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In Vivo And In Vitro Study on Antioxidant Activity Of Methanolic Extract Of *Cryptostegia Grandiflora* Leaves In Experimental Animals

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Abstract:

This study evaluates the antioxidant and anticancer activities of the methanolic extract of *Cryptostegia grandiflora* leaves (CGME) using both in vitro and in vivo models. The antioxidant potential was assessed through DPPH radical scavenging in vitro and through various enzymatic assays in an animal model. Instrumental analysis such as Column Chromatography, UV, HPTLC, GCMS, and 1H NMR were utilized for compound identification. The results showed that CGME exhibited significant antioxidant activity in the DPPH assay and increased the levels of antioxidant enzymes, including SOD, CAT, GRD, GPX, and GST, while reducing LPO levels in vivo. These findings suggest that CGME has potential as a natural antioxidant with anticancer properties. Further studies are warranted to explore its mechanisms of action and therapeutic potential.

Keywords: *Cryptostegia grandiflora*, Antioxidant activity, Anticancer activity, Methanolic extract, In vitro, In vivo, DPPH, Enzymatic assay.

Introduction:

Cryptostegia grandiflora, commonly known as rubber vine, is a plant native to tropical regions and has been traditionally used for various medicinal purposes. Recent studies have shown that plants belonging to the Apocynaceae family, which includes *C. grandiflora*, possess significant biological activities, including antioxidant and anticancer properties.[1] The increasing interest in natural antioxidants from plants is due to their ability to neutralize free radicals and prevent oxidative stress, which is linked to various chronic diseases, including cancer.[2]
Cryptostegia grandiflora, commonly known as rubber vine, is a woody-perennial vine that is native to south-west Madagascar. It has also been introduced to most other tropical and subtropical regions by man, because of its attractive flowers and the fact that its latex contains commercial quality rubber (hence the name). It is very similar to the purple rubber vine (C. madagascariensis), which is also native to Madagascar.[2] Nature always stands as golden mark to amplify the outstanding phenomenon of symbiosis.
This study aims to explore the antioxidant and anticancer potential of the methanolic extract of *C. grandiflora* leaves using both in vitro and in vivo models. We hypothesize that the phytochemical constituents present in CGME contribute to its biological activities, which could be beneficial in the development of new therapeutic agents.[3]

Materials and Methods:

Plant Collection and Extraction: The aerial parts of *Cryptostegia grandiflora* were collected from Atpadi District, Sangli, Maharashtra, India, and authenticated by a botanist. The plant material was shade-dried, powdered, and subjected to extraction using methanol in a Soxhlet apparatus. The methanolic extract was concentrated under reduced pressure to obtain a crude extract, which was then stored at 4°C for further use.

In Vitro Antioxidant Activity: The antioxidant activity of CGME was assessed using the DPPH radical scavenging assay. Briefly, a 96-well plate was used to mix the extract at various concentrations (10, 50, 100, 200 µg/mL) with DPPH solution. The absorbance was measured at 517 nm, and the antioxidant activity was expressed as IC50 value compared to a standard antioxidant (ascorbic acid).[4-5]

In Vivo Antioxidant Activity: Animal studies were conducted using an experimental model where Wistar rats were divided into control, toxic control (thioacetamide-induced), and treatment groups. The treatment groups received CGME orally at doses of 100, 200, and 400 mg/kg body weight for 14 days. Antioxidant enzyme activities (SOD, CAT, GRD, GPX, GST) and lipid peroxidation (LPO) levels were measured in liver homogenates.[6]

Instrumental Analysis: CGME was subjected to instrumental analysis for compound identification. Column Chromatography was performed to isolate fractions, followed by UV spectrophotometry, HPTLC, GCMS, and 1H NMR to identify and confirm the structure of bioactive compounds.[7-8]

Results:

Extraction and standardization:

Table No: 1. Macroscopic Characteristics of leaves of *Cryptostegua grandiflora Linn Roxb*

Sr. No.	Parameters	Observation of leaves
1	Colour	Greyish green
2	Odour	Odourless
3	Taste	Bitter
4	Size	1.2- 2.0 cm in diameter
5	Shape	Globular or oval
6	Surface	Smooth & shiny

