purified enzymes exhibited plasma-clotting activity mediated by a thrombin-like mechanism [18].

It also possesses aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, phenylalanine and lysine [19]. Several studies have been made on the latex of *Calotropis procera* are said to reportedly possess anti helminthic, antileprotic anti-rheumatism, anti-cold activity [20]

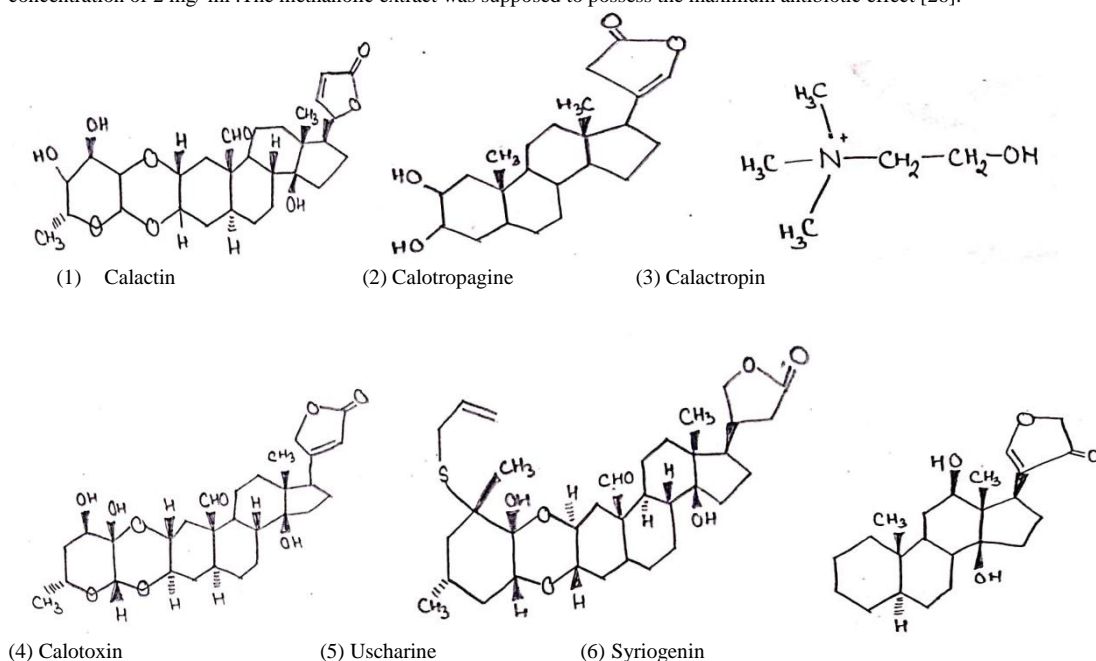
The Albino rats were evaluated with latex of *Calotropis procera* for the protection against isoproterenol 20mg/100g induced for myocardial infarct. The prior treatment with the ethanolic extract of *Calotropis procera* latex at a dose of 300 MG /KG body weight when given orally three times a day for consecutively 30 days reduce significantly the elevated markers enzyme level in serum and heart harmonized in isoproterenol in our induce myocardial infarction [21]

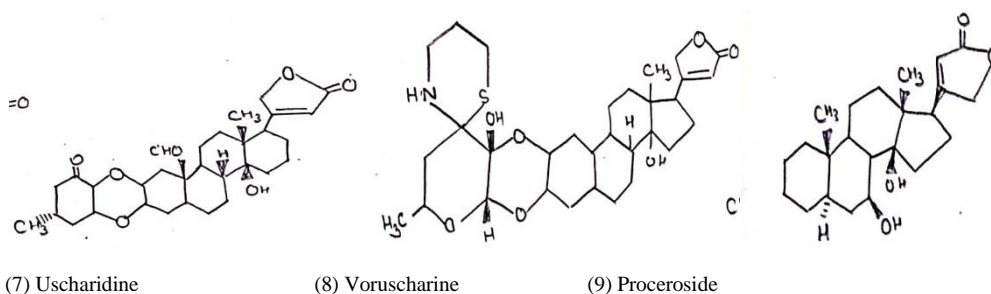
The latex of *Calotropis procera* has been studied to possess anti diarrheal activity when studied against the rats it has been observed that the administration of single dose of dry Latex reduces the frequency of the reaction by a significant percentage when compare to the animals treated with castor oil.[22]

