

The Chemical Study of Calotropis

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ABSTRACT

Calotropis (*Asclepiadaceae*) commonly known as “madar” is a useful medicinal plant. The two species i.e. *Calotropis gigantea* and *Calotropis procera* are to a great extent having a very similar chemical properties, but the colours of their flowers are different. The pH of latex of these two species has been found different in the present study. The temperature effects have been noticed on their pH values which is varying from 7.2 to 8.1 between the temperatures 25 °C to 45 °C and then remains constant for *Calotropis gigantea*. The milky latex contains hydrocarbons, fatty acids, sterols and terpenels. Seven spots have been observed on the TLC plates; out of which 3 were identified as calotoxin, uscharin and calactin. Aluminum, calcium, cadmium, cobalt, chromium, copper, iron, magnesium, nickel, lead, and zinc metal elements were investigated in the latex and similarly in the leaves and bark from the AA spectra. The amount of magnesium was found the highest in the latex of both species but calcium was found highest in leaves and bark of both species. Copper, chromium and lead were not found at all in latex but a very little amount of copper and lead were found in leaves and bark. The atomic absorption spectrophotometer was used to investigate the metals which were measured in the order of ppm.

Keywords: *Calotropis*; Madar; AAS; Metals

1. INTRODUCTION

The calotropis, the widely distributed has only two species – *Calotropis gigantea*, and the *Calotropis procera*. Both the species are found throughout the world, but the calotropis procera is more common which has purple flower while the *Calotropis gigantea* has whitish flower. Physically the main difference between the two species that are easily differentiated is their flowers' color while in bud, or bloomed condition. So, it is hard to recognize the species if the plant is not having flower. It is possible to know the species by only pH test of the plants' milky latex that is collected from the cut part of stem. This latex comes out very slowly. The calcium oxalate crystals lend to the milky color in the latex of many common plants as *Calotropis*.

A brief account of the properties of latex of both species was presented by Verma and Verma [1]. Further Murti and Sheshadri studied some properties of calotropis gigantea [2], Dhar and Singh published a paper on the chemistry of calotropis procera [3], Gupta et. al [4] reported the presence of two pentacyclitriterpenes. Edeoga, Okwu and Mbaebie studied a few chemical constituents [5]. The present study has been taken into the account of a more rigorous properties of latex, leaves and bark of both species.



Fig. 1. Flowers of *Calotropis procera* (purple).



Fig. 2. Flowers of *Calotropis gigantea* (white).



Fig. 3. Fruits of *Calotropis sp.*

The *Calotropis* has several uses from the ancient time. Tannin used as dyestuff: A macerated bark extract can be used for de-hairing hides and tanning. The bark and the latex are widely used as arrow and spear poisons. The latex is cardiotoxic with the active ingredient being calotropin. Latex of *C. procera* is 80 % effective in inhibiting the activity of the tobacco mosaic virus. The leafy branches are said to deter ants. Medicine: Compounds derived from the plant have been found to have emeto-cathartic and digitalic properties.

The principal active medicines are asclepin and mudarin. Other compounds have been found to have bactericidal and vermifugal properties. The latex contains a proteolytic enzyme called calotropaine. An infusion of bark powder is used in the treatment and cure of leprosy and elephantiasis. It is inadvisable to use bark that has been kept for more than a year.

The root bark is an emetic, the flower a digestive, and a tonic is used for asthma. Bark and wood stimulate lactation in cattle. Roots (extremely poisonous) are applied for snakebite. The milky sap is used as a rubefacient and is also strongly purgative and caustic.

The latex is used for treating ringworm, guinea worm blisters, scorpion stings, venereal sores and ophthalmic disorders; also used as a laxative. Its use in the treatment of skin diseases has caused severe bullous dermatitis leading sometimes to hypertrophic scars. The local effect of the latex on the conjunctiva is congestion, epiphora and local anaesthesia. The twigs are applied for the preparation of diuretics, stomach tonic and anti-diarrhoeics and for asthma.

Also used in abortion, as an anthelmintic, for colic, cough, whooping cough, dysentery, headache, lice treatment, jaundice, sore gums and mouth, toothache, sterility, swellings and ulcer.

2. EXPERIMENTAL

2. 1. Methodology

pH measurement of *Calotropis* latex

The latex of the *Calotropis* of both the species were tested and was found completely varying in pH values. The *Calotropis procera* latex had an acidic nature with pH of 4.2 at room temp of 25 degree centigrade. The *Calotropis gigantia* latex had a completely basic nature with a pH of 7.2 at 25 degree centigrade. The pH changes with temperature were studied in *Calotropis gigantia* species as given below:

Table. 1. pH variation in *Calotropis gigantia* (with white flowers).

Temperatures	pH values
25 degree Centigrade	7.20
32 degree Centigrade	7.82
35 degree Centigrade	7.92
40 degree Centigrade	8.10
46 degree Centigrade	8.10

The above table shows the pH fluctuation with temperature changes in *Calotropis gigantia* but it does not lose its basic nature. The increase in temperature increases latex alkalinity up to a pH of 8.10 and then becomes constant in *calotropis gigantia*.

2. 2. Procedure to dissolve the *Calotropis* dry latex

The dry latex of *Calotropis* is very difficult to dissolve all its constituents at a single time. This happens because being a plant latex is somewhat like rubber, it contains tannins (a long polymer bound with alkoids, gum, proteins, polysaccharide) and lots more. There are polar and non-polar both types of constituents in a dry latex of a plant. So we needed to dissolve both the polar and non-polar constituents, and for doing so we had to use both polar solvents and non-polar solvents for complete dissolving of the dry latex. Further the dry latex appears as a settled material with brownish liquid above it.