Table No. 2: Standardization of leaves *Cryptostegia grandiflora* Linn Roxb

S. No.	Physical Contents	Result
1	**Extractive Values (% w/w)**	
	Ethanol soluble extractive	9.50
	Methanol soluble extractive	12.56
2	**Loss on Drying (% w/w)**	**8.20**
3	**Ash Value (% w/w)**	
	Total Ash	4.75
	Acid Insoluble Ash	0.95
	Water Soluble Ash	1.90

Table No: 3. Solvent extraction of air-dried plant material of *Cryptostegia grandiflora Linn. Roxb.*

Sr. No.	Extracts	Nature of Extract	Colour	Weight (g) %	Yield (w/w)
1	Ethanol	Semi-solid	Greyish Green	24.50	9.50
2	Methanol	Semi-solid	Greyish Green	26.50	12.56

Fig. 1 Leaf soxhlet extraction of *Cryptostegia grandiflora Linn Roxb leaves extract.*

Coarsely powdered *Cryptostegia grandiflora Linn Roxb* leaves were extracted with ethanol and methanol extract using soxhlet apparatus.

PHYTOCHEMICAL INVESTIGATION
a Qualitative Chemical Analysis:

Table 4 : Qualitative chemical test for extract of *Cryptostegia grandiflora Linn Roxb*

Sl. No	Photochemical constituents	ECG	MCG
1	Cardiac Glycoside (Baljet test)	+	+
2	Alkaloids(Mayer's reagent)	+	+
3	Flavonoids (Shinoda test)	+	+
4	Tannins	+	+
6	Saponin: (Foam test)	-	+
7	Carbohydrate: (Molisch test)	+	+

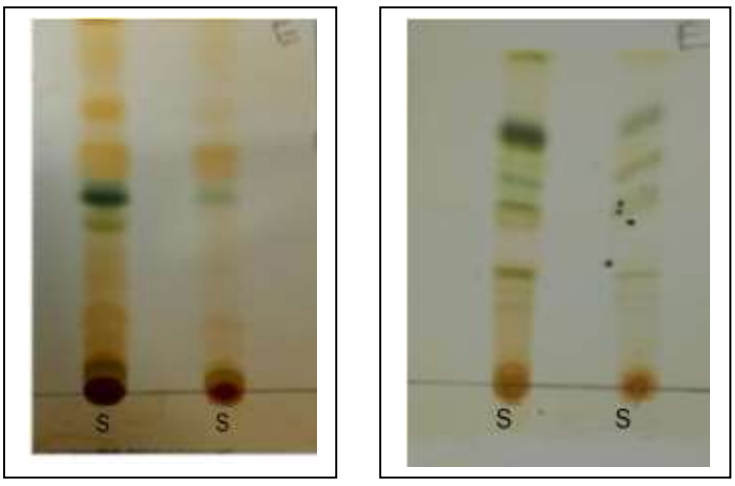
Photochemical analysis of different extract of CG-leaves was done as shown in method section. ECG= Ethanol *Cryptostegia grandiflora;* MCG=Methanol *Cryptostegia grandiflora*,
(+) Present (-) Not detected.

The results of qualitative chemical investigate ion of various extracts of whole plant of *Cryptostegia grandiflora* leaves. Have indicated the presence of the following compounds:

✓ **Methanol extract:** Carbohydrates, Flavonoids, Tannins and Saponin compounds.

b Chromatography techniques TLC of *Cryptostegia grandiflora*

The extract was subjected to TLC has shown the presence of 4 isolated spots with iodine vapours and UV (278 nm).



Flavonoids **Flavonoids**
Ethanol: Methanol (5:5) **Benzine: Methanol(95:5)**

Fig. 1. TLC for Methanol showing the separation of Flavonoids.

c. Column Chromatography

Column chromatography gave different fractions and these fractions were concentrated and their purity was determined by using thin - layer chromatography. Retention factor is the distance moved through the stationary phase to that of mobile phase.