Dry latex of *Calotropis procera* has the potential for anti-cancer effect due to its differentiable targets and non-interference with regular pathway of apoptosis. Dry latex treatment of mice showed a complete protection against hepato carcinogenesis. No adverse effect was observed in these animals. [23] It has been observed that daily administration of oral dose of latex in Diabetic rats with the dosage of 100 and 400 mg/Kg decreases the Glucose level and consequently increases the hepatic glycogen level of the treated animals, it has also been reported that the restoration of the daily water consumption and body weight has seen. [24]

Inflammatory capabilities has also been observed by air pouch and pedal oedema models of inflammation, when the rats were treated with aqueous subcutaneous injection of dry latex into plantar surface of the paw induced inflammation, the maximum inflammation was obtained for an hour and was maintained for the next hour, the response of the inflammation was determined by the increase in vascular permeability as it reached its Maxima in 15 minutes [25].

The latex has been observed for the antibacterial activity by the Agar diffusion method when streptomycin was taken as a positive control with a concentration of 2 mg/ ml. The methanolic extract was supposed to possess the maximum antibiotic effect [26].



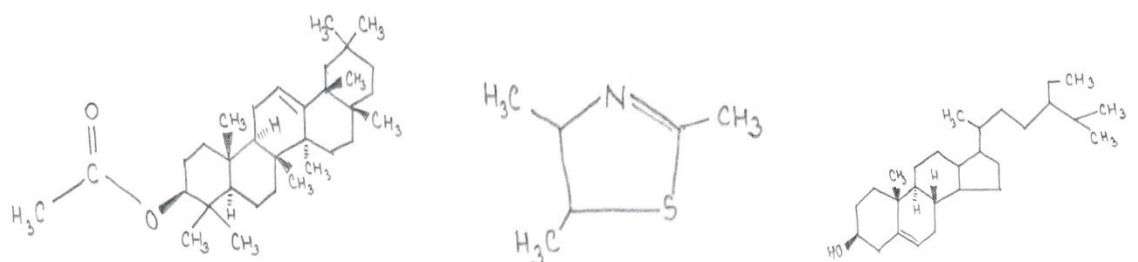
Figures- 1-9, Compounds Isolated from Latex of *Calotropis procera***LEAVES**

The leaves of *Calotropis procera* possess cardiac glycosides, and sterols and terpenoids. Two major cardiac glycosides that has been isolated from the leaves are calotropin and calotropagenin, whereas terpenoids were mainly alpha-acetate (10), beta-amyrin, 3- thiozoline (11). The only sterol that is present in the leaf is Beta sitosterol (12) Ratinoside (13) Ursolic acid (15), alpha amyline (16), were also isolated from the leaves of *Calotropis procera*. The leaves of plant possess antihelmethetic, antirheumatism, cytotoxic, antiasthmatic, antioxidant activity. [27]

Anti helmethetic activity of the leaf has been valuated against Indian earthworm *Pheritomapostuma*, 70% hydro ethanolic extract in 12.5 mg/ ml concentration showed death and paralysis in 29.05 m and 18.58 m respectively. This effect depends totally on the concentration taken. [28]

Cytotoxicity against hepatic to has been observed by the leaf extract. [28]

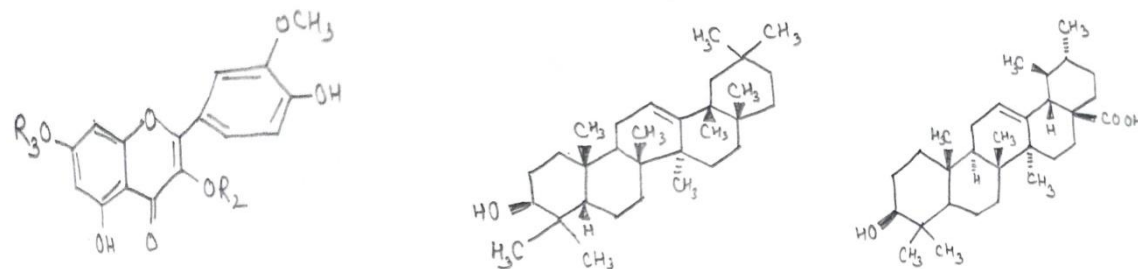
Antioxidant potential of *Calotropis procera*'s methanolic and aqueous extract of leaves by using scavenging activity of the stable (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) DPPH free radical. Half- maximal inhibitory concentration (IC₅₀) of the methanolic extract was 110.25 mg/ml that represents that aqueous extract has strong antioxidant effect. [29]