The settled material is like the small granules of lightly grayish or slightly creamish or say dull colour in appearance. So, the bond between them has to be broken. But lastly a way I found to dissolve it. The material (dry latex) was dissolved in a test tube with 3ml toluene, 2ml acetic acid, then 2 ml hexane.

This gave a slightly detergent like appearance in the test tube with lots of very small bubbles. There were two layers formed - one upper one was a clear solution mixture while lower one was slightly light bluish with bubbles and some very fine brown particles like dust which could be seen very carefully. So we added now about 1 ml HCl to the mixture and then brown small particles disappeared. The complete procedure is as

Dry latex (10 gm) + Toulene (3 ml) + Acetic acid (2 ml) + Hexane (2 ml) + HCl (1 ml)



A mixture separated into 2 layers
 [1st Upper layer as a clear solution] &
 [2nd lower layer of light bluish solution]

The toluene (moderately polar) was used to dissolve the moderately polar molecules to least polar molecules as possible. Then the acetic acid (very polar solvent) was taken to dissolve the highly polar molecules, if any. The hexane (non-polar) was used keeping in mind to dissolve the non-polar molecules that were bonded. Lastly the hydrochloric acid as to dissolve the brownish particle like dirt that gave some woody or hard appearance.

The selection of the polar and non-polar solvents were chosen because that when the acid was added firstly and polar and non-polar solvents later, the acid stopped the other reactions to occur and the dissolving did not work. The dry latex was to dissolve to some extent in dichloroether, or acetic acid, hexane, o-xylene (used to clear solvent mixture in test tube after dissolving mainly took place) but the complete dissolving result was not achieved if one dissolved something there, the other got left behind. But finally the above procedure gave a good result and dissolved the Dry Latex completely. The above dissolved dry latex was left as such for a week and then the test tube content was observed. The bluish detergent like liquid that was present had changed to beer like brownish nice colour.

2. 3. Absorption properties of dissolved dry latex of *Calotropis*

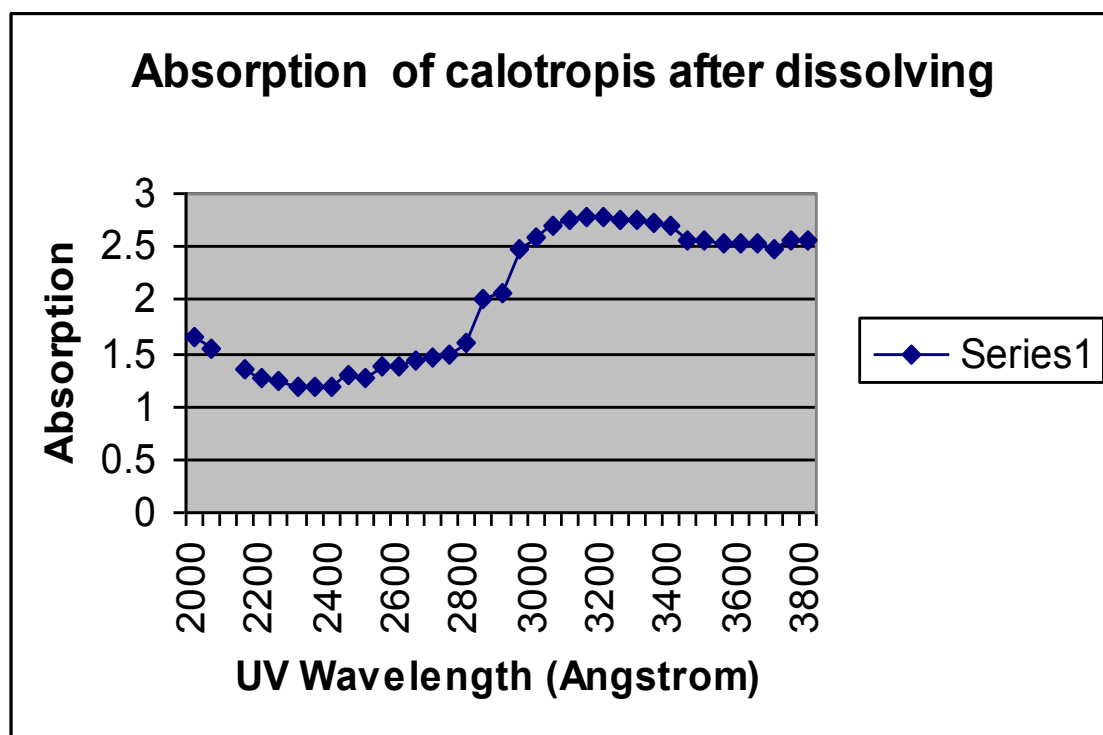


Fig. 4. Line graph of dissolved dry latex of *Calotropis* (immediately after dissolving).