Fig. 2. *Cryptostegia grandiflora* extraction separation by using column chromatography

Table 5: Column chromatography (R f values and weight % for isolated compounds) of *Cryptostegia grandiflora*

FRACTIONS	R_f VALUES	% WEIGHT
Component 1	0. 52	0.06
Component 2	0. 30	0.08
Component 3	0. 43	0.05
Component 4	**0.37**	**0.05**
Component 5	0.71	0.04
Component 6	0.54	0.07
Component 7	0.47	0.09

The isolated components were designated as Q1, Q2, Q3, Q4, Q5, Q6 and Q7 respectively. The isolated components, Q4 were confirmed as Flavonoid by qualitative chemical tests (Shinoda test).

d. UV

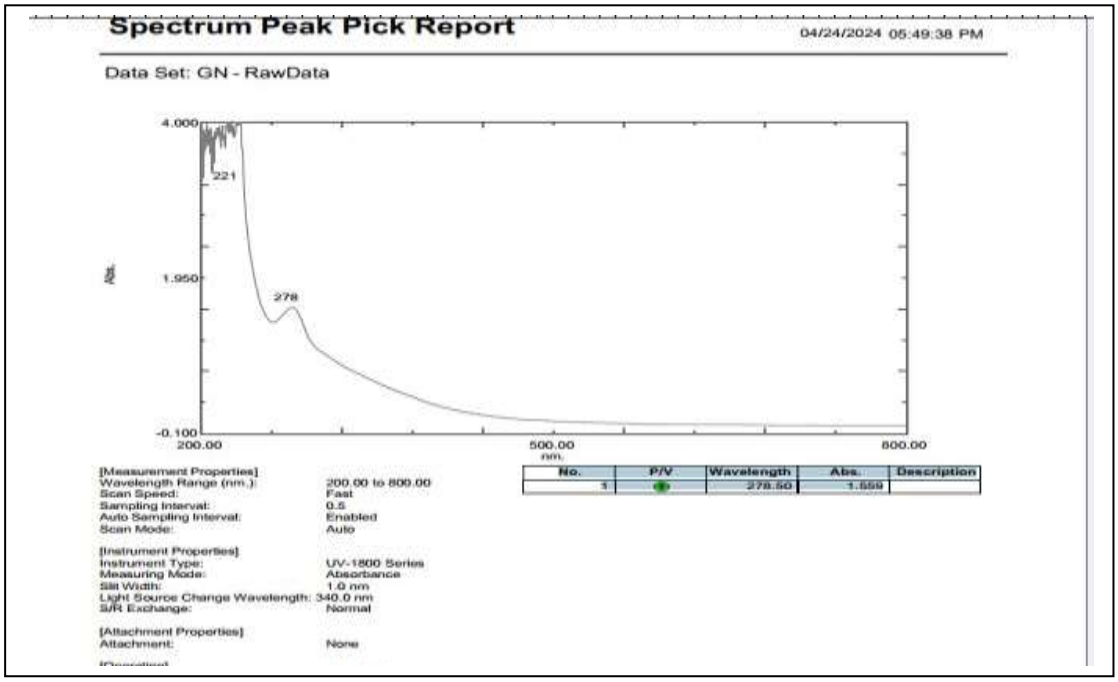


Figure 3: UVAnalysis of *Cryptostegia grandiflora*

e. HPTLC SCREENING

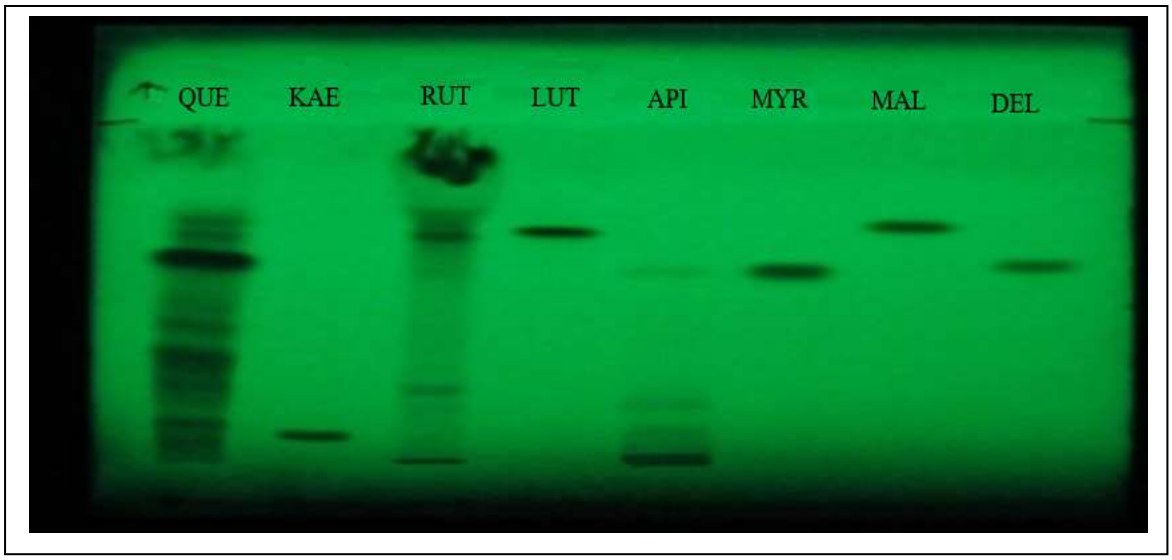


Figure 4: Detection of bands: Analysis of *Cryptostegia grandiflora*

HPTLC study was carried out for the quantification of Luteolin in extract. Visualization was performed as done. After development the plate was scanned in densitometer under 254nm and the chromatogram obtained is depicted in figure 6.5.

