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Analysis Of Phytochemicals, Protein And Anti-Microbial Activity From The Latex Of *Calotropis Procera*

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Abstract

Calotropis procera is a perennial shrub with soft wood that is evergreen. It is a member of the Apocynaceae family and Asclepiadoideae subfamily. GC- MS (Gas chromatography-Mass Spectroscopy) is used to detect compounds. On performing GC-MS, 52 peaks for various substances are produced. The quantitative analysis of Methanolic, Ethanolic, and Crude extracts of *Calotropis procera* is done for secondary metabolites such as flavonoids and phenols, is carried out by using Gallic acid and Rutin as a standard. Various methods are used for the qualitative test for Alkaloids, Flavonoids, Phenols, Tannins, Saponins, Cardiac glycosides, Resins, Terpenoids. Latex, milky and sticky secretion form *Calotropis procera* has performed antimicrobial action against certain bacteria. The latex of *Calotropis procera* responds positively to Amylase assay. It displays the free space.The sample's concentration of each phytocompound is measured, and latex exhibits antibacterial actiontowards microorganisms. The growth of bacteria is prevented. The Lowry method and SDS-PAGE were also used for protein estimation and separation.

Keywords: Amylase assay, Antimicrobial, Calotropis procera, Latex, GC-MS,

1.Introduction

Calotropis procera is a perennial tracheophyte shrub with soft wood. The common names for *Calotropis Procera* include Akund, Apple of sadom, Auricular tree, Giant milkweed, Mudra, Rooster tree, Rubber bush, small crown flower, Sadom milkweed, and Swallow wort [English], Aak, Akada [Hindi] is a kind of upright, xerophytic shrub. That is native to many parts of Africa and Asia and is a member of the Asclepiadaceae family (Mohammad et al., 2017). Older pharmacopoeias have highlighted the therapeutic value of Calotropis, which is highly advised in the treatment of a number of diseases including Leprosy, Hepatic and Splenic disorders, Dropsy, worms, and Hypertrophy. Latex is applied on swollen, aching joints to get relief from pain. In India, the milky latex is often employed as a purgative. (Kumar et al., 2019). It is believed that *Calotropis procera* is even more dangerous than Cobra venom and is more toxic than *Calotropis Gigantea*. Considering that latex contains Calactin, Calotropin, Gigantin and Calotoxin all of which are toxic and caustic (Barkha et al., 2021).

The motivational factor of this study is, there is lack of availability of information about Proteins present in latex of *Calotropis procera*. As a result, initial bioassays evaluating these Proteins'ability to repel insects were completed in our lab. The findings suggested that laticifer proteins might be used in insect defence mechanisms. Test this theory, partial biochemical and enzymatic characterization was carried out to learn more about the Presence and biological functions of proteins in the latex of *Calotropis Procera*, which is thought to be even more toxic than *Calotropis gigantea* and more poisonous than cobra venom. Because the Cobra and other dangerous snakes can't stand the smell of this plant, snake charmers in Bengal use it to keep Cobras under control. It demonstrates its widespread use in Sudanese, 12 Arabic, and Indian traditional medical systems for the treatment of a variety of ailments. (Barkha et al., 2021)

Calotropis procera is an Ayurvedic plant with important medicinal properties helps in the treatment of various diseases. It is found in most areas of the world with a warm climate in dry, sandy and acidic soils. It is an erect, tall, highly branched and perennial shrub or small tree that grows to a height of 5.4 m, with milky latex (Ramos et al., 2007).

The milky latex was used in traditional medicines to treat worms, Colic piles, Ulcers, Toxin, Ulcerative colitis, Liver, Abdominal glands, and many inflammatory disorders (Oliveira et al., 2010). Clinical and pathological effects of latex *Calotropis procera*have been thoroughly studied (Batista et al., 2011). The discovery of medications from medicinal plants for the treatment of microbial disease is the focus of lot of studies today.

On centrifugation latex were separated into three different layers Natural Rubber was found in the top layer,Serum-containing latex present at the bottom andLipids-rich layer found at the middle layer. The chemical makeup of latex is extremely complex; however, it contains 25 to 35% of natural rubber poly (cis-1, 4-isoprene), which is thought to be the main component of natural rubber latex. This isoprene is a highly unsaturated hydrocarbon with an average molecular weight of 10^6 Da (Mohamed et al., 2017).

