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Preliminary Phytochemical screening, in vitro antisolar, lipid peroxidation and caseinolytic activity of *Calotropis gigantea., L.*

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ABSTRACT

Calotropis gigantea or Giant Indian Milk weed, belongs to Asclepiadaceae. Calotropis is drought resistant, salt tolerant to a relatively high degree, grows wild up to 900 meters throughout the country. Malayali tribes in Javaadhu hills of Tiruvannamalai., Tamilnadu used the flowers for the treatment of snake bite, cold and asthma [1]. Tribal people of Alirajpur district, Madhya Pradesh used the latex for pain and swelling [2]. The phytochemical screening of methanol:chloroform extract(1:1) and HAECG of *Calotropis gigantea* leaves revealed the presence of alkaloids, carbohydrates, cardiac glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, amino acids and the absence of anthraquinone glycosides. The fresh & dry latex showed prominent absorbance at 200-290nm (UV-C). Methanol: chloroform (1:1) extract showed prominent absorbance at 200-290nm (UV-C) and good absorbance at 320-400nm (UV-A). The inhibitory concentration (IC_{50}) of ***Calotropis gigantean*** (leaves) against ascorbic acid was found to be 68.48µg/ml in comparison with ascorbic acid 14.91 µg/ml. Crude extract of *Calotropis gigantean*, latex and papain exhibited caseinolytic activity in a dose dependent manner. Papain showed an average increase in absorbance of 0.03 at 660 nm/hr. *C.gigantea* showed an average increase in absorbance of 0.02 at 660nm/hr. Latex of *Calotropis gigantea* exhibited moderate caseinolytic activity when compared to the papain.

Keywords: *Calotropis gigantea*, Caseinolytic, Anti-solar, Anti-oxidant phytochemical**.**

INTRODUCTION

Calotropis gigantea L commonly known as Giant Indian Milk weed, belongs to the family Asclepiadaceae, drought resistant, salt tolerant to a relatively high degree, grows wild up to 900 meters found throughout the country. The flowers of this plant are used for the treatment of jaundice, inflammation ulcer and asthma (1). The latex is used for treatment of stings, toothache, caries, leprosy, ringworm infection, syphilius, tumors, rheumatism, anti septic, vermifuge and purgative, possess digitalis like action, infanticide and abortive properties. Fresh leaves are used for treating convulsions, rheumatic pains [2]. Powdered root is used for the treatment of elephatiasis, leprosy and dysentery [3]. The flower infusion is used for the treating rheumatism,

intestinal worms and epileptic attacks (4). In India Siddis and Gowlistribals of Western Ghats of Karnataka, used for the treatment of skin infections, reduces labour pain during child birth [6]. Malayali tribes of Javaadhu hills, Tiruvannamalai, Tamilnadu used the flowers for the treatment of snake bite, cold and asthma [7]. Tribal people of Alirajpur district, Madhya Pradesh, India, used the latex for pain and swelling [8]. In Unani system of medicine used to treat piles, ache, worms skin disease, paralysis asthma and dropsy [9]. People of Hainai islands, China used for the treatment of asthma, cough, carminative, leprosy, anthelmithic [10]. The literature review of latex revealed the presence of gigantin [11]. Large number of cardiac glycosides had been isolated [12-14]. Further, flavonol glycosides such as calotropiside along with isohamnetin-3-o-rhamnoside, rhamnoglucoside, isohamnetin-3-o-glucoside and taxosterylacetate[15]. The plant exhibited various pharmacological activities such as anti-microbial [16], analgesic [17], wound healing [18], CNS activity [19], anti-diarrhoeal [20], anti-pyretic [21], anti-inflammatory [22], hepato-protective [23], vasodilation effect [24), anti-oxidant [25). Pro coagulant activity [26], hemostatic, anti-diabetic [27], in vitro clot dissolving activity [28]. The literature review revealed this plant does not show any extensive studies in prelimnary phytochemical screening and in vitro antioxidant effect.

An exertion was undertaken to investigate the prelimnary phytochemical screening, anti solar and caseinolytic studies for this plant.

