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Phytochemical analysis, TLC and UV studies of *Calotropis gigantae* leaves extract

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Abstract:

Background: *Calotropis* genera comprise of two species, with 90% inhabiting southern Asian country and are most endemic to the India, Indonesia, Malaysia, Thailand, and Srilanka, China. **Objective:** The present project attempts to study the phytochemical and TLC and UV studies *calotropis gigantea* leaves extract.

***Calotropis gigantean* L. Material and Methods:** The latex of ***Calotropis gigantean* L.**is subjected to various extractions using ethanol and distilled water. The extracts were subjected to phytochemicals were screening and antimicrobial study was performed against different bacterial species i.e. *E .coli, S.aureus, S. dysenteriae, B. subtilis* using disc diffusion method. **Result:** The aqueous extracts and ethanolic extracts show presence of alkaloid, tannin, flavonoid, saponin and cardiac glycosides. The UV studies show the absorbance of the different concentration of the phytochemical extract.

Keywords: *Calotropis gigantea;* phytochemical screening; antimicrobial activity; ethanolic extract;

INTRODUCTION:

In the ayurvedic system different herbal extracts have been used for centuries because many constituents with more than one mechanism of action are considered to be beneficial [1]. The kingdom of plants is a source of a vast spectrum of medicinally active compounds. Plants have synthesized these compounds using different metabolic pathways. Therefore, it is essential to establish a quick and reliable method for screening plant extracts. Plants, with a huge number of bio active ingredients, are the primary suppliers of natural herbal medicines that are effective against different diseases, including cancer, and these bio active components are associated with the biological activities of plants[2].Over the last few decades, the importance of herbal medicines for the treatment of various diseases has risen exponentially because a vast number of people belonging to diverse cultures rely on the use of phytomedicines due to a shortage of primary health facilities. Calotropis contain some of the sesquiterpene which are also having biological activities against cancer, inflammation, parasitic,bacterial.fungal,viral infection and other functional disorders[3].According to the

World Health Organization reports on phyto medicines, more than 25 percent of the drugs prescribed in recent years come from various plant sources. Herbal medicines which refers to the medicinal product of whole plant is one of the best sources for extraction of chemo prevention agents as they are nontoxic in nature and causeless or no side effect to the patients. *Calotropis gigantea* L.(Apocynaceae) is commonly known as "Arka" or giant milk weed. It is a large shrub or small tree growing 3-4 m tall. The milky juice of the plant was used against cancer, arthritis and inflammation. [4-5].

Material & method:

Plant Collection

Calotropis gigantea L. plants were collected from Barasat, West Bengal, India. The leaves are collected from the plant. The leaves were allowing to shade dried for 15-20 days. Dried leaves were chopped and grounded into a fine powder. It was stored in air tight container for further use.

Preparation of extracts

The leaves aqueous solution and alcoholic solution with ethanol was prepared. Then these two mixture were left for three days in 4-8℃ with shaking. The filtrate was collected and spread these filtrate in petridis. The petridis were placed upon water bath and leave it for evaporation. After evaporation the remaining extracts were used for next tests [6].

Physiochemical investigation

Moisture Content

The percentage of active chemical constituents in crude drugs is mentioned in air dried basis, Hence the moisture content of a drug should be determined and should also be controlled. The moisture content of a crude drug should be minimized to prevent decomposition of crude drugs either due to chemical change or microbial contamination.

The moisture content is determined by heating 1gm of drug at 105^0 C in an oven to a constant weight. After 30 minutes of heating the moisture content was found to be 12% of the powdered drug.

Ash value determination

Ash values are helpful to determine the quality as well as purity of a crude drug, especially when the drug is present in powdered form. The object of ashing crude drugs is to remove the traces of organic matter which may be interferes in an analytical determination.

Calculation-weight of the crude drug=3.88gm

Weight of the crude drug after charring=2.95gm

ASH VALUE= (3.88-2.95) gm=0.93gm

% of ash value= (0.93/3.88)%=0.23%

The Acid Insoluble Ash (AIA) content is the proportion of a sample that is not hydrolyzed by 72% sulfuric acid and is not subsequently volatilized upon the incineration of this Acid Insoluble Residue.

Calculation-weight of the ash before acid digestion=1.55gm

weight of the ash after acid digestion =1.20gm

so acid insoluble ash=1.55-1.20gm=0.30gm

% acid insoluble ash=(0.30/1.55)%=19.34%

Qualitative Phytochemical Analysis

The different qualitative chemical tests were performed for establishing the profile of given extract of its chemical composition. The crude drug was re-dissolved in methanol and subjected to various phytochemical analyses. The following tests were performed on the extracts to detect various phyto constituents present in them [7].