Numerous poisonous and caustic chemicals, including Calotropin, Calotoxin, Calcin, and Gigantin, are found in the latex of the *Calotropis procera* plant. (Kurichen et al. 1989) Contrary to the popular belief, *Calotropis* latex is highly toxic to the Corneal endothelium but mildly hazardous to the Corneal epithelium when it accidentally enters into our eyes it causes Ocular toxicity. There was Corneal edema that causes various degrees of Descemet's folds andaggressive kerato-conjunctivitis, which results in Cornealdeterioration but no pain. (Biedner et al.1977). *Calotropis procera* extract has antiproliferative effects both in vitro and in vivo (Magalhaes et al. 2010). Reactive oxygen species are crucial for controlling Cell signalling, Autophagy, and Homeostasis (Wang et al.2012). Human cancers can be suppressed by Cardiac glycosides found in the latex of *Calotropis procera* (Newman et al., 2007).

2. Materials and methods Plant material and extraction

2.1 Sample preparation for GC-MS

Latex extract preparation: Known quantity of fresh latex (1 ml) was mixed with methanol (1 ml). Then mixtures were placed in shaker overnight, filtered through the Whatman's filter paper and further used for GC-MS analysis.

GC-MS analysis: Thermo Scientific's Triple Quadrupole GC-MS (Trace 1300 GC, Tsq 8000 triple quadrupole MS) was used to conduct the GC-MS analysis of the methanol extract of *Calotropis procera* latex using a TG 5MS (30m X 0.25mm, 0.25m) column. With an injection volume of 1µl, Helium was employed as the carrier gas at a flow rate of 1 ml/min. Ion source temperature was 230 °C, while injector temperature was maintained at 250 °C. The Mass Spectra transfer line temperature of 280 °C was kept at 50 °C isothermal in the oven(Sharma et al.2016)**.**

2.2 Phytochemical Screening of extracts:

Crude extracts were used for preliminary phytochemical analyses**.** The following qualitative tests for metabolites were done as follows:

2.2.1 Test for Alkaloids: Wagner's test: About 1 ml of extract was taken and few drops of Wagner'sreagent was added and the formation of a Reddish-Brown precipitate indicated the presence of Alkaloids.

2.2.2 Test for Saponins: Crude extract was mixed with 5ml of distilled water in a test tube and was shaken vigorously. The formation of stable foam was considered as an indication for the presence of Saponins.

2.2.3 Test for Phenols: Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A Blue-Green or Black coloration indicated the presence of Phenols.

2.2.4 Test for detection of Tannins: 1 ml of the extract was taken in a test tube, and then 1 ml of 0.1% ferric chloride containing 0.1 N HCl was added. Appearance of Blue-Black coloration indicates the presence of Tannins.

2.2.5 Test for Cardiac glycosides: Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A Brown ring at the interphase indicated the presence of Cardiac glycosides.

2.2.6 Test for Flavonoids: Crude extract was mixed with few fragments of Magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which indicated the presence of Flavonoids.

2.2.7 Test for Carbohydrates: Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

2.2.8 Test for Protein &Amino acids: Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin,Violet colour appeared suggesting the presence of Amino acids and Proteins.

2.2.9 Test for Terpenoids: Crude extract was dissolved in 2ml of Chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A Greyishcolour indicated the presence of Terpenoids.
2.2.10 Test for detection of Quinones: To 1 ml of plant extract, 1 ml concentrated H_2SO_4 was added. Blue-Green or Red coloration indicates the presence of Quinones (Geetha et al., 2014).

2.3.Quantitative phytochemical analysis:
Methanolic, Ethanolic and Crude extracts was used for preliminary phytochemical analyses**.** The following quantitative tests for metabolites were done as follows:

2.3.1 Total Phenolic content: The Folin–Ciocalteutechnique was used to calculate the latex extract's total Phenolic content (Kaur et al., 2002). 200 µl of crude extract (1 mg/mL) were diluted to 3 mL with distilled water, thoroughly mixed with 0.5 mL of Folin–Ciocalteureagent for 3 min, and then 2 mL of 20% (w/v) Na_2CO_3 were added. After another 60 minutes in the dark, the mixture was tested for absorbance at 650 nm. The standard used was Gallic acid (1 mg/ml). There were three copies of each test run. Gallic acid equivalent (mg/g of the isolated chemical) was used to calculate the results using the standard curve (Malik et al. 2015).

2.3.2 Total Flavonoid content: The total Flavonoid content is determined using a slightly modified colorimetrymethod. A 2 mg/2 ml suitably diluted sample solution aliquot of 0.5 ml was combined with 2 ml of distilled water, then with 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of a 10% $AlCl_3$ solution was added, and the combination was then given 2 ml of 4% NaOH solution. Water was added right away to make the final volume 5 ml, and after that, the mixture was properly stirred and allows to kept for an additional 15 minutes. At 610 nm, the mixture's absorbance was measured in comparison to water blank. The analysis was carried out in triplicate, and the Rutin results were presented (Geetha et al., 2014).