MATERIALS AND METHODS

Collection and authentication of plant

Fresh leaves and latex of *Calotropis gigantea* were collected from medicinal garden, College of Pharmacy campus, Madurai Medical College, Madurai during the month of April-2019 and was authenticated by Dr.D.Stephen,Msc.,Ph.D. Professor, Department of Botany, American college, Madurai. The herbarium of this specimen was kept in the department for the further reference.

Preparation of Hydroalcohol (70%) & Methanol-Chloroform Extract of *Calotropis gigantea* L. (HAECG) / M-CECG.

Procedure

Fresh leaves were collected, shade dried, powdered coarsely and was defatted with petroleum ether (60-80˚c) and filtered. The residue was dried and extracted with hydroalcohol (70%) and methanol:chloroform (1:1) separately by maceration until the complete extraction of the powder was filtered and concentrated under reduced pressure to obtain a solid residue HAECG and MCEG.

Phytochemical studies

Hydroalcoholic extract of *Calotropis gigantea* (HAECG) and MCECG extract of *Calotropis gigantea* L. (Leaf) were subjected to qualitative chemical analysis for the identification of secondary metabolites and was determined as per (Harborne; 1998) [29] and the results are displayed in table 1.

Determination of anti-solar activity

Procedure

Fresh and dry latex (2 drops) were dissolved in a mixture of chloroform: glacial acetic acid (1:1) so as to prepare different concentrations (2-8µg/ml) and was scanned(200-400nm) under UV spectrophotometer method was adopted as per Gharge VG et al., [30]and the absorbance was recorded and the results are depicted in **Fig:** 1and 2 and tabulated in **Table** 2-4**.**

In vitro antioxidant activity

Effect of HAECG on lipid peroxidation levels

Procedure HAECG was subjected to in-vitro antioxidant studies. It includes lipid peroxidation inhibition. In-vitro antioxidant activity was determined for HAECG as per DananjayaPerera et al.,2018.(31]Lipid peroxides formed in the egg yolk was used as the lipid-rich source. Briefly, fresh egg yolk emulsion was diluted to 10% v/v with 11.5% w/v KCl. Egg yolk emulsion (0.5mL), different concentrations (0.5ml) of plant extracts(100-500µg/ml) and ascorbic acid(different concentrations), aqueous trichloroacetic acid (200%,1.5ml) and 6.7% w/v thiobarbituric acid (1.50ml) were added respectively. The reaction mixture was then vortexed thoroughly and incubated at 95°C in water bath for 1 hour. The mixture was cooled and centrifuged at 3000 rpm for 10 min. Absorbance of the upper layer was measured at 532 nm and percentage inhibition was calculated with the following formula.

%Inhibition of lipid peroxidation = 1- Absorbance of sample/Absorbance of negative X 100.

The results are depicted in fig and tabulated in Table 5 and fig 7.

Determination of caseinolytic activity

Isolation of papain from *Caricaya papaya* and crude enzyme from *C.gigantea*latex

Latex was subjected to caseinolytic activity as per Rajesh et al., (25) The latex was collected, diluted with equal volume of 10 mM phosphate buffer (pH 7.0) and kept overnight at 4-8ºC, centrifuged at 12,000g for 20 min at 4-8ºC. The clear supernatant was decanted and dialyzed against 10 mM phosphate buffer (pH 7.0). The supernatant was subjected to protein precipitation by 80% ammonium sulphate. The precipitated pellet was dissolved in 10 mM phosphate buffer (pH 7.0) and dialyzed against the same buffer to remove ammonium sulphate. The protein concentration in the supernatant was used as crude enzyme source.