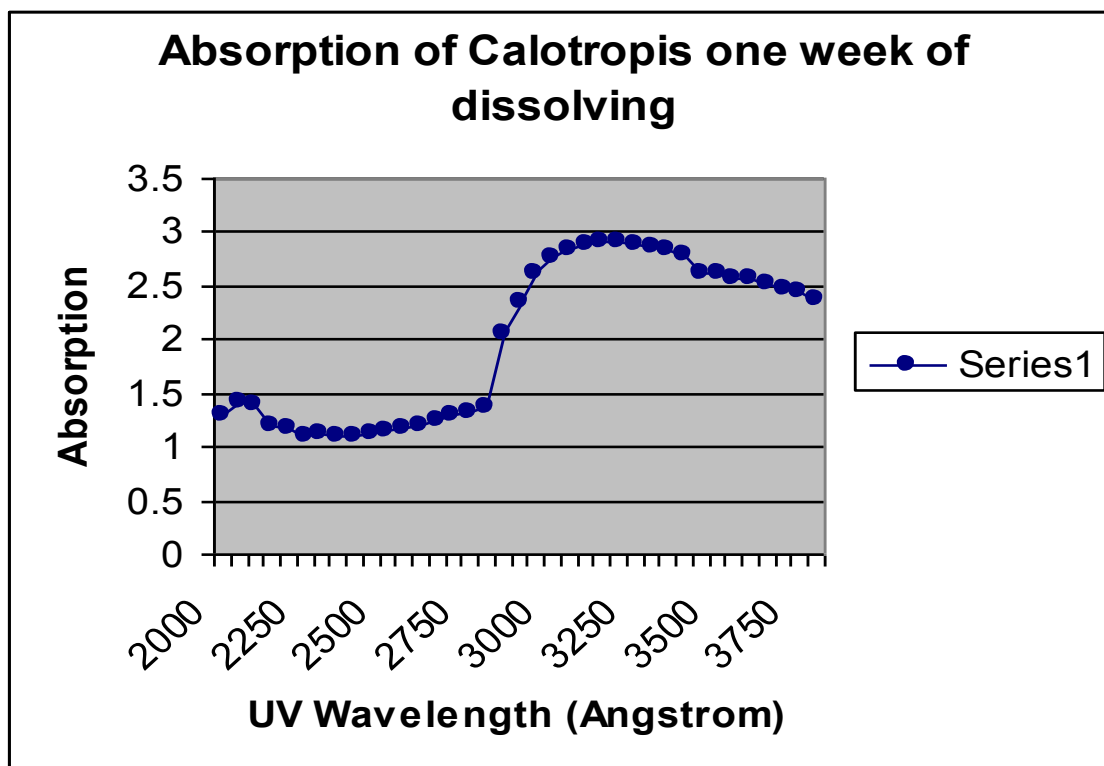


Fig. 5. Line graph of the dissolved Dry latex of *Calotropis* (after one week of dissolving).

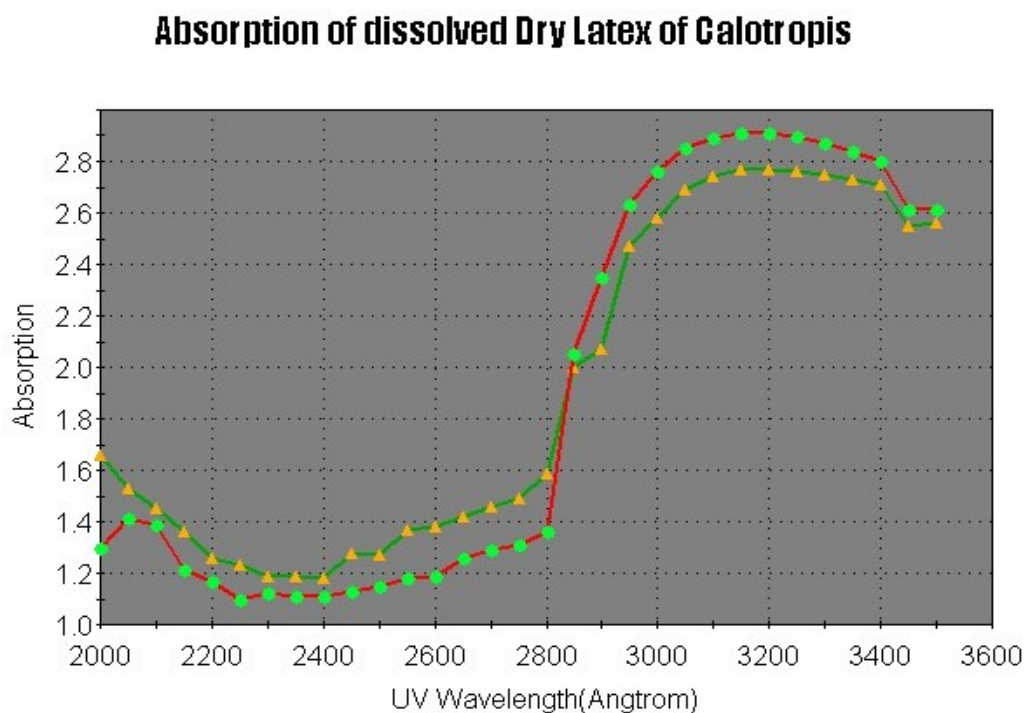


Fig. 6. Comparative line graph of the dissolved dry latex of *Calotropis* (just after dissolving and after one week later).

The green line graph with yellow triangles, show the absorption of the dissolved ved dry latex of *Calotropis*, just after the dissolving is done, when the lower mixture in the test tube was light blue and upper part was clear solution like transparent. The red line graph with green circles shows the absorption of the dissolved dry latex of *Calotropis* after leaving for a week. This spectrometric absorption is done after the lower mixture changes its color from light blue to brownish.