2.4. Antimicrobial Activity of crude extract of *Calotropis procera*:
For testing the antibacterial activity of the provided sample against the sample, the disc diffusion method is used. After a 24-hour incubation period, the test organism is spread out on an agar plate, and an antibiotic medication diffuses from the disc to stop the growth of the cells around it. Clear ringssurrounding the antimicrobial discs will determine the antimicrobial activity of latex. The pace at which a certain medication diffuses through the medium, how susceptible an organism is to the drug, how many organisms are inoculated on the plate, and how quickly they grow are all factors that affect the extent of the zone of inhibition. Therefore, it is crucial that the test be conducted in a properly standardised manner so that the figures displayed on the chart provide accurate results (Reddy et al., 2008).

2.5. Assay for Amylase activity:
1 ml of enzyme sample, add 1 ml of 1% starch solution and incubate it for 37 degrees Celsius for 30 min in water bath. A blank is maintained with 1ml of 1% starch solution and add 1ml of D/W. immediately after

incubation add 1 ml of 2N NaOH to stop enzyme reaction. Add 1 ml of DNS reagent to each tube and place them in boiling water bath for 10 min and cool in tap water. Observe formation of orange to reddish color in a tube. In alkaline solution, reducing sugar from enediols are readily oxidized to its perspective sugar acids by the oxidizing agent 3,5 dinitro salicylic acid to form orange red colour complex. The absorbance is measured at 540nm aliquots of standards that is Maltose were taken in a series of six test tubes (0.0 to 1 ml) and make up to 1ml volume by D/W. 1ml DNSA were added in each test tube and keep it in boiling water bath for 15 min and measure the absorbance at 540 nm. (Sumner et al., 1921)

2.6. Protein extraction:

At room temperature, the latex and modified buffer are aggressively vortexed for 30 minutes. After that, the sample was sonicated on ice for 5 minutes at room temperature with amplitude of 40% and pulse duration of 2 seconds. After that, the sample was centrifuged at 14500 rpm and 40 c for an hour. Total pure latex was recovered from the intermediate phase. Repeat centrifuging the rubber latex. The pallet, rubber particle, and upper phase transfer to fresh centrifuged tube were thrown away. Where Sodium azide act as a antifungal agent, was made. The extract was kept at 4°C for 2 hrs. The Protein from the crude extract was precipitated by a gradient of saturated Ammonium sulphate solution. The solution was centrifuged at 12000 rpm for 20 minutes to get the protein pellet. This pellet was resuspended in minimum volume of buffer and was set on dialysis in a 3kDa membrane for 24 hrs against buffer. The dialyzed protein was stored at 4°C for further purification. (Kumar et al., 2019).

2.7. Estimation and Separation of Proteins:

The purified latex sample was quantified by using Lowry method for protein (Lowry et al., 1951) and SDS PAGE for Protein Separation (Laemmili et al., 1970)

3. Results

3.1 GC-MS analysis of latex of *Calotropis Procera*:

(Fig.1) Shows the GC-MS chromatogram of methanolic extract of latex of *Calotropis procera.* Total 52 compounds were found in GC-MS analysis methanolic extract of *C. procera*. Latex found to be rich in CO_2 and another abundant compound wereAcetic acid and Methyl ester, Hexadecane, Sulfurous acid, Cyclohexyl-methyl heptyl ester, Methacrylic acid, Heptadecyl ester with retention time 1.107, 1.297, 24.954, 34.079, 45.412. Percent area occupied by these compounds was more than other compounds (Table 1).

Fig 1.GC-MS chromatogram of methanolic extract of *Calotropis procera* latex

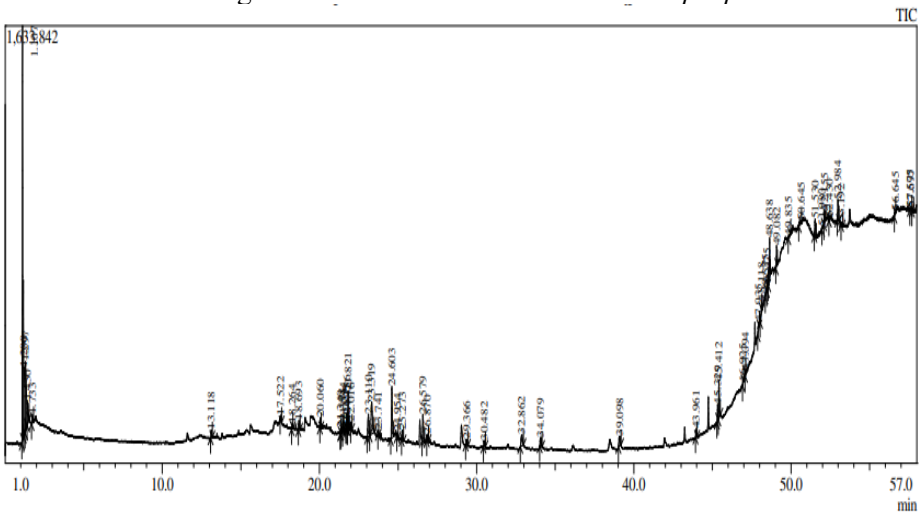

Table 1.GC-MS analysis of methanolic extracts of *Calotropis procera* latex.