Procedure

Different concentrations (1-4 mg/ml) of crude extract and papain was treated with 0.4 ml Casein (2% in 0.2 M Tris–HCl buffer, pH 8.5), incubated with at 37.8ºC separately for 2 h. The reaction was stopped by adding 1.5 ml of 0.44 M TCA and allowed to stand for 30 min. The mixture was centrifuged at 1500g for 15 min. An aliquot (1 ml) of the supernatant was mixed with 2.5 ml of 0.4 M sodium carbonate and 0.5 ml of 1:2 diluted Folin's reagent and the color developed was read at 660 nm. One unit of enzyme activity was defined as the amount of enzyme required to increase in absorbance of 0.01 at 660 nm/h at 37.8ºC. Activity was expressed as units/h at 37ºC. The results are tabulated in table 6 and depicted in fig 8 and 9.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Methanol:chloroform(1:1) extract and HAECG of *Calotropis gigantea(*leaves) were subjected to qualitative chemical analysis and its results were displayed in the table 1

Table 1 Preliminary Phytochemical Screening of (HAECG) &MCECG

S.No	Tests	M-CECG	HAECG
1	**ALKALOIDS**	POSITIVE	POSITIVE
2	**CARBOHYDRATES**	POSITIVE	POSITIVE
3	**STEROLS**	POSITIVE	POSITIVE
4	**CARDIAC GLYCOSIDES**	POSITIVE	POSITIVE
5	**ANTHRAQUINONE GLYCOSIDES**	NEAGATIVE	NEGATIVE
6	**TANNINS AND PHENOLIC COMPOUNDS**	POSITIVE	POSITIVE
7	**SAPONINS**	NEAGATIVE	NEGATIVE
8	**FLAVANOIDS**	POSITIVE	POSITIVE

Phytochemical screening of methanol:chloroform extract(1:1) and HAECG of *Calotropis gigantea* leaves revealed the presence of alkaloids, carbohydrates, cardiac glycosides, sterols, tannins, phenolic compounds, flavonoids and the absence of anthraquinone glycosides and saponins.

Anti-solar activity

Fresh and dry latex were screened for its antisolar effect expressed the maxima absorbance at 220 and 320nm reveals the phytoconstituents in the latex are responsible for this effect. The results are displayed in Fig 1 and 2 and Table 2.

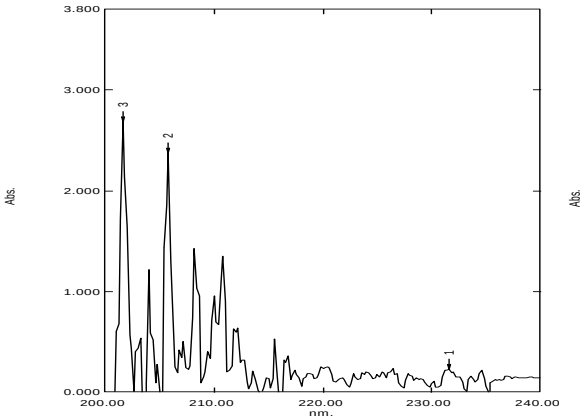

Fig 1: Absorption UV Solar Activity spectra of dry latex

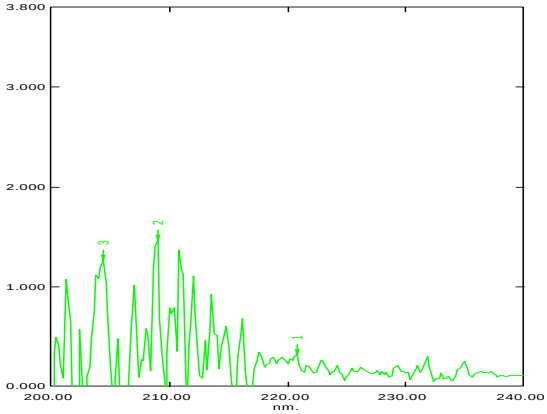

Fig.2 Absorption UV-spectra of fresh latex

Table :2 Determination of UV Solar Activity spectra of dry latex/ fresh latex

S.NO	Lambda Maxima (λmax)	ABSORBANCE(nm)
1	220	0.317 (fresh latex)
2	231.60	0.224 (dry latex)