Detection of Alkaloids

a) Mayer's Test:

To a few mL of filtrate, a drop or two of mayer's reagents was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

b) Hager's Test: Alkaloids give yellow precipitate with Hager's reagent (Saturated solution of picric acid).

c) Wagner's Test:

To a few drops of filtrate, few drops of wagner's reagent was added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive.

Alkaline reagent test:

To the test solution add few drops of sodium hydroxide solution. Intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicates the presence of flavanoids.

Zinc hydrochloride test:

To the test solution add mixture of zinc dust and conc. Hydrochloric acid. It givesb red colour after few minute.

Leucoanthocyanidine test:

Leucoanthocyanidine gives red color in strong acidic media. While in weak acidic media nutralised or Ionised base is formed which imparts blue colour.

Detection of saponins:

Foam Test:

The extract (50 mg) was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 10 min. A two cm layer of foam indicated the presence of saponins.

Detection of Anthraquinone glycosides:

Borntrager's test:

Boil the test material with 1 ml of sulphuric acid in a test tube for five minutes. Filter while hot. Cool the filtrate and shake with equal volume of chloroform. Separate the lower layer of chloroform and shake it with half of its volume of dilute ammonia. A rose pink to red colour is produced in the ammoniacal layer.

Modified Borntrager's test: Boil the test material with 2 ml of dilute sulphuric acid. Treat it with 2 ml of 5% aqueous ferric chloride solution for 5 minutes, shake it with equal volume of chloroform and continue the test procedure as Borntrager's test.

Detection of Hydroxy-anthraquinones:

Treat the sample with potassium hydroxide solution red colour is produced.

Detection of Cardiac glycosides:

Liebermann Buchardle's test:

Extract of powered drug when treated with acidic anhydride and concentration sulphuric acid given bluish green colour.

Baljet's test:

Treat the test solution with picric acid, orange colour formed.

Detection of Tannins:

Ferric chloride test: Treat the extract with ferric chloride solution, blue colour appears if tannin is present.

Gelatin Test: To the test solution add 1% gelatin solution containing 10% sodium chloride. Precipitate is formed.

Table 1: Phytochemical screening of the ethanolic and aqueous extract

Tests	Ethanolic Extraction	Aqueous Extraction
Detection of Alkaloids:		
Mayer's Test	+ve	+ve
Hager's Test	+ve	+ve
Wagner's Test	+ve	+ve
Test For Flavonoids:		
Alkaline reagent test	-ve	-ve
Zinc hydrochloride test	+ve	+ve
Leucoanthocyanidine test	+ve	+ve
Test forSapnonin:		
Foam test	(-)Ve	(+)Ve
Test for Anthraquinone Glycoside:		
Borntrager's test	(-)Ve	(-)Ve

Modified borntrager's test	(+)Ve	(+)Ve
Test for Hydroxyanthraquinon	(+)Ve	(-)Ve
Test For Cardiac Glycosides:		
LibermannBuchardle's test:	(+)Ve	(+)Ve
Baljet's test	(+)Ve	(+)Ve
Ferric chloride test	(-)Ve	(-)Ve
Gelatin Test	(+)Ve	(-)Ve

Thin Layer Chromatography (TLC)

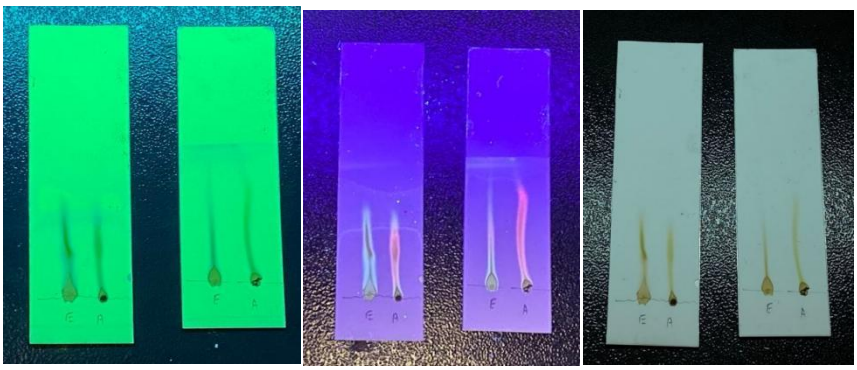
Thin layer chromatography was performed with various crude extracts such as such as hexane, chloroform, ethyl acetate and methanol of *Calotropisgigantea*. For each extracts.The ratio of the solvent is 3:2 and 2.5:2.5 of chloroform and methanol, chloroform and ethanol

TLC plate should be 8cm long in which spots were kept at above 1 cm. Silica Gel plate is used to separate the compounds. Based on the band that appear in TLC plate Retention factor (Rf) value can be calculated[8].