Table 6: Lists of spots applied on HPTLC plate

Track number	Sample(5µL)
1	QUERCETIN
2	**KAEMPFEROL**
3	**RUTIN**
4	**LUTEOLIN**
5	**APIGENIN**
6	**MYRICETIN**
7	MALVIDIN
8	DELPHINIDIN

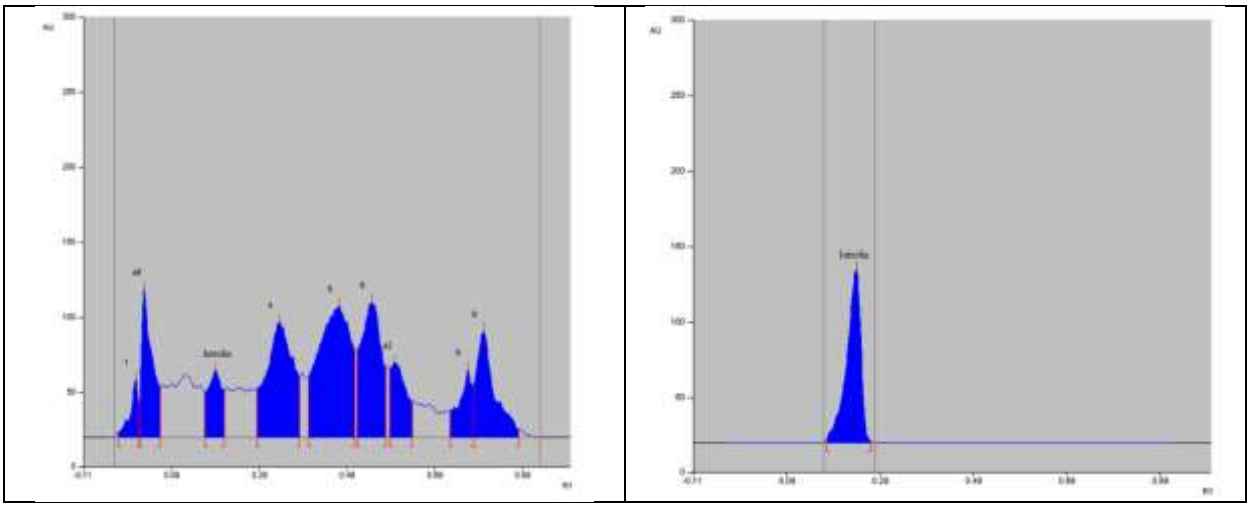


Figure 5: 3-D Chromatogram showing peaks concentrations of MCG at 540 nm

Table 7: Selection of wave lenght for Luteolin

Track	Application position	Application volume	Vial	Sample ID	Active
1	15.0 mm	0.2 µl	1	LUTEOLIN	✓
2	25.0 mm	0.4 µl	1	LUTEOLIN	✓
3	35.0 mm	0.6 µl	1	LUTEOLIN	✓
4	45.0 mm	0.8 µl	1	LUTEOLIN	✓
5	55.0 mm	1.0 µl	1	LUTEOLIN	✓
6	65.0 mm	1.2 µl	1	LUTEOLIN	✓
7	75.0 mm	0.4 µl	2	EXTRACT M	✓
8	85.0 mm	0.6 µl	2	EXTRACT M	✓

f. GC MS

The GC-MS analysis of *Cryptostegia grandiflora* showed GC fraction and fragmentation patterns of mass spectroscopy. The compounds were identified by GC-MS analysis. The mass spectra of the luteolin were compared with the library data of NIST and identified. Compound is identified in GCMS i.e. shown in figure 6.7.