The green lined graph is for absorption at the day when the Dry latex of the *Calotropis* was dissolved. The triangles on the line of the graph shows the absorption at particular UV wavelength. Given .In the green line graph we see that the absorption decreases from a start of 1.66 at 2000 Angstrom wavelength till reaches a minimum absorption of 1.18 through being a little steady between 2300-2400 Angstrom wavelength. After showing an absorption of 1.18 it rises again and goes on increasing continuously. A rise is seen between wavelength 2400Angstrom to 2800 Angstrom. After a wavelength of 2800 it shows a certain frequent rise to an absorption value reaching 2.0. Then increases slowly to 2.07 to 2850 Angstrom and after that rises again quickly to 2.47 at 2950 Angstrom. After this wavelength goes on to increase from 2950-3450 Angstrom and goes to form somewhat circular graph as the absorption increases and then shows a very slight variation and then comes down .During this absorption comes to maximum of 2.77 at 3150 and 3200 Angstrom. This 2.77 is the peak absorption for the dissolved dry latex . Later there is a decrease in the absorption for higher UV wavelength.

This graph shows much absorption in the region of UVA ultraviolet wavelength of 3150-3400 Angstrom. It shows some absorption in the UVC region of UV wavelength (germicidal or short wavelength).This shows that the dry latex has some germicidal properties. The short wavelength at 253.7 nm is called germicidal wavelength and it is wavelength at which micro-organisms are killed. But still some micro-organisms are more resistance to UV then others. Molds are more resistance to UV then bacteria. So some organisms need higher intensity and time exposure to kill the same amount micro-organism and show some amount of killing. Temperature and humidity also effect the killing rate.

The second reading of absorption was taken after a week, when the dissolved liquid showed a change in the color in the lower part from light bluish to brownish. This experiment was performed to check whether the color change had taken place in the dissolved dry latex has some chemical changes. The experiment was done in the same procedure as in previous case. The graph shown by red line graph starts absorption of 1.3 at 2000 Angstrom and rises to 1.41 and then starts to decrease and shows a very slight fluctuations between a UV wavelength of 2250 to 2450 from 1.10 absorption to 1.13. Then shows gradual increases with slight decreases at some points of UV wavelength. After a UV of wavelength of 2800 Angstrom it shows a big rise in absorption from 1.36 to 2.05 and then goes in increasing order and reaches a peak of 2.91 and again starts decreasing forming a somewhat parabola. After at UV wavelength of 3400 Angstrom it shows a clear decrease.

2. 4. Comparision of the two graphs

The comparison from the two graphs as shown above we found that both are running in same shape with an exception at the beginning. This perhaps is due to the chemical changes that occurred due to color change from light blue color to brownish color within a week.

A point can be seen in both graphs that after a UV wavelength of 2000 Angstrom the absorption rises much. So absorption in both cases is higher when UV of higher wavelength is passed through the dissolved liquid .

Line graph of the dissolved Dry latex of *Calotropis* (after one week of dissolving). Comparative line graph of the dissolved dry latex of *Calotropis* (just after dissolving and after one week later).

2. 5. Properties viewed from the graphs

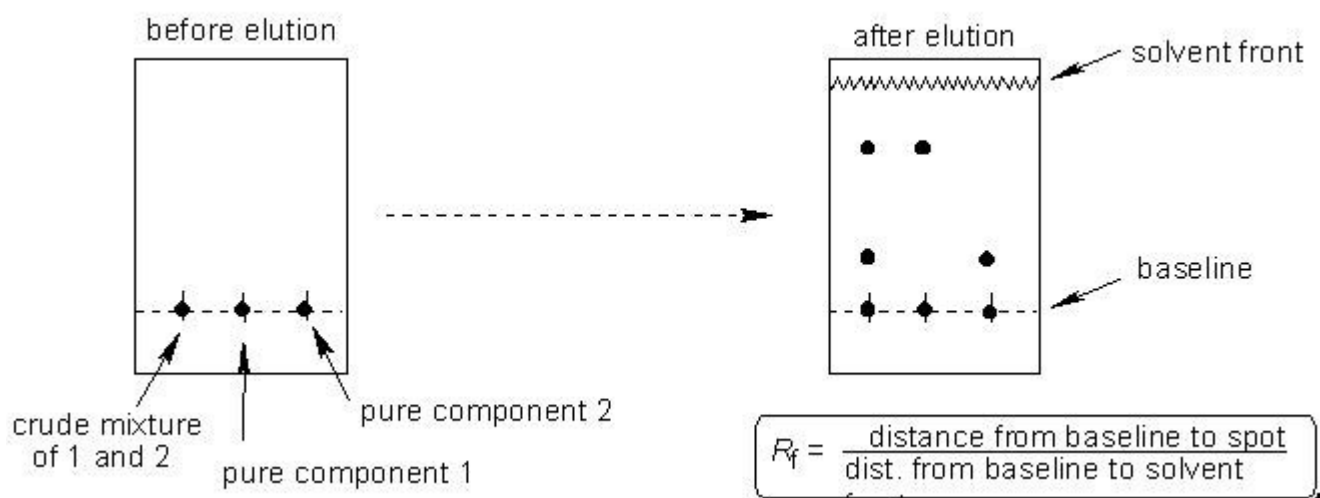
- (1) This Shows an absorption in UVC range(i.e. 2000 to 2800 Angstrom). This region of the UV wavelength is considered as the Germicidal .The absorption in this region is used in Dermatology.
- (2) In UVB, absorption is much high and has been in increasing order with wavelength. The absorption at this UV wavelength is related to Sun tanning. So *Calotropis* can be a source to protect skin from harmful Sun rays as it has such a useful property.
- (3) It shows absorption also better in the UV wavelength ranging from 3200-3800 Angstrom. Absorption in this area refers to UV curing.
- (4) Also shows a better absorption in the UV wavelength ranging from 3400-3800 Angstrom So we see that *Calotropis* has absorption fluctuating in the invisible region of spectrum having electromagnetic radiation having wavelength from 2000-4000 Angstrom.