Absorption Spectra Of Methanol:Chloroform(1:1)Extract

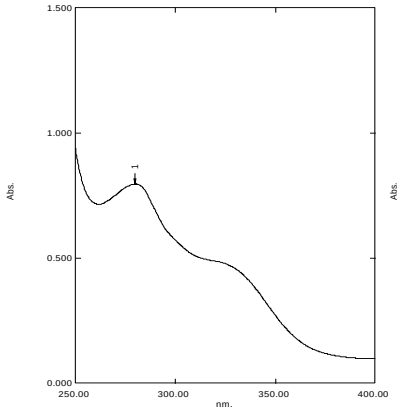

Fig 3: UV-spectrum (2µg/ml)

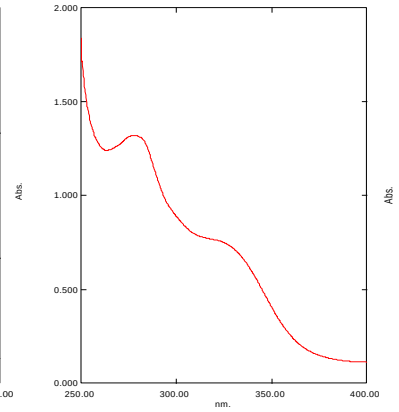

Fig 4:UV-spectrum (4µg/ml)

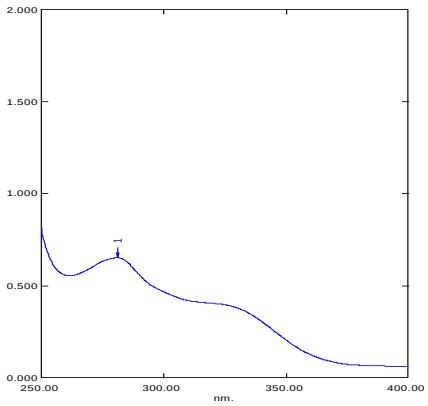

Fig 5: UV-spectrum (6µg/ml)

Table : 3 Determination of UV Solar Activity spectra methanol:chloroform(1:1)extract

S.NO	Concentration (µg/m L)	Lambda Maxima (λmax)	Absorbance (nm)	Inference
1	**2**	281nm	0.652	**UV-C ****
		340nm	0.323	**UV-A***
2	**4**	280nm	0.725	**UV-C ****
		330nm	0.498	**UV-A***
3	**6**	279.6 nm	0.797	**UV-C****
		330nm	0.486	**UV-A***

UV-A*-320-400/ .
UV- C-200-290 nm**

Qualitative investigation indicated the presence of flavonoids in the extract. Flavonoids are the coloured pigments mainly found in the leaves and they are well known for their attractive colours and pharmacological activities. It also absorbs light and helps to protect the photo sensitive substances in the leaves and thus play a key role in the defense mechanism of plants. Absorption of UV radiation is the main characteristic for identification of flavonoids in natural sources. The results showed strong to moderate absorption of UV radiation along the whole range and this ability may be due to the

presence of flavonoids. The results obtained showed the ability of fresh & dry latex and methanol:chloroform(1:1) extract of *Calotropis gigantea* to absorb UV radiation and hence proved its UV protection ability. The fresh & dry latex showed prominent absorbance at 200-290nm(UV-C).Methanol:chloroform(1:1)extract showed prominent absorbance at 200-290nm(UV-C) and good absorbance at 320-400nm(UV-A).

INVITRO ANTIOXIDANT ACTIVITY

Determination of lipid peroxidation effect of HAECG

Table 4: Determination of Lipid Peroxidation Effect of HAECG

S.No	Concentration Ascorbic Acid & HAECG (µg/mL)	Percentage Inhibition of Ascorbic Acid	Percentage Inhibition of HAECG
1	100	54.56±0.11	50±0.075
2	200	63.56±0.05	62±0.081
3	300	55.56±0.10	64±0.171
4	400	75.67±0.11	70±0.157
5	500	79.76±0.09	75.56±0.166
	Ic_{50}	14.91µg/mL	68.48µg/mL