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	Name
1	1.107	1.060	1.135	3110920	24.2018	1550763	36.68	Carbon dioxide
2	1.200	1.185	1.260	990834	7.7083	256349	6.06	n-Hexylmethylamine
3	1.297	1.260	1.400	1395003	10.8526	258641	6.12	Acetic acid, methyl ester
4	1.430	1.400	1.525	320204	2.4911	84982	2.01	n-Hexane
5	1.733	1.685	1.805	124153	0.9659	32097	0.76	2-Butanone, 3-methyl-
6	13.118	13.075	13.180	82784	0.6440	30035	0.71	D-Limonene
7	17.522	17.500	17.595	50432	0.3923	16945	0.40	3,5-Dibutoxy-1,1,1,7,7,7-hexamethyl-3,5-bis(t
8	18.264	18.225	18.305	43457	0.3381	20045	0.47	1-Bromo-3,7-dimethyl-2,6-octadiene
9	18.693	18.650	18.760	122078	0.9497	35806	0.85	Cyclohexasiloxane, dodecamethyl-
10	20.060	20.025	20.105	75731	0.5892	36576	0.87	2-[(p-Trimethylsilyloxy)phenyl]-2-[(p-trimethy
11	21.340	21.315	21.370	31558	0.2455	20972	0.50	Heptadecane, 2,6,10,15-tetramethyl-
12	21.428	21.370	21.470	55445	0.4313	18636	0.44	Decane, 1-bromo-2-methyl-
13	21.514	21.475	21.540	55220	0.4296	34528	0.82	Cycloheptasiloxane, tetradecamethyl-
14	21.695	21.665	21.725	44379	0.3453	16260	0.38	Tetradecane, 1-chloro-
15	21.756	21.725	21.785	138890	1.0805	56837	1.34	Dodecane, 2,6,11-trimethyl-
16	21.821	21.785	21.900	319796	2.4879	142033	3.36	Hexadecane
17	22.016	21.980	22.075	71345	0.5550	25594	0.61	Bis(pentamethylcyclotrisiloxy)tetramethyldisil
18	23.110	23.030	23.190	353739	2.7520	83369	1.97	Tetradecane, 1-chloro-
19	23.319	23.190	23.480	711240	5.5332	118787	2.81	Diethyl Phthalate
20	23.741	23.715	23.765	32748	0.2548	17212	0.41	Cyclooctasiloxane, hexadecamethyl-
21	24.603	24.535	24.725	732577	5.6992	191175	4.52	Hexadecane, 1-chloro-
22	24.954	24.920	25.000	37721	0.2935	17205	0.41	Hexadecane
23	25.273	25.220	25.350	139563	1.0857	37791	0.89	2,4,4,6,6,8,8-Heptamethyl-1-nonene
24	26.579	26.525	26.685	364485	2.8356	100516	2.38	Hexadecane, 1-chloro-
25	26.870	26.820	26.930	76348	0.5940	24108	0.57	Trichloroacetic acid, tetradecyl ester
26	29.366	29.305	29.435	83900	0.6527	21427	0.51	Hexadecane, 1-chloro-
27	30.482	30.435	30.555	90960	0.7076	21970	0.52	Sulfurous acid, cyclohexylmethyl pentadecyl e
28	32.862	32.780	32.970	251188	1.9541	41826	0.99	Methacrylic acid, tetradecyl ester
29	34.079	34.010	34.165	173420	1.3491	35577	0.84	Sulfurous acid, cyclohexylmethyl hexyl ester
30	39.098	39.015	39.180	190917	1.4853	35285	0.83	Octadecane, 1-chloro-
31	43.961	43.915	44.020	82597	0.6426	30260	0.72	Phosphite, tris(2,4-dimethylpent-3-yl-
32	45.329	45.285	45.370	145974	1.1356	61718	1.46	2,4,4,6,6,8,8-Heptamethyl-1-nonene
33	45.412	45.370	45.495	416767	3.2423	137937	3.26	Methacrylic acid, heptadecyl ester
34	46.925	46.915	46.990	27590	0.2146	9975	0.24	Heptadecafluorononanoic acid, pentadecyl est
35	47.094	47.070	47.115	29024	0.2258	14634	0.35	1-(Trimethylsilyl)-1-propyne
36	47.935	47.875	47.985	105802	0.8231	29425	0.70	Sulfurous acid, cyclohexylmethyl tetradecyl es
37	48.118	48.035	48.150	112675	0.8766	44053	1.04	Dihexadecyl phosphate
38	48.375	48.355	48.460	57084	0.4441	13437	0.32	[1,1'-Bicyclohexyl]-4-carboxylic acid, 4'-penty
39	48.475	48.460	48.550	53963	0.4198	12069	0.29	[(1-methyl-2-piperidinocarbonyl)vinyloxy]-4-r
40	48.638	48.550	48.685	383402	2.9827	148767	3.52	Sulfurous acid, cyclohexylmethyl heptyl ester
41	49.082	49.035	49.130	206021	1.6028	76867	1.82	Heptyl octacosyl ether
42	49.835	49.820	49.970	34784	0.2706	8277	0.20	E-10,13,13-Trimethyl-11-tetradecen-1-ol aceta
43	50.645	50.495	50.655	84223	0.6552	11284	0.27	1-Cyclohexene-1-butanal, .alpha.,2,6,6-tetrame
44	51.530	51.480	51.610	211714	1.6471	66042	1.56	Sulfurous acid, cyclohexylmethyl heptadecyl e
45	51.980	51.960	52.090	31723	0.2468	8763	0.21	Methyl 2-hydroxy-eicosanoate
46	52.155	52.090	52.205	113200	0.8807	39556	0.94	3,3-Diethylpentadecane
47	52.430	52.405	52.480	41158	0.3202	10841	0.26	Ethyl Oleate
48	52.984	52.930	53.050	274265	2.1337	79920	1.89	Sulfurous acid, cyclohexylmethyl pentadecyl e
49	53.192	53.175	53.275	42344	0.3294	9981	0.24	.beta.-L-Arabinopyranose 1,2:3,4-bis(butaneb
50	56.645	56.570	56.660	48098	0.3742	12402	0.29	Chloromethyl undecanoate
51	57.575	57.565	57.685	33488	0.2605	8620	0.20	2H-Cyclodeca[b]pyran, 3,4,5,6,7,8,9,10,11,12
52	57.697	57.685	57.800	47151	0.3668	10100	0.24	1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentameth
				12854082	100.0000	4228275	100.00	