2. 6. Thin layer chromatography

In thin layer chromatography solid phase (i.e. absorbent) is coated with a Silica or Alumina. The coating acts as a binder on one side. The solid phase used may be glass, aluminum or plastic. The glass palte was used as solid phase. A thin film of silica gel is coated on the glass plate. The mixture were to separate to spots is dissolved completely using any polar, non-polar, or acids. A mark is made by a pencil about 1cm height from the lower edge of the end of plate. The dissolved liquid of dry latex is spotted on this line, by the help of fine capillary, 2 or more spots can be placed on this line, but the distance between the two spots should be at least 2 cm. This distance maintains the spots from over lapping or mixing while separating. A solvent or mixture is selected and kept in a wide mouth container having a top cover to it. The plate is placed in this container such that the liquid solvent does not touch the line marked with spot with a pencil. This solvent is called as eluant and flows and goes up by capillary action on the plate. When the solvent goes up it takes the compound present in the spot on the line to some distance and the compounds separate and appear as spots. Sometimes these spots appear as greasy appearance, yellow, white, brown or even red. Different spots are seen at different distance from the line marked. The number of spots that come on the silica plate depends on the solvent used, and number of compounds present in the dissolved liquid to be spotted on the line on the plate.

The compounds that are closer to the line marked having spots marked to it on the plate, show less movement and are more polar than the compounds that make movement as spots further from the lower line marked. Several factors determine the efficiency of a chromatographic separation. One important property of the solvent used in as moving phase is its ability to be itself absorbed on the absorbent. If the speed on movement of solvent over the plate is very slow, it means the separation of the spots on the plate would not be much. Movement of the spots of compounds on the silica plate varies also with selection of solvent. The spots that come on the silica plate also have particular shape and colour. The distance traveled by the solvent is marked with a pencil and the spots are seen by help of iodine crystals vapour kept in a closed chamber and UV-lamp and marked as the shape the make. One should note look directly facing UV lamp as will damage eyes.

The distance traveled by the spots and distance traveled by the solvent is noted. The R_f value of each spot is calculated. R_f value of a spot is the distance traveled by the spot from the line marked to the distance traveled by the solvent (moving phase) from the line marked on the plate. This R_f value we get is compared to the R_f value chart. This value gives us the knowledge of the compound present in the dissolved liquid of dry latex we use. The number of spots that separate also depend on how nicely the dry latex is dissolved. The dissolving should be like clear solution through which one can see. No problem dissolved liquid may be colored. Sometimes the R_f value of 2 more spots may be very close. The spots in this condition will overlap each other by edges or more



As mentioned that the No. of spots that come on the silica plate may vary on the basis of solvent selected as moving phase. The solvent selection is done at random just to see which moving phase gives a better number of spots. The moving phase selected may be a single particular solvent or a mixture of 2 or more solvents in some proportion. Selection of the eluant is done keeping in mind the polarity and non-polarity of the solvent.

2. 7. TLC for *Calotropis* dry latex

The dry latex also gets dissolved in hexane + diethyl ether + o- xylol. The dissolved mixture is always dissolved forming two layers ,mostly above slight whitish or colorless and lower colored. But we came to know that it did not dissolve totally, something was left un-dissolved. All was dissolved but some cotton (or fine fibre) like thing with a few fine brown dots was floating un-dissolved. This could be seen when seen carefully.

The dissolved Dry latex in hexane + diethyl ether + xylol dissolves somewhat completely giving upper whitish liquid and lower particular sediment. This was separated in 2 parts to upper first part more xylol was added to give clear solution and to lower part Acetic acid + acetone was added to give clear dissolved. But this way of dissolving in TLC gives lesser number of spots. This also showed movement with moving phases as Hexanes, Toluene, (Carbon Tetrachloride, Chloroform etc.). The movement in which Chloroform taken as moving phase, takes one and half hours more two hours for moving phase to rise over the

silica plate. When we used polar solvent as moving phase, the more better number of spots were seen away from initial spot marked and movement was faster.

As we did not get much spots in above case and thought something remain undissolved. So, now we tried to dissolve the *Calotropis* latex by a different better process and found that the process described in topic above “dissolving of *Calotropis* dry latex”.

Dry latex (10 gm) + Toluene (3 ml) + Acetic acid (2 ml) + Hexane (2 ml) + HCl (1 ml)



A mixture separated into 2 layers
[1st layer as clear solution &]
[2nd lower layer of light bluish solution]

This dissolved mixtures obtained are separated in 2 parts and both parts are spotted by help of fine capillary on the line marked by a pencil 1cm from lower edge of TLC plate. Distance between the two spots marked is kept at least 2cm. So that the spots formed by the two marks do not overlap. This silica plate was now placed in a container containing selected solvent (i.e. eluant) and was cover and left. The eluant was left to rise on the plate. Both the spots marked so rarely similar spots (as are part of same dissolved latex) but show spots that moved different distances (i.e. had different Rf values). There was a very unique spot, long and had a greasy appearance and had several compounds overlapping which was easily coming to seen. This large spot developed from the spot marked with the lower light blue dissolved part of dry latex dissolved. Several silica plates were spotted time to time and placed in other moving phase to see what spots we get. The Rf value was calculated. Several times we got similar Rf values in different phases. Some are mentioned below:

Rf values when water selected as moving phase:

(Observed 6 different spots)

Rf values are 0; 0.25; 0.29; 0.52; 0.74; 0.88

Rf values when petroleum ether was used as solvent;