(10) Alpha-acetate

(11) 3-thiozoline

(12) Beta sitosterol



(13) Ratinoside

(14) Alpha-amyrin

(15) Ursolic acid

Figures- 10-15, Compounds Isolated from Leaves of *Calotropis procera***MATERIAL AND METHODS**

Preparation of plant extract - Generally the standard process which is used in the preparation of plant extract is as follows. Leaves of *Calotropisprocera* dried in shade in order to avoid direct contact of sunlight for this pulverization method is used. The dried leaves are macerated in the liquid i.e hexane, isopropyl, ethyl acetate, ethanol, methanol, acetone, for 48 hours. The latex is collected in sterile plastic/glass bottle by squeezing the apex and tips of leaves and kept in refrigerator at 4 °Celsius [30]. The latex dried under shade at ambient temperature to remove the chlorophyll content then take measured amount of latex and extracted with petroleum ether in the separating funnel after the formation of two separate layers of petroleum ether and residue the same is repeated with other solvent. [30]

Test on organism- Studies reveals that the antibacterial effect on the extract of leaves and latex were mainly performed on these bacteria's namely, *Shigella boydii* [31], *Streptococcus pyogen*, *Streptococcus pneumoniae*, *Staphylococcus albus* [32], *Staphylococcus epidermis*, *Staphylococcus saprophylics*, *Shigella dysenbria*, *Plesiamonas shigelloides*, *Vibrio cholera*, *Shigella sonneria*, *Shigella flexneria*, *Pseudomonas aeruginosa* [33], *Escheria coli*, *Salmonella typhii*, *S.aureus*, *S.flexerni*, *E.facalis* [34].

Biochemical standards and photo chemical screening test were reported to use the following methods. [35], [36], [37], [38], [39], [40], [41], [42], [43], [44]

1. Test for carbohydrates: Molisch's test: Benedict's solution test
2. Test for alkaloids: Dragendorff's reagent: Mayer's reagent: Hager's reagent
3. Test for proteins and amino acids: Biuret test: Ninhydrin solution test
4. Tests for steroids: Liebermann-Burchard reaction: Salkowaski reaction
5. Test for flavonoids: Shinoda's test: Lead acetate solution test
6. Test for glycosides: Bontrager's test
7. Test for Phenols: Ferric chloride test
8. Test for Amino acid and protein: 1% ninhydrin solution in acetone test
9. Test for Saponins: Foam test
10. Test for Sterols: Liebermann- Burchard test
11. Test for Tannins: Braymer's test
12. Test for Terpernoids: Salkowki's test
13. Test for Quinones: A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration) confirms the presence.

ANTIBACTERIAL TEST [45]

Antibacterial activity on hexane, petroleum ether, ethyl acetate, isopropyl alcohol, ethanol, chloroform, methanol, diethyl ether and aqueous extract of *Calotropis procera* studied over by the researchers and they mainly used disc diffusion method for the antibacterial determination.

In Disc diffusionmethod a petri-disc of 90 mm diameter is taken and media of MHA is prepared and the test organism is spread uniformly in the petri-disc, now sterile disc are placed in the media infused with the extract that needs to be evaluated for antibacterial activity, negative control (pure solvent) and positive control (chloroemphicol) were taken as a reference to compare the result, this is placed inside incubator for 24 hours at 37 degree celsius temperature the zone of inhibition is measured with respect to a control.