3.2 Qualitative analysis for secondary metabolites:

The latex extract was screened for the presence of various bioactive compounds. The screening revealed the presence of Cardiac glycosides, Saponins, Phenolic compound, Terpenoids, Alkaloids, Flavonoids, Tannin and in some extracts with a note that the concentration of the various classes of secondary metabolite varies amongst the extract evaluated.

Table 2. Shows thepresence or absence of Phytocompounds in latex of *calotropis procera*

Phytocompounds	Methanolic extract of *Calotropis procera*
Alkaloids	+
Phenols	+
Flavonoids	+
Terpenoids	+
Tannin	-
Saponin	+
Cardiac Glycosides	+
Carbohydrates	+
Proteins	+
Quinones	-

Note: (+) sign shows presence and (-) sign shows absence of phytocompound

3.3 Quantitative Phytochemical analysis

3.3.1 Total Flavanoid content: The amount of Flavonoid found in the Ethanolic, Methanolic and Crude extract was determined using Rutin as a standard. All samples used in quantification were those that showed the presence of flavonoids tested in phytochemical screening**.**

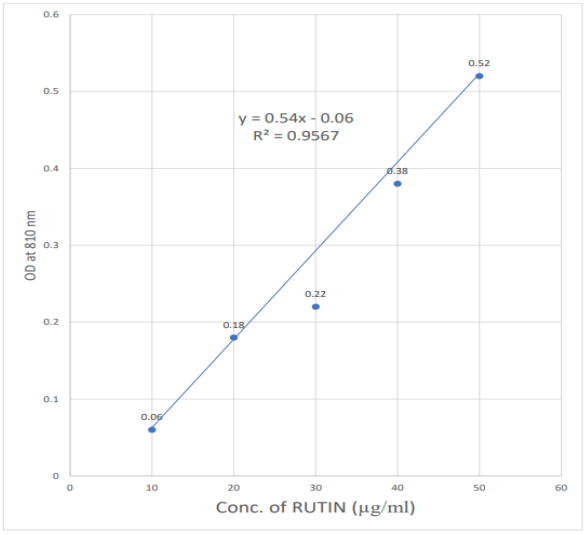