(Observed 14 different spots)

Rf values are 0; 0.03; 0.08; 0.13; 0.21; 0.24; 0.29; 0.49; 0.57; 0.66; 0.82; 0.84; 0.89; 0.93; 0.968

Rf values when acetone was used as solvent:

(Observed 7 different spots)

Rf values are 0.08; 0.18; 0.24; 0.33; 0.4; 0.79; 0.93

Rf values when a combination of chloroform, benzene, ethanol was used:

(Got 9 different spots)

Rf values are 0.05; 0.15; 0.37; 0.49; 0.68; 0.83; 0.86; 0.90; 0.98

Rf values when a combination of chloroform, methane, Carbon tetrachloride is solvent:

(Observed 1 spot)

Rf value is 0.39

2. 8. Phytochemical constituents

Some plants are of a great importance due to their special constituents. These constituents have chemical substances that produce definite physiological action in human body when consumed in some fixed amount. Generally in plants these bioactive constituents are alkoids, tannins, flavonoids, and phenolic compounds (Hill, 1952). For the phytochemical procedure, we used the leaves and stem including flowers.

These are air-dried and grinded into fine powder. For the test we soak 100 g of dried powder sample in 200 ml of distilled water for 12 hrs. This extract is filtered by filter paper. The filtrate is used to perform the experiments. But even the dried extract (100 g) can be boiled in water in a beaker with a top cover and this can be filtered after cooling and be filtered through Watmann filter paper. This filtrate can be kept to use for the phytochemical constituent detection.

Phytochemical screening chemical tests are carried out on the aqueous extract and on the powdered specimen as described below.

The grinded part of the plant had wood like particles, brown small particles, white finner particles and brownish powder. When we dissolved the substance (powder) in distilled water, something was sticking to the wall of the beaker, it had somewhat greasy and cotton fibre (very fine) like in appearance. When the water was put into the beaker, there was a light brownish (color as black tea with lemon) liquid below with fine dark brown and white particles and rest things came above to float. The floating part had wood like particles, some a bit larger blacker particle than below one.

The above was heated on a hot plate placed and while heating the beaker was covered so that nothing should evaporate out of it. If any volatile thing evaporates it comes back as drop below into liquid back. The boiling started after a bit of time. The bubbling was slower and later increased even though the liquid does not run out.

This boiling of the liquid was always below the upper particle layer. Later, now the upper layer was seen to have got lots of small bubbles in it but the particles there did not show vertical movement. Lots of vapour was formed and was falling into the boiling liquid. But the boiling liquid did not rise to fall out of beaker due to boiling. Something was seen to evaporate along with the vapour and sticks to all the area of vapour on beaker wall and the lid used to cover the beaker. This thing had a fibre like appearance when observed when the lid was dried.

This cotton like thing was always noticed while dissolving the Dry Latex of the *Calotropis*. So, the beaker area empty and the lid used to cover the beaker both had this fibre like appearing material on it. This material when washed with water does not leave the lid just by falling water, but has to be cleaned by rubbing carefully. It took 30 min for 50 ml of the liquid to boil and make the upper particles to come to lower and show movement. Lots of small bubbles were present at the edges of the outer surface of liquid to the beaker wall. After 45 min. all the particles present at surface settled down as sediments in the liquid mixture boiling. This boiled extract was cooled placing the beaker in a plastic container containing water and filtered later.

The lid of the beaker was removed at the time of filtering. While cooling the beaker is still covered as to get back if anything evaporated to vapour in form of drops back to liquid. The filtrate after boiling and cooling becomes a bit more brownish solution. The following tests were done a day later keeping filtrate in a closed container of glass. (Here we have used *Calotropis procera* for the experiment, that plant which has purplish colored flowers).

2. 9. Observations of the phytochemical screening on the aqueous solution of *Calotropis* extract

Test For Tannins:

0.5 g (powdered sample of *Calotropis* dried extract) is boiled in 20 ml water in test tube. To its filtrate add 0.1 % of Ferric Chloride. This gives a Brownish green color to the mixture. This result also comes if we use the filtrate of the extract boiled above. This brownish green color shows the presence of Tannin in the plant.

Tannins:

These are astringent, having a bitter test that bind and precipitate proteins. Tannins have a high molecular weight from 500 to 3000. These are generally large polyphenolic compounds containing sufficient hydroxyls and other suitable groups (as carboxyls), that form complexes with proteins and other macromolecules. Tannins are generally found in woody plants. Tannins are of two types - hydrolyzed tannins or condensed tannins. The tannins seen to bind to proteins, starches, cellulose and minerals. This property of tannins to bind to proteins, is used in leather industry for tanning of leather.

Test For Saponin:

The filtrate (10 ml) of aqueous plant extract was taken and 5 ml of distilled water is added. This mixture now in the test tube is vigorously shaken for a while. Permanent froth is found. When to this froth 3 drops of olive oil was added and shaken vigorously, we got an emulsion. This shows that *Calotropis* has Saponin in it.

Saponin:

These are glycosides of steroids, steroid alkaloids (steroids with nitrogen function) found in plant. Saponins are glycosides with a distinctive foaming characteristic. This provides waxy coating to the plant parts and helps in terms of protection. These are amphiphilic in nature and thus dissolve in water and form froth as soap gives with water. Generally the saponins are useful but some are poisonous to humans and may cause skin rashes if swallowed. Some saponins help in controlling cholesterol.