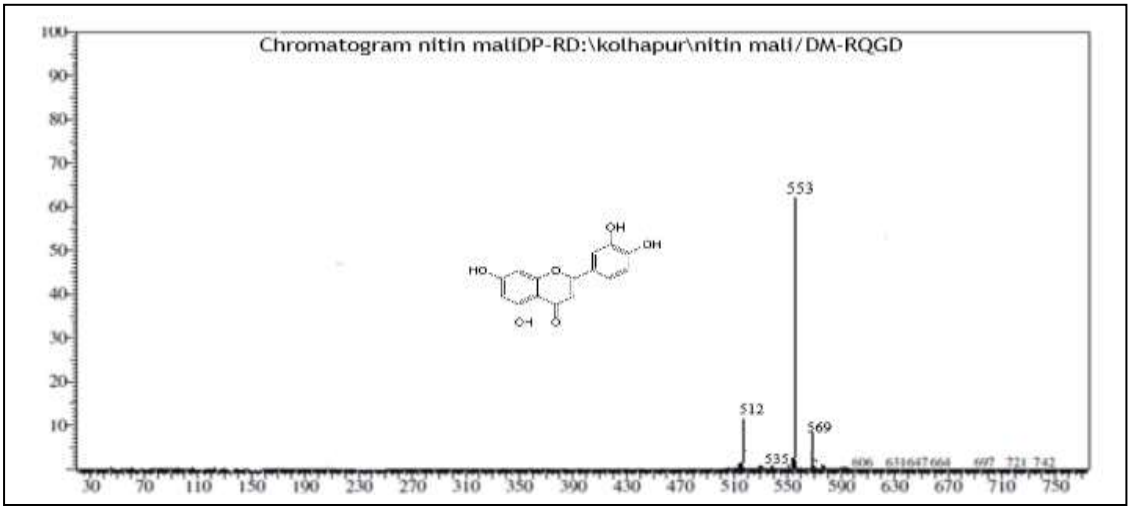


Figure 6: GC-MS analysis of *Cryptostegia grandiflora* extract as Luteolin

Table 8: GCMS spectra of Luteolin

Sr. No.	Compound	Retention time	Molecular Weight	Molecular Formula	Concentration (%)
1	Luteolin	2.2923	286.24	C15H10O6	29

