Phytochemical and antibacterial screening of leaves and latex of *Calotropis procera:* A comparison

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Abstract- The objective is to compare and investigate the antibacterial and phytochemical screening of leaves and latex extract of *Calotropis procera.* Extract of petroleum ether, ethanol, chloroform and water was made and all the mentioned extract were then evaluated for the phytochemical and the antibacterial activity. The antibacterial activity of leaf and latex extract has been reported with three bacteria namely *Escheria coli, S. aureus, B. cereus*. The results revealed that the best extraction solvent for antibacterial activity of leaf and latex extract is of alcohol followed by chloroform and water. The results revealed that the latex extract of *Calotropis procera* has better antibacterial effect as compare to the leaf extract of *Calotropis procera.*

Keywords: *Antibacterial, Phyto-chemical, Screening, Evaluated*

Introduction

India has been known for use of herbal drugs of the plant origin since the ancient past. Looking back at times, we could find the people have been much dependent on herbs as they are beneficial. The home remedies and DIY have been the most common and favourite way of dealing with diseases for us as we've practised 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 of various medicinal plants. Medicinal plants are precious and renewable source for new drugs. W.H.O. has claimed that 80% of the world's population rely on the herbal method of treatment of various diseases. There are about 500,000 plant spices which have estimated but only a small amount has been investigated phyto-chemically. More than 130 drugs in the world's market come from higher plants either directly or synthetically[1–5]. 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 is the home of several medicinal plants not facts says so but always the science has given approval to this very fact. Himalayan Region with background information on their family, habit and nativity. A total of 190 invasive alien species under 112 genera, belonging to 47 families have been recorded. Among these, the dicotyledons represent by 40 families, 95 genera and 170 species;

monocotyledons represent 7 families, 17 genera and 20 species. The analysis of invasive species reveals that 18 species have been introduced intentionally, while the remaining species established unintentionally through trade[7].

Calotropis procera is a plant of Asclepiadaceae family and it is a large broadleaf evergreen plant with a strong odour, abundant in the tropical regions of Asia and Africa, which is commonly known as Milkweed Apple and many other names. *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 possesses potential antimicrobial, anthelmintic, anti-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 *Calotropis procera* has been known for important indigenous medicinal uses due to its laxative, antisyphilitic and analgesic action[9]. *Calotropis procera* flowers causes temporary paralysis of red stomach worm in sheep and notably reduces egg count percent of gastrointestinal nematodesin 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 *Calotropis procera* 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 research work is to analyze the various solvent extracts obtained from the leaf, seed and stems bark of *Calotropis procera* and to qualitatively screen them for phytochemicals using standard tests. Successful extraction, determination and isolation of biologically active components from plant material are largely dependent on the type of solvent[14].

Material and Methods

Collection: The leaves and latex of *Calotropis procera* has been collected from local areas of Balawala, Dehradun. The plant is identified at that place by the means of standard key and description.

Preparation of plant extract: Leaves of *Calotropis procera* were dried in shade to avoid direct contact of sunlight and pulverization method is used which is as follows. The dried leaves were macerated in the liquid such as hexane, isopropyl, ethyl acetate, ethanol, methanol, acetone for 48 hours. The latex was collected in sterile plastic/glass bottle by squeezing the apex and tips of leaves and kept in refrigerator at 4 ˚Celsius[15].

The latex was then dried under shade at ambient temperature with the yield of 20 gram/100 ml. To remove the chlorophyll content, the sample was extracted with petroleum ether. 20 ml of latex then further was 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 solvents also.

Test organism- To study antibacterial effect of the very plant, three different bacteria's were taken namely, *Escheria*

coli, S. aureus, B.cereus from the department.

Photo-chemical Screening[16-25]

1. Test for carbohydrates

Molisch's test: Take 2-3 ml of extract and added few drops of 95% naphthol solution in alcohol. After shaking, conc. H_2SO_4, was added from the sides of the test tubes. Appearances of violet ring at the junction of two layers indicate the positive test for reducing sugar.

Benedict's solution test: Equal volume of Benedict's reagent and extract were mixed in the test tube. Heat it in boiling water bath for 5 mins. Appearance of red coloured solution indicates the positive test for reducing sugar.

2. Test for alkaloids

Dried extract was dissolved in dilute HCl. Filtered and subjected the filtrate to the following tests.

Test with Dragendorff's reagent: Took 2-3 ml of filtrate added few drops of Dragendorff's reagent. Formation of orange brown precipitates reveals the positive test for alkaloids.

Test with Mayer's reagent: Took 2-3 ml filtratered, add few drops of Mayer's reagent. Formation of cream coloured precipitates reveals the positive test for alkaloids.