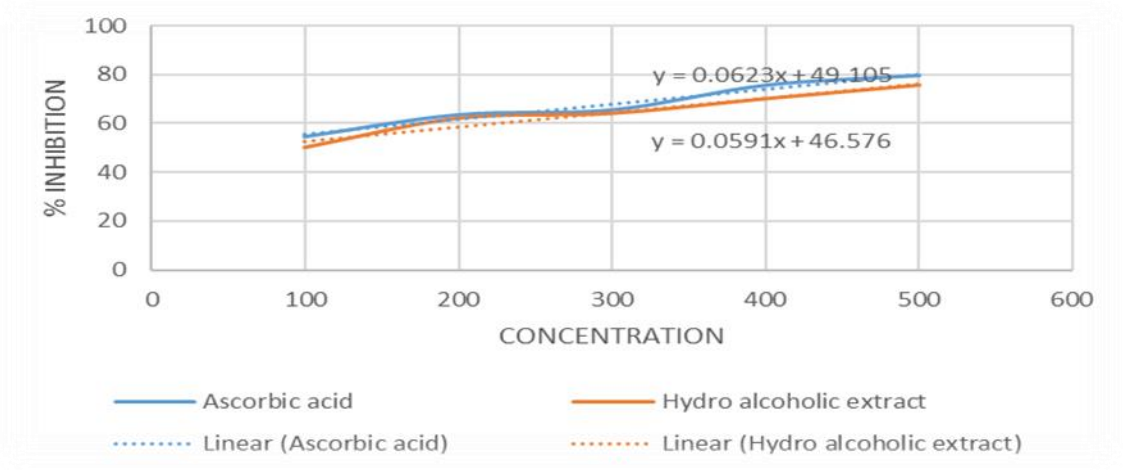

Fig 7. Determination of Lipid Peroxidation Effect of HAECG

As a result, lipid peroxides are formed causing membrane damage. Inhibition of lipid peroxidation therefore can prevent the cell membrane damage increasing membrane stability.

The inhibitory concentration (IC_{50}) of hydro alcoholic extract of the leaves of *Calotropis gigantea* was compared with the standard anti-oxidant ascorbic acid. The inhibitory concentration (IC_{50}) of ***Calotropis gigantea***(leaves) against ascorbic acid is found to be 68.48µg/ml in comparison with ascorbic acid 14.91 µg/ml. The effect of the extract was lesser than ascorbic acid.

Caseinolytic activity

Isolated casein was generally used as a substrate for proteases and assayed as caseinolytic activity. Caseinolytic activity of crude *Calotropis gigantea* latex was compared with that of standard papain.one unit of enzyme activity was defined as the amount of enzyme required to increase in absorbance of 0.01 at 660nm/hr at 37ºc.crude extract of *Calotropis gigantea* latex and papain exhibited caseinolytic activity in a dose dependent manner. Papain showed an average increase in absorbance of 0.03 at 660 nm/hr. *C.gigantea* showed an average increase in absorbance of 0.02 at 660nm/hr.latex of *Calotropis gigantea* exhibited moderate caseinolytic activity when compared to the papain.

Table 6: Determination of Caseinolytic Activity of Papain/*C.Gigantea*

S.No	Papain/ *C.gigantea*latex (mg/ml)	Papain (absorbance)			*C.gigantea latex* (absorbance)		
		0 hr	1st hr	2nd hr	0 hr	1st hr	2nd hr
1	5	0.24	0.27	0.31	0.10	0.12	0.14
2	10	0.26	0.29	0.32	0.12	0.14	0.16
3	15	0.27	0.30	0.33	0.14	0.16	0.18
4	20	0.28	0.31	0.34	0.16	0.18	0.20