Phytochemistry [46]

When the leaf extracts of acetone, petroleum ether, chloroform, ethyl acetate, ethanol, methanol, isopropanol, hexane, petroleum ether and water were analysed or tested for the presence of alkaloids, flavonoids, saponins, terpenes, tannins, stair instance glycoside, phenolic compounds, amino acid, carbohydrate, steroid, quinone it was found that the different extract shows positive test for the certain fight of constituent as shown in table-1

RESULTS

Table-1 Phytochemical Screening of *Calotropis procera* leaf extract [46, 47]

Solvent	%yiel d	Alkaloid s	Flavnoid s	Saponin s	Terpene s	Tannin s	glycosid e	Phenoli c	Aminoaci d	Carbohydrat e	Quinon e
Acetone	9.68	+	-	+	-	-	+	-	-	-	-
Diethylethe r	3.01	-	-	++	-	+	-	-	-	-	-
Chloroform	3.6	+	-	++	++	+	-	-	-	++	-
Ethylacetat e	5.6	-	-	-	-	-	+++	+	-	-	-
Ethanol	6.8	+++	-	+++	-	+	++	+	-	+	-
Methanol	8.9	+	+	++	-	+	+	+	+	+	+
Isopropano l	2.5	+	+	+	+	+	+	-	-	-	-
N hexane	1.2	-	+	+	-	-	++	-	-	-	-
Pet.Ether	0.76	-	-	-	+	-	+	-	+	-	+
Water	6.0	+	-	-	-	+	+	+	-	+	-

+ = Present

- = Absent

Table-2 Phytochemical screening of latex of *Calotropis procera* [48]

Ingredient	Ethanol	Aqueous
Reducing sugar	+	–
Tannins	+	+
Steroid glycoside	+	+
Resin	–	+
Alkalioids	–	–
Saponins	+	+
Flavonoids	+	+

Table-3 Inhibition diameter [48]

Plant extract	S.aureus		S.flexerni		E.coli		E.facalis		S.typhii	
	Leaf	Latex	Leaf	Latex	Leaf	Latex	Leaf	Latex	Leaf	Latex
Methanol	12.5±0.5	11±0.2	-ve	10.01	-ve	-ve	10±0.1	-ve	8±0.6	7±0.6
Ethanol	11.2±0.7	11±0	24±0.5	-ve	11±0.5	7±0.1	-ve	-ve	16±0.4	-ve
Ethanol70%	11±0.1	13±0.1	-ve	12±0.1	9±0.1	12±0.4	-ve	9±0.4	13±0.0	15±0.1
Water	11±0.2	12.05±0.5	-ve	9±0.3	10±0.1	7±0.6	10±0.1	-ve	8±0.6	7±0.6
Chloroform	-ve	10±0.0	8±0.5	-ve	9±0.5	9±0.4	-ve	-ve	9.1±0.1	-ve
Chloroamphicicol	14±0.3		17.3±0.1		13±0.06		15±0.1		11±0.4	

ANTIBACTERIAL EFFICACY [49], [50]

The zone of inhibition diameter varies for different extract as antibacterial effect of the plant *Calotropis procera* varies for different microbes. Pure solvent are used as negative control. *S.aureus* was the bacteria that was very sensitive to every latex and leave extract to the plant as can be concluded by the data. *Calotropis procera* for ethanol extract of leaves and 70% ethanol extract and leave extract for *E coli* (17mm and 15 mm respectively) with MIC of 20 mg/ml. *E.facalis* is resistant to ethanolic extract, while *S. flexerni*, *S.typhii*, *E.coli* were sensitive to some of the extracts mention above. Ethanol extract of leaves and ethanol 70% extract of latex on leaves on salmonella in a typhoid 1615 and 13 mm with mic of 20 and 50 mg/mm respectively and *E coli* (11 12 and 9 mm) with MIC of 20 and 40 mg/ml respectively

Only ethanol and 70% ethanolic extract of *Calotropisprocera* recorded and zone of inhibition than chloroamphicicol in *S. typhii*.

Discussion/Conclusion

Drug resistant antibiotic has increased alarmly all over the globe as they are becoming as they are being used indiscriminately. Nature has been a supplier of medicines and medicinal agents for years and an impressive number of modern drugs have been isolated from natural sources [45]. In vitro and bacterial assay has been reported as the first step toward the development of novel therapeutic agents studies have been recorded for the antibacterial activities of aqueous and other extract of *Calotropis procera* against human pathogen it has been seen in the earlier studies that the extract of ethanol shows maximum zone of inhibition because ethanol itself possess some antibacterial activity, some work has also reported that methanolic extract shows greater Zone of inhibition then hexane and ethylacetate.

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