Test for Hager's reagent: Took 2-3 ml of filtrate, add few drops of Hager reagent. Formation of yellow coloured precipitates revealed the positive test for alkaloids.

3.Test for proteins and amino acids

Biuret test: Took 2-3 ml of aqueous extract added 4% NaOH and few drops of 1% CuSO4 solution. Violet or pink colour was formed, proteins are present.

Ninhydrin solution test: Heated 3 ml of extract and 3 drops of 5% ninhydrin solution in boiling water bath for 10 mins. The development of violet colour showed the presence of amino acid.

4. Tests for steroids

Liebermann-Burchard reaction: Mixed 2 ml of extract with chloroform. Added 1-2 ml of acetic anhydride and 2 drops of conc, sulphuric acid from the sides of test tubes. Development of green colour revealed the positive test for the steroid.

Salkowaski reaction: Took 2 ml of extract, 2ml of chloroform and 2ml conc. sulphuric acid. After shaking appearance of red colour in chloroform layer and greenish yellow fluorescence in acid layer revealed the positive test for steroid moiety.

5. Test for flavonoids

Shinoda's test: Took 2ml of extract, 2ml ethanol, few drops of conc. HCI and little amount of magnesium turning. Appearance of pink colour revealed the positive test for flavonoids.

Lead acetate solution test: Took small quantity of extract added lead acetate solution. Appearance of yellow colour precipitate revealed the positive test for flavonoids.

6. Test for glycosides

Bontrager's test: Took 2-3 ml of extract, added dilute H_2SO_4 boiled it and filtered, then added equal volume of chloroform to appearance of red colour in ammonia layer revealed the positive test for anthraquinone glycosides.

filtrate. After shaking chloroform layer was separated.

Result and Discussion

Antibacterial test[26]

Antibacterial activity of chloroform, ethanol, aqueous extract o*f Calotropis procera* were determined by Agar well diffusion method.

Phytochemistry[27]

When the leaf and latex extract of petroleum ether,chloroform,, ethanol, and water was analysed or tested for the presence of alkaloids, flavonoids, saponins, tannins, stair instance glycoside, carbohydrate and amino acids, it was found that the different extracts showed positive test for the certain constituent as shown in Table-1and 2

When the yield of extract of leaf was observed, it was found that the maximum yield was found in the water extract that is about 7.55%, followed by ethanol 6.15%.The minimum amount of extract was formed by the petroleum ether that is 1.31%.

When ethanol, chloroform and aqueous extracts of *Calotropis procera*`s leaf was subjected to the phytochemical test, the results observed were that the petroleum ether extract was rich in glycoside and amino acid whereas ethanol and chloroform extracts were rich in tannins, carbohydrates, reducing sugar, alkaloids and saponins also some alkaloids found abundantly in aqueous extract.

Table-1 Phytochemical screening of *Calotropis procera* leaf extract

Ingredient	Ethanol	Pet. ether	Aqueous	Chloroform
%Yield	1.66	0.87	0.32	0.56
Reducing sugar	++	+	++	-
Tannins	+++	+	++	+
Steroid glycoside	++	-	+++	+
Resin	_	+	+	+
Alkaliods	_	+	_	_
Saponins	+	+	+	+
Flavonoids	++	+	++	+

+ = Present - = Absent

Table-2 Phytochemical screening of *Calotropis procera* latex extract

Solvent	%yield	Alkaloids	Flavonoids	Saponins	Tannin	Glycoside	Amino acid	Carbohydrate
Chloroform	2.1	+	_	++	+	_	_	++
Ethanol	6.15	+++	_	+++	+	++	_	+
Pet.Ether	1.31	_	_	_	_	+	+	_
Water	7.55	+	-	-	+	+	-	+

+ = Present - = Absent

From the table-2, it has been observed that the ethanolic extract of the latex was obtained in maximum amount i.e 1.66% followed by Pet.ether (0.87%).The minimum % yield was obtained in aqueous extract i.e 0.32%.

When the latex extract of *Calotropis procera* was subjected to phytochemical investigation of mainly ethanol, chloroform and aqueous extract, it was found that the ethanol extract was rich in reducing sugar, tannins, steroids, flavonoids and loaded with saponins whereas chloroform is mainly rich in tannins and saponins. Aqueous extract has tannins, steroids, resin, saponins and flavonoids[27, 28].