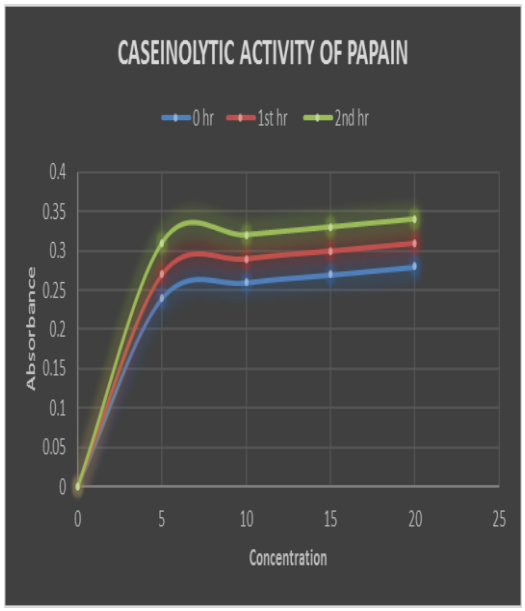

Fig 8 Determination of Caseinolytic activity of Papain

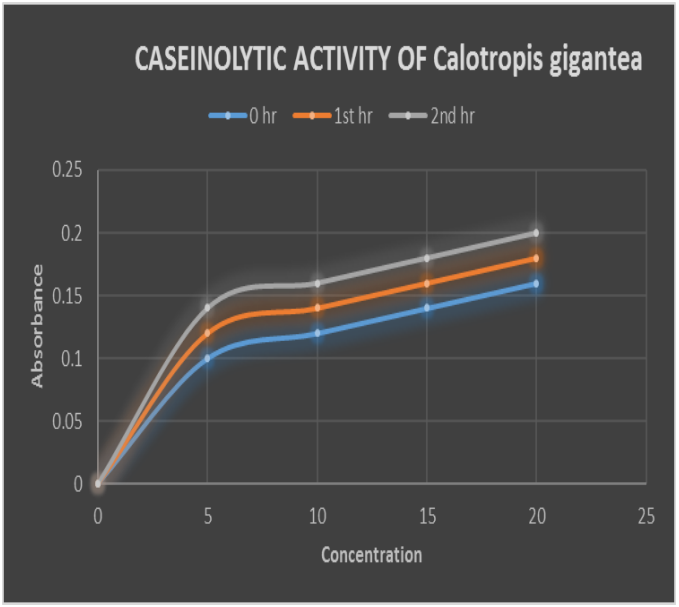

Fig 9 Determination of Caseinolytic activity of *C.gigantea*

CONCLUSION

The phytochemical screening of methanol:chloroform extract(1:1) and HAECG of *Calotropis gigantea* leaves revealed the presence of alkaloids, carbohydrates, cardiac glycosides, sterols, saponins, tannins, phenolic compounds, flavonoids, amino acids and the absence of anthraquinone glycosides. The phytoconstituents are capable of Uv radiation which directly evidence the uv screening formulation can be developed. Both extracts were also screened for anti solar showed the absorbance in a linear manner at 280nm and 330 nm which imparts the extract contains UV absorption core. It elevates that UV screening cosmetics can be designed and developed in o rder to meet the challenges of the synthetic cosmetics. The inhibitory concentration (IC50) of hydro alcoholic extract of the leaves of *Calotropis gigantea* was compared with the standard anti-oxidant ascorbic acid. The inhibitory concentration (IC50) of ***Calotropis gigantea***(leaves) against ascorbic acid is found to be 68.48µg/ml in comparison with ascorbic acid 14.91 µg/ml.

As a result, lipid peroxides are formed causes membrane damage. Inhibition of lipid peroxidation can prevent the cell membrane damage increasing membrane stability. The inhibitory concentration (IC_{50}) of hydro alcoholic extract of the leaves of *Calotropis gigantea* was compared with the standard anti-oxidant ascorbic acid. The inhibitory concentration (IC_{50}) of ***Calotropis gigantea***(leaves) against ascorbic acid is found to be 68.48µg/ml in comparison with ascorbic acid 14.91 µg/ml. The effect of the extract was lesser than ascorbic acid. The antisolar activity of both dry and fresh latex, hydroalcoholic extract reflects the efficacy of vats range of biological activities correlating the chemical constituents and unwanted effects in some cases. Therefore the plant validates all the traditional medicine claim, which paves the way to the isolation of active compounds, clinical trials and formulation

development can be considered for further or future studies researches along with the mechanistic approach.

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