Test For Flavonoids:

Powdered plant extract was added to 10ml of ethyl acetate and was heated in water bath. The mixture was filtered. To this filtrate 1ml of dil Ammonia solution was added. We have a yellow colouration. Even this can be done taking aqueous filtrate of plant added with 10 ml of Ethyl acetate. This is heated and the test tube is placed in water container and 1 ml of dil. Ammonium solution is added. This will give yellow coloration This shows flavanoid is present.

Flavonoids:

Refer to a plant secondary metabolite. These have antioxidant property. These provide health benefit as acting against cancer and heart disease. Also known as Bioflavonoids. These are found to give pigmentation to the flower of the plant as yellow, red or bluish and also protect against microbes and insects. This have very low toxicity to other compounds in the plant extract.

Test For Steroids:

To 0.5 g ethanolic extract we added 2 ml of Sulphuric acid. Then 2 ml of acetic anhydride was added. There was no change of color from violet to Blue or green. So, we see a negative result for Steroids. No steroid present in *Calotropis* extract.

Test For Terpenoids:

The aqueous filtrate of the plant extract is taken in a test tube and then 2 ml of chloroform is added and mixed well (be careful not to fall content out of test tube). The test tube is placed in a water container to keep cool. Now 3 ml of Conc. sulphuric acid is added from the side of the test tube. We observed a clear Reddish brown coloration at the interface. This proves the presence of Terpenoids in the plant extract. This a positive result for presence of Terpenoids. Leave this test tube in the water and after 30 min observe it a more clearer wider reddish brown coloration develops at the interface.

Terpenoids:

Also called as Isoprenoids. These are naturally occurring organic chemicals (hydrocarbon) somewhat close to terpenes. These are derived from 5-carbon isoprene units assembled and modified in many ways. All differing in functional groups. This a lipid found in all living things. Plant Terpenoids are used for aromatic qualities. This property makes a traditional herbal medicine by its antibacterial, antineoplastic and other medicinal values.

Test For Cardiac Glycosides:

To 5 ml of the extract filtrate added 2 ml of glacial Acetic Acid and 1 drop Ferric Chloride solution. Then 1ml conc. Sulphuric acid is added from the side of test tube. This gives a more browner ring at the interface then the mixture above the interference and in the Acetic acid layer a greenish ring is seen as a thin layer with a slightly very light green color. The rings are clearly visible and stay. This proves presence of Cardiac Glycosides in the *Calotropis* plant extract we have.

All these experiments of the phytochemical screening is done keeping the test tube in water in a container. So the reaction cools and nothing boils and throws through out of the test tube.

3. DISCUSSION

3. 1. Fungicidal property of *Calotropis*

Fungicidal property of a substance refers to its effect of that substance to kill fungi. These substances which can kill the fungi are of great importance to many fields of medicinal and agricultural areas. Their are several plants in nature that help us to get fungicidal liquids. These liquids can be used to control the different fungi. As a result controls several plant and animal diseases caused by fungi group members.

Experiment was performed on by selecting fungi spore on area containing fungi hyphae in large numbers. This selected part was taken off the culture and was placed on a slide and viewed under microscope firstly under 100X and then under 40X resolution. The fungal hyphae cells were clearly visible with their cell wall. Later a drop of the liquid that is present as brownish color on top, in the dry latex of was placed upon this hyphae on slide under the microscope viewed again. The slide prepared still had the same type of view as was previously viewed. But after 10 minutes a complete change was seen in the structure of the cells that formed hyphae of the fungi. During this observation the slide was set to view a particular area and was under continuous observation to see the speed of change and the change. The speed of change was not fast as it took about 10 minutes, but the change was quite clear. It was observed that the cell wall got broken and the area became more denser in color due to losing off the content of the cells. This with time get a bit denser in color and with more broken walls.

Here, for the above experiment we used the fungi "*Aspergillus flavus*" from a culture medium previously done. This is one of the very harmful fungi, which causes serious diseases not only in plants but animals also. In the plants as crops and grains this causes common diseases in peanuts and corn. In animals this pathogen is associated with to the disease *Aspergillosis* of lungs, and Corneal disease, Otomycotic, and Nasoorbital infections. *Aspergillus flavus* is also able to produce aflatoxin (a carcinogenic substance) that can cause cancer. Mycotoxins are also said to be produced by *Aspergillus flavus*.

This proves that the *Calotropis* latex content is having the antifungal property and can kill several fungi. So there is a possibility of the latex to be a cure to several diseases of fungal origin. It can be a source to be used to form some medicine for animal use and sprays for plant

3. 2. Antibacterial property of Calotropis dry latex contents

The experiment was performed on *E.coli* (a very harmful gram-ve bacteria) causing diseases. The main target is the bacterial cytoplasmic membrane. After chlorhexidine has caused extensive damage to the cytoplasmic inner membrane, precipitation or coagulation of protein and nucleic acids occurs. Damage also occurs to the outer membrane in gram-negative bacteria and the cell wall in gram-positive cells. Chlorhexidine also damages the cytoplasmic membrane of yeasts and prevents the outgrowth, but not the germination, of bacterial spores. If chlorhexidine is hydrolyzed, small amounts of carcinogenic para-chloraniline may develop (87); this chemical has been found even in manufactured chlorhexidine solutions. At temperatures above 70 °C, chlorhexidine is not stable and may degrade to para-chloraniline. An upper limit for para-chloraniline has been set in the British Pharmacopoeia at 0.25 mg per 100 mg of chlorhexidine).