Fig 2. Standard graph for Flavonoids

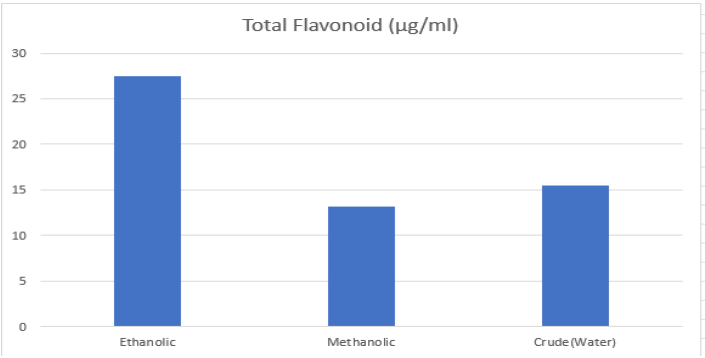

Fig 3. Amountof Flavonoid in µg/ml

3.3.2 Total Phenolic content: The amount of Phenols found in the Ethanolic, Methanolic and Crude extract were determined using Gallic acid as a standard. All samples used in quantification were those that showed the presence of Phenols tested in a phytochemical screening.

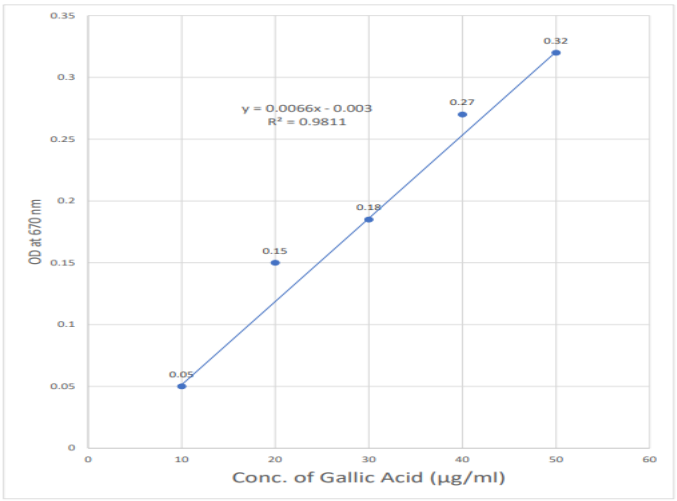

Fig 4. Standard graph for Phenols

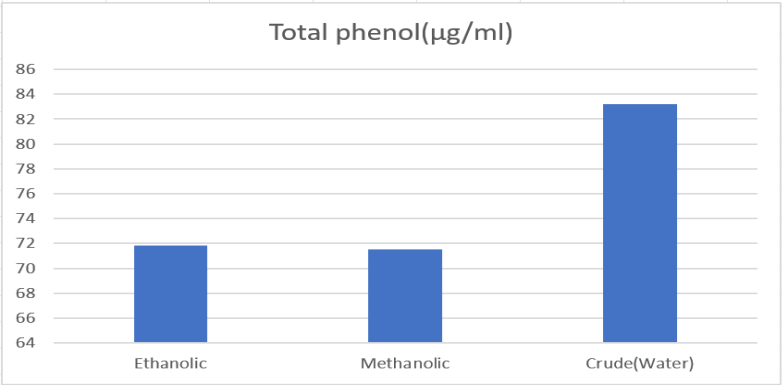

Fig 5.Amountof Phenols in µg/ml

3.4 Antimicrobial activity of latex against bacteria:

Ethanolic extract of latex of *Calotropis procera* is used to check antimicrobial activity of Bacteria against B. Subtilis, Agrobacterium tumefaciens and B licheniformis. The zone of inhibition shown by these bacteria were measured considering its diameter that is 0.8cm, 0.4cm, 0.45cm.

B. subtilis *Agrobacterium tumefaciens* *B. licheniformis*

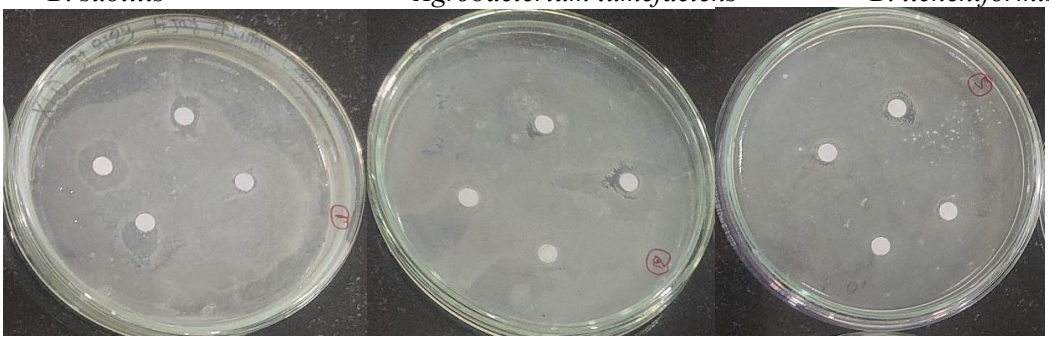

Fig 6. Antimicrobial activity of latex against*B. subtilis, Agrobacterium tumefaciens, B. licheniformis*at 37ºc temperature.