Zone of inhibition

The Zone of inhibition (*S.aureus)* bacteria petrolum ether extract of leaves and latex of calotrapic procera .It are shown in (Table-3) the concentration that we took were 50 mg per ml and 100 mg per ml of solvent extract for *S.aureus* bacteria only. It has been observed that the maximum zone of inhibition was found for the ethanol followed by petroleum ether latex extract when taken in 100 mg/ml concentration, followed by chloroform leaf extract when taken in 100mg per ml concentration which was 13mm, 11mm and 10mm respectively. When the results were compared to the standard that is chloramphenicol it has been observed that it showed a zone of inhibition of 15 mm for petroleum ether extract and 19 mm for ethanol where as it showed 15 mm of zone of inhibition for chloroform extract.

Similarly when zone of inhibition was observed for the bacteria *Bacillus cereus*(Table-4) the different extract of solvent of leaf and latex were treated in different concentration for the bacteria, the zone that has been observed for *Bacillus cereus* bacteria is very less as compared to *S.aureus* the maximum zone of inhibition has been shown by the petroleum ether latex extract when taken in 50 mg per ml concentration that is 6 mm whereas the ethanol extract in 50 mg/ml showed of zone of inhibition of 5 mm. Whereas the petroleum ether latex extract when taken in 20 mg/ml concentration it showed zone of 4 mm other than that chloroform and petroleum ether leaf extract showed, zone of negative, the standard chloramphenicol showed zone of inhibition of 17 mm, 15 mm and 14 mm for petroleum ether, ethanol and chloroform respectively.

When the zone was observed for *E.coli* bacteria, (Table-5) extracts were taken in the concentration of 20 mg/ml and 50 mg per litre, the maximum zone was found for ethanol leaf extract that is 6 mm at the concentration of 50mg/ml and ethanol leaf extract that is 5 mm for 50 mg per ml concentration. The chloroform extract of latex and leaf, when taken in 20mg/ml concentration showed no zone of inhibition. It has been seen that extract of *Calotropis procera* is less effective against *E.coli* bacteria by observation.

The *S. aureus* showed zone of 11mm for both leave and latex ethanol extract, whereas the water extract for leaf and latex showed a zone of 11 and 12 mm while chloroform leaf extracts showed zero zone of inhibition while latex shoes 10 mm of zone. Chloramphenicol showed zone of 14 mm when taken in 25 mg/ ml[29].

The Zone of inhibition for *Bacillus cereus* when recorded by others was found that the zone of aqueous extract of leaf when

taken in 30 mg/ml concentration was found to be 14.32mm and for methanol it was 18.24mm[30].

Similarly for *E.coli* bacteria the ethanol showed zone of 11mm and 7mm for leaf and latex extract.Water extract showed on of 10mm and 7 mm for both leaf and latex while the standard showed are zone of 13 mm[29]

Table-3 Zone of Inhibition (Bacteria - S.aureus) - Petroleum ether extract of Leaves and Latex of *Calotropis procera*

	Bacteria - S.aureus											
	Pet.ether latex		**Pet.ether leaf**		**Ethanol latex**		**Ethanol leaf**		**Chloroform latex**		**Chloroform leaf**	
Con.(mg/ml)	50	100	50	100	50	100	50	100	50	100	50	100
ZOI in (mm)	-ve	11	4	5	5	8	-ve	8	2	6	7	10
Chloroamphenicol 25mg/ml	15mm				19mm				15mm			

Table-4 Zone of Inhibition (Bacteria - Bacillus cereus) – Petroleum ether, chloroform and ethanol extract of Leaves and Latex of *Calotropis procera*

	Bacteria - Bacillus cereus											
	Pet.ether latex		**Pet.ether leaf**		**Ethanol Latex**		**Ethanol leaf**		**Chloroform latex**		**Chloroform Leaf**	
Con.(mg/ml)	20	50	20	50	20	50	20	50	20	50	20	50
ZOI in (mm)	4	6	-ve	3	2	5	1	2	1	2	-ve	1
Chloroamphenicol 25mg/ml	17mm				15mm				14mm			

Table-5 Zone of Inhibition (Bacteria – E. coli) - Petroleum ether, chloroform and ethanolextract of Leaves and Latex of *Calotropis procera*

	Bacteria - E.coli											
	Pet.ether latex		**Pet.ether leaf**		**Ethanol Latex**		**Ethanol leaf**		**Chloroform latex**		**Chloroform Leaf**	
Con.(mg/ml)	20	50	20	50	20	50	20	50	20	50	20	50
ZOI in (mm)	1	4	-ve	2	3	5	2	6	-ve	5	-ve	1
Chloroamphenicol25mg/ml	15mm				17mm				13mm			