At lower concentrations, chlorhexidine has a bacteriostatic effect against most gram-positive bacteria (e.g., at 1 µg/ml), many gram-negative bacteria (e.g., at 2 to 2.5 µg/ml), and bacterial spores. At chlorhexidine concentrations of 20 µg/ml or more, a bactericidal effect can be expected as well as activity against yeasts. The actual effective concentration against *Burkholderia cepacia* and *S. aureus* varies with different supplements from 0.004 to 0.4 % (factor 100), and the actual killing time also varies with different supplements (phenylethanol or edetate disodium) from < 15 min to > 360 min (465). In most studies, concentrations for rapid inactivation are well in excess of MICs, e.g., for *S. aureus*, *E. coli*, *Vibrio cholerae*, and yeasts. When used in a liquid soap, chlorhexidine usually has a concentration of 4 % and exhibits a bactericidal activity against various gram-negative and gram-positive bacteria. In some comparative studies using suspension tests chlorhexidine (4 %) was found to be less effective against MRSA than against methicillin-susceptible *S. aureus*, which has raised concerns about the suitability of the active agent in the prevention of transmission of MRSA. This concern has been confirmed with enterococci. Against Enterococcus species and VRE, chlorhexidine (4 %) was found to be essentially ineffective in suspension tests if neutralization of residual activity is excluded. In a comparison with a nonmedicated hand wash product, a chlorhexidine-based scrub yielded a lower reduction of different antibiotic-resistant test bacteria such as MRSA, VRE, or high-level gentamicin-resistant enterococci. Chlorhexidine has no sporicidal activity. The data on mycobactericidal activity are not unambiguous but do indicate the relevance of a threshold concentration of chlorhexidine. In one report, 4 % chlorhexidine was described as having very good activity against *Mycobacterium smegmatis* (reduction of > 6 log₁₀ units within 1 min), whereas another study with *Mycobacterium tuberculosis* suggested a low activity of 4 % chlorhexidine (reduction of < 3 log₁₀ units within 1 min).

Chlorhexidine at 1.5 % did not reveal sufficient activity against *Mycobacterium bovis*, and chlorhexidine at 0.5 % had no activity against *Mycobacterium avium*, *Mycobacterium kansasii*, or *M. tuberculosis* within 120 min .

3. 3. Effect of *Calotropis* on the white fungal like structures stuck to flowering plants

The *Calotropis* bad smelling liquid that comes on the top of the dry latex stored in test tube. When this liquid was dropped 2-4 drops on the fungal like appearing structures stuck to the flowering plants leaves, stem and sometimes the roots. These white structures completely got killed leaving only a dead hollow porous appearance there. The spongy fungal like structure completely lost its fungal look. Actually these are insects with fungal like growth over them. This killing occurred within 24 hrs.

3. 4. Effect of *Calotropis* dry latex on yellow spot disease of citrus

The brown liquid obtained in the dry latex of *Calotropis* was applied several times on the selected yellow spots present on the leaves of orange plant. After a month the all these spots applied with the liquid were seen to have disappeared. This yellow spot diseases is a very well known popular disease of the citrus plant.

3. 5. Elemental analysis

The metal elements analysis were performed using the Atomic Absorption Spectroscopic Technique. The samples were dissolved in the mixed solution of nitric acid and the hydrochloric acid. The hydrogen peroxide was then used to clear the samples. These samples were then used to run the AA spectrum of all samples i.e. latex, leaves and bark. The following table shows the presence of the metal elements in these samples. It has been observed that their presence differ to a great extent.

Tabel 2. Elemental composition of Latex,leaves and bark of *Calotropis gigantea* (white flower) and *Calotropis procera* (purple flower).

Elements Tested	Samples description					
	<i>Calotropis Gigantea</i>			<i>Calotropis Procera</i>		
	Latex	Leaves	Bark	Latex	Leaves	Bark
Al	300.00	nd	nd	200.00	nd	nd
Ca	300.00	3987	1774	340.00	3694	1650
Cd	100.00	4.54	2.27	120.00	2.10	2.12
Co	690.00	4.20	3.62	80.00	2.54	2.52
Cr	nd	nd	nd	nd	nd	nd

Cu	0.00	5.75	11.96	0.00	6.01	7.23
Fe	1000.00	164.81	61.44	50.00	72.69	38.32
Mg	40000.00	1544.00	1401.00	39400.00	11.32	529.00
Mn	27.00	26.20	3.43	17.00	8.07	nd
Ni	50.00	nd	nd	50.00	nd	nd
Pb	0.00	0.55	0.31	0.00	0.36	0.33
Zn	106.00	11.77	19.60	23.00	11.62	8.93
K(%)	nd	3.85	3.67	nd	3.82	4.29
N(%)	nd	3.82	1.97	nd	4.46	1.44
P(%)	nd	0.34	0.46	nd	0.58	0.29

nd = Not detected

Values are in (mg/kg)

The above table is very much self explanatory. The amount of magnese was found highest amount in latex of both species but the Calcium was found highest in leaves and bark and of low level in latex of both species. The level of chromium was completely absent in all samples. The iron was of medium amount in latex of *Calotropis gigantea* but very low level in other samples.

4. CONCLUSIONS

The main findinds can be concluded categorically as

- (1) The pH of the latex of both species is behaving differently with the the variation of temperatures.
- (2) Antibacterial property has been found with latex of both species.
- (3) The presence of tannins, saponin, flavonoids, terpenoids and cardiac glycosides were observed but no steroids.
- (4) The magnese has the highest amount in latex of both species but the Calcium has the highest amount in leaves and bark.
- (5) Chromium and lead were absent in all samples.
- (6) Some different features have also been found in latex, leaves and bark.

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