Table 3.Bacteria showing zone of inhibition in cm

Bacteria	Zone of inhibition(diameter) in cm
B. Subtilis	0.8
Agrobacterium tumefaciens	0.4
B. Licheniformis	0.45

3.5. Amylase assay:

The qualitative analysis of enzyme assays showed that Blue-Black colour appeared by flooding plate with Lugol's reagent due to the presence of starch indicating absence of Amylase production whereas colourless zone was appeared around the amylase producing colonies due to Starch hydrolysis.

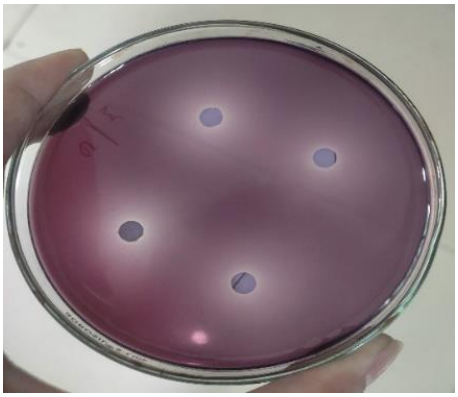

Fig 7. Amylase test positive for the Methanolic extract of latex of *Calotropis procera*

Table 4. Different fractions of latex showing enzymatic activity

Extract	Enzymatic activity µg/ml/min	Specific enzyme activity µg/ml/min
Crude	1620	179.005
Ethanolic	800	88.39
Methanolic	750	82.87

3.6. Protein quantification by Lowry method:

Protein was tested against standard protein curve that shows the 1.6-2.0mg/ml (Kumar *et al.,* 2019). According to this studythe protein concentration of in the latex of *Calotropis Procera* tested against standard protein curve at 660 nm was found to be 9.05µg/ml.

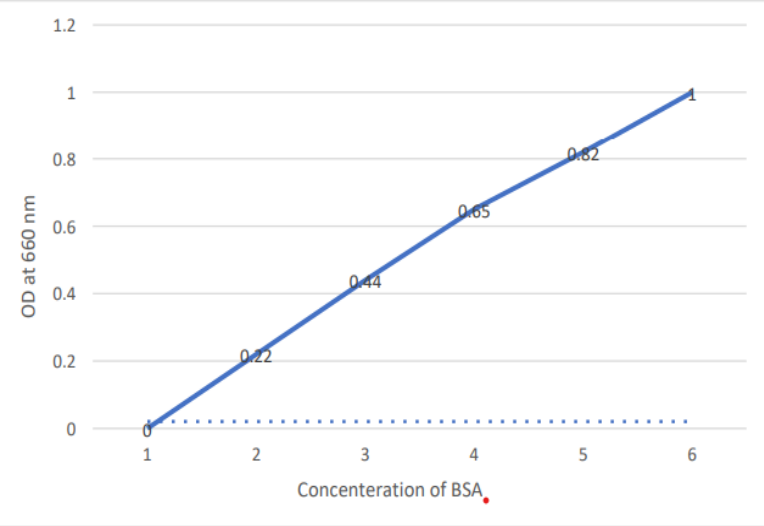

Fig 8. Standard curve for BSA

3.7. Protein Separation using SDS PAGE

On performing 10% SDS PAGE four bands of separated protein were seen according to their molecular weight that is 139, 73,65, 50 kDa for 1st, 2nd, 3rd, and 4th band.