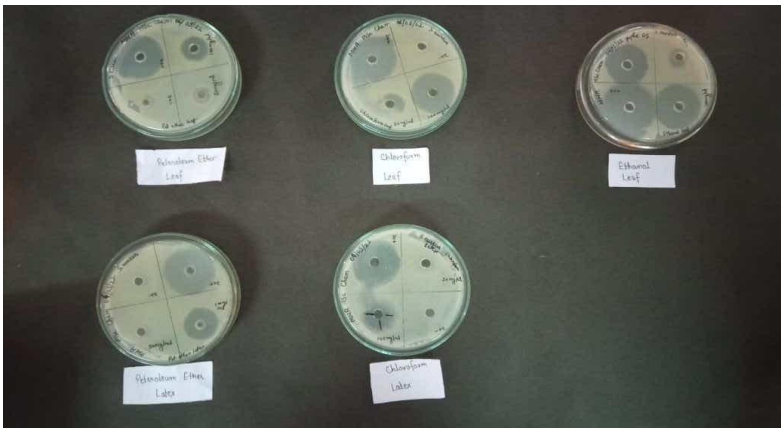

Figure-1 Zone of Inhibition for *Staphylococcus aureus*

Figure-2 Zone of inhibition for *Bacillus cereus*

Figure-3 Zone of Inhibition for *E. Coli* bacteria

Conclusion

80 grams of leaves were taken for the practical which were macerated for 48 hours in the respective solvent to get the extract of the solvent,which were used according to the polarity .The percentage yield of leaf extract of petroleum ether was found to be 1.31%, the alcohol was 6.15 %, chloroform was 2.1%, water extract was 7.5%.Water content has the maximum percentage yield.About 40 ml of latex is used and the percentage yield was found maximum for ethanol equals to 1.66%, followed by petroleum ether extract equals to 0.87%.

The zone of inhibition for *Staphylococcus aureus* for petroleum ether latex extract was shown as that 50 mg/ml, give no zone of inhibition whereas 100 mg/ml of Petroleum ether extract give a zone of inhibition of 11 mm, whereas when petroleum leaf extract was taken in the concentration of 50 mg per ml and 100 mg per ml, they give only zone of inhibition of 4 and 5 mm respectively .The ethanol latex and ethanol leave extract of 50 mg per ml give a zone of inhibition of 5 and 7 mm while when taken in 100 mg per ml it gives the zone of inhibition of 8 mm and 10 mm respectively. Chloroform latex extract when taken in 50 mg per ml gives a zone of inhibition of 2 mm, while when it is taken in 100 mg per ml it give zone of inhibition of 6 mm. Chloroform leaf extract when taken in 50 mg per ml gives a zone of inhibition of 7mm and while the 100 mg per ml concentration gives the zone of exhibition of 10 mm.

For the *Bacillus cereus* bacteria it has been found that petroleum ether latex extract when taken in concentration of 20 mg per ml and 50 mg per ml gives a zone of 4 mm and 6 mm respectively, while when petroleum ether extract of leave was taken in the concentration 50 mg/ml, gives the zone of 2 mm and 3 mm ,whereas ethanol latex extra when taken in 20 and 50 mg per zone of exhibition of 2mm and 5mm respectively ,whereas for leaf it is negative and 2 mm for 20mg/ml and 50 mg/ml respectively. For chloroform extract of latex when taken in 20 mg per ml gives 1mm of zone of inhibition while when taken in 50 mg per ml give 1 mm as zone of inhibition.

For *Escherichia coli*, bacteria when zone of any inhibition was observed for petroleum ether latex when taken in concentration of 20 mg per ml and 50 mg per ml was 4 and 6 mm respectively, whereas for petroleum ether leaf extract, it was found to be 2 mm and 8 mm for 20 mg/ ml and 50 mg/ ml respectively. The ethanol latex and ethanol leaf extract for 20 mg per ml and 50 mg per ml is 5 and 2 for 20 mg, 2 and 6 for 50 mg per ml respectively. The chloroform latex extract for 20 mg/ml shows no zone of inhibition whereas 50 mg /ml shows a 5 mm zone of inhibition, as the latex when taken in 20mg/ml gives no zone of inhibition whereas when the amount is increased upto 50 mg/ml, it gives a zone of 1mm.

From the above results, we can finally conclude that the *S.aureus* bacteria stated showing zones at a concentration higher than 20mg/ml whereas for the other 2 bacteria smaller concentration was good enough to get a zone .Hence, we can conclude that for *S.aureus* the smaller concentration of leaf and latex was not enough for antibacterial effect.

When the *B.cereus* and E.coli were observed for antibacterial activity it has been found that smaller concentration is

effective for antibacterial activity as it started showing zone in 20mg/ml concentration. It can be concluded from the above result that the leaf has greater antibacterial activity as it shows zone greater for the same concentration of latex.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the products.

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