Rf = distance travelled by the compound / distance travelled by solvent front

TLC profile *of Calotropi sgigantea* L. on Methanol crude extract solvent system

Chloroform: Ethanol 2.5:2.5 and 3:2



TLC profile of *Calotropisgigantea* L. on Methanol crude extract solvent system

Chloroform: methanol 2.5:2.5 and 3:2.

Visible light | Long UV | Short UV

Table 2:Rf value calculation from the TLC plate

	Visible light		**Long UV**		**Short UV**	
Chloroform : methanol						
Eth extract	Rf = 0.833	Rf=0.916	Rf=0.75	Rf=0.80	Rf=0.77	Rf=0.88
Aq extarct	Rf=0.63	Rf=0.727	Rf=0.659	Rf=0.704	Rf=0.545	Rf=0.636
Chloroform: ethanol						
Eth extract	Rf=0.8	Rf=0.91	Rf=0.9	Rf=1	Rf=0.833	Rf=0.9
Aq extarct	Rf=0.64	Rf=0.757	Rf=0.689	Rf=0.734	Rf=0.575	Rf=0.696

UV spectroscopy :

UV spectroscopy or UV visible spectrophotometry (UV-VIS or UV/VIS) refers to absorption spectroscopy ore reflectance spectroscopy in part of the ultraviolet and full adjacent visible region of electromagnetic spectrum. This means it uses light in the visible and adjacent ranges. The absorbance or reflectance in the visible range directly affect the perceived colour of the chemicals involved. In this region of the spectrum atoms and molecules undergo electronic transition. Absorption spectroscopy is complementary to fluorescence deals with transition of electron from the excited state to ground state, while absorption measures transition from ground state to excited state [9].

Methods:-

1) For ethanolic solution:-

We have taken 0.26 g of ethanolic extract that was dissolved in 100ml of ethanol which is the concentration of 0.26% or 0.26 mg/ml then 1 ml of that solution was taken which was having a concentration of 0.26 mg/ml and then again another 1 ml of that solution was taken which is having a concentration of 0.0026 mg/ml[10].

Table 2: Concentration vs absorbance of the ethanolic extract

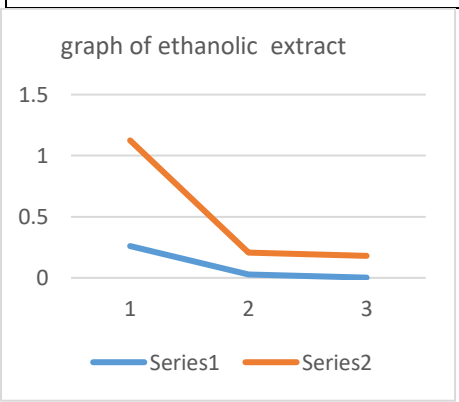
Conc(mg/ml)	Abs
0.260	1.127
0.026	0.409
0.002	0.280

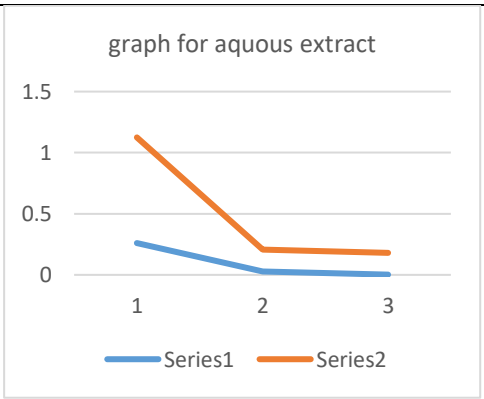
2) For aqueous solution: -

We have taken 0.26 g of aqueous extract that was dissolved in 100 ml of aqueous sol which is the concentration of 0.26% or 0.26 mg/ml then 1 ml of that solution was taken which is having a concentration of 0.26 mg/ml and then again another 1 ml of that solution was taken which is having a concentration of 0.0026 mg/ml.

Table 2: Concentration vs absorbance of the aqueous extract

Conc(mg/ml)	Abs
0.260	1.125
0.026	0.207
0.002	0.180





Results

After performing the experiment the *Calotropis gigantean* found to be having the above physiochemical parameter like moisture content, ash value and phytochemical alkaloid, tannin, flavonoid, saponin, cardiac glycosides and the TLC and UV studies of different extract has been shown, the results are compared with the standard data [11].

Conclusion

It is estimated by the World Health Organization that about 80 % of the population in developing countries rely on herbal medicines for primary health care needs [12]. Recently botanical and traditional uses of organic compounds, especially which are obtained from plant origin, had received greater affection because of their efficacy and safety for human consumption. We are identified the phytochemical compounds for respective extracts after phytochemical screening, TLC studies and UV studies. We will go for the further enzymatic study with this extract to found the anti-snake venom activity [13].

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