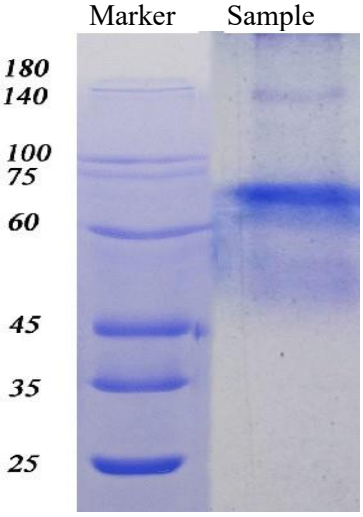

Fig 9.10% SDS PAGE for latex of *Calotropis procera*

4. DISCUSSION

The GC-MS chromatogram of the M ethanolic latex extract of *Calotropis procera* displayed 52 peaks for various chemicals. The majority of the chemicals were found in latex (Fig. 1). Additionally, latex was also discovered to be rich in CO_2 Phenol,2,5-bis(1,1-dimethylethyl) $C_{14}H_{22}O$, Z-1,6-Tridecadiene ($C_{13}H_{24}$), L-Glutamic acid ($C_5H_9NO_4$), Hexadecane ($C_{16}H_{34}$), 1-[(T-butyl) dimethyl silyl thin] Butane ($C_7H_{15}F_3O_3SSi$) at

retention time 49.16, 49. 57, 49.87, 52.89. According to published research, Chloroform is a superior solvent solution used for extracting compounds from *C. gigantea*. However, the current work supports the use of Methanol in the extraction of compounds from *C. gigantea*. (Sharma et al., 2016)

By comparing the chromatograms of the latex from *Calotropis procera* and *Calotropis gigantea*, both of which belongs to same familyAsclepiadaceae. In the GC-MS study of the *C. Procera*Methanolic extract, a total of 52 compounds were discovered. Acetic acid and Methyl ester, Hexadecane, Sulfuric acid, Cyclohexyl methyl heptyl ester, Methacrylic acid, and Heptadecyl ester with retention time were also discovered to be plentiful in latex, in addition to CO_2. They were also abundantly present. These compounds occupy a greater percentage of the total area than other compounds. According to this paper (Sharma et al., 2016), common chemicals that are present in the chromatogram of methanolic extract of latex of *Calotropis procera* and *C. gigantea* are Methyl ester, Decane, and Hexadecane which displays various peaks, retention time, and percentage of the region covered by those compounds is different in both *Calotropisprocera*and *Calotropis gigantea.*(Table 1)

Above qualitative study shows that Methanolic extract of latex contains Alkaloids, Flavonoids, Phenols, Terpenoids, Cardiac glycosides, Carbohydrates, Proteins, Saponin, but it shows negative tests for Quinones and Tannins. According to (Hassan et al., 2017) The Methanolic extract contains Alkaloids, Flavonoids, Phenols, Cardiac glycosides, Carbohydrates, Proteins, Saponin, but it shows negative tests for Terpenoids and Tannins. As shown in (Table 2). Quantitative analysis of Methanolic, Ethanolic and Crude (water) extract of latex of *Calotropis procera* for Phenols and Flavonoids in µg/ml(Fig2),(Fig 4). Amount of Flavonoid is high in Ethanolic extract as compared to Methanolic and Crude (Fig3) and Phenolic content of crude extract is high as compared to Methanolic and Ethanolic shown in (Fig5).

Antimicrobial activity against the three bacteria out of seven shows the positive results. Those three bacteria are *B. subtilis, Agrobacterium tumefaciens and B. Licheniformis* shows clear zone of inhibition (Fig 6) that is 0.8cm, 0.4cm, 0.45cm (Table 4).Amylase activity of latex sample against the crude extract of latex of *Calotropis procera*shows clear zone (Fig 7) and DNSA method is used to measure the enzymatic activity of latex. The enzyme activity and the specific enzymatic activity is more for the crude extract of latex of *Calotropis procera* (Table 4)

Purified protein was quantified by using Lowry method. A polynomial nonlinear equation describing the standard curve generated for Protein curve that shows the 1.6-2.0mg/ml (Kumar et al., 2019). Similarly, Protein was quantified by using Lowry method. The OD measured at 660 nm determined for BSA protein concentrations ranging from 50 to 250 µg/ml. Nonlinear equation describes the Standard curve(Fig 8). The protein concentration of latex of *Calotropis Procera* tested against standard protein curve at 660 nm is 9.05µg/ml. On performing SDS PAGE the molecular weight of 1st, 2nd, 3rd and 4th bands of proteinwas 139, 73,65, 50 kDa respectively. (Fig 9) (Kumar et al., 2019).

5. Conclusion

Examination of phytochemical of plant latex of *Calotropis procera* identified 52 compounds. Qualitative analysis of latex of *Calotropis procera* shows the positive for secondary metabolites such as Alkaloids, Flavonoids, Saponins, Cardiac glycosides, Phenols and Terpenoids and quantitative analysis shows the concentration of Phenols and Flavonoids that is in µg/ml. The compound present in latex shows the antimicrobial, and amylase activity. Protein concentration also estimated. On performing SDS-PAGE four bands of proteins were observed.

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