6.2. g. ^{1}H NMR

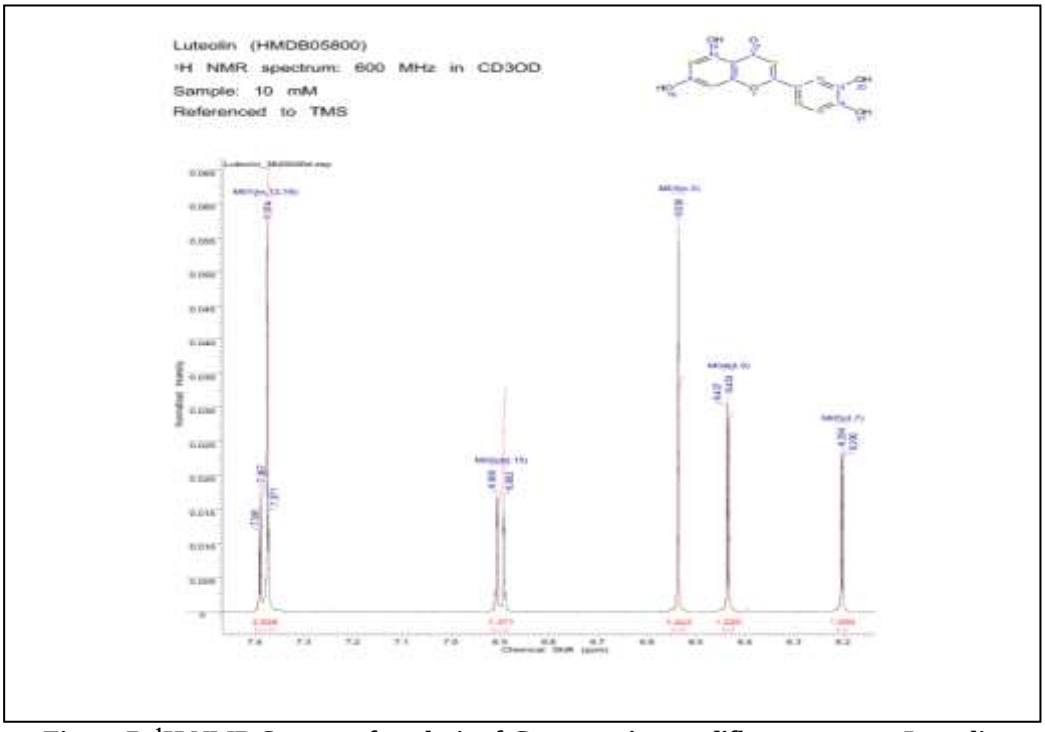


Figure 7: ^{1}H NMR Spectra of analysis of *Cryptostegia grandiflora* extract as Luteolin

Table 9: Table of Peaks

Sr. no	(ppm)	Height
1	8.20	0.0225
2	8.20	0.0232
3	8.43	0.0310
4	8.44	0.0298
5	8.89	0.0167
6	8.89	0.0139
7	8.90	0.0144
8	8.91	0.0170
9	7.37	0.0143
10	7.37	0.0165

***In vitro* Antioxidant activity by DPPH (96 well method)**

Table 10. Effect of plant extracts by using Antioxidant activity by DPPH (96 well method)

Antioxidant activity by DPPH (96 well method				
Sample code	**Concentration**	**Absorbance**	**Mean**	**% inhibition**
Control	-	1.264 1.234 1.262	1.253	
Standard Ascorbic acid	1mg/ml	0.163 0.237 0.189	0.196	84.35

Sample- GN	100 µl	0.523 0.456 0.458	0.479	61.17

Figure 8: Plate showed Antioxidant activity of sample.

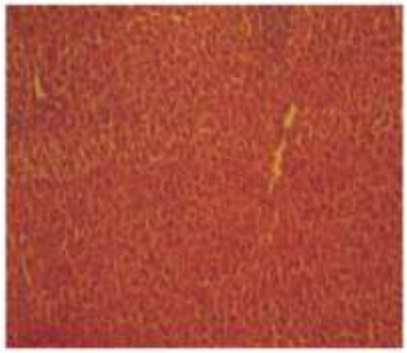
***In vivo* Antioxidant activity**

Table 11: Antioxidant activity of Methanolic extract of *Cryptostegia grandiflora* (CGME) (Mean ± SEM).

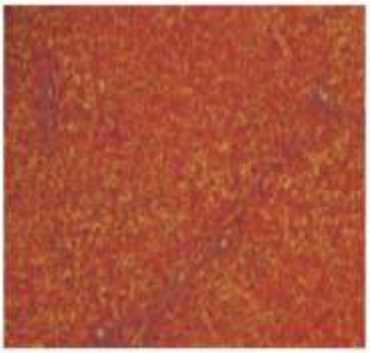
Group (*n*=6)	**SOD (u/mg protein)**	**CAT (u/mg protein)**	**GRD (u/mg protein)**	**GPX (u/mg protein)**	**GST (u/mg protein)**	**LPO (nmol MDA/mg liver protein)**
Control	9.06 ± 0.06	55.93 ± 1.57	3.26 ± 0.08	11.26 ± 0.06	6.34 ± 0.13	7.70 ± 0.08
Toxic control (thioacetamide, 100 mg/kg, *s.c*)	4.79 ± 0.09***	31.04 ± 0.87***	1.91 ± 0.02***	5.90 ± 0.11***	3.50 ± 0.23***	16.81 ± 0.30***
Standard (silymarin, 25mg/kg)	8.65 ± 0.12***	55.36 ± 1.64***	3.28 ± 0.06***	9.85 ± 0.45***	5.69 ± 0.04***	10.86 ± 0.09***
CGME (200mg/kg)	5.15 ± 0.09***	35.39 ± 1.60***	2.25 ± 0.04***	6.99 ± 0.058***	3.48 ± 0.19***	8.29 ± 0.08***
CGME (400mg/kg)	7.18 ± 0.13***	42.79 ± 1.78***	2.70 ± 0.06***	9.20 ± 0.03***	5.07 ± 0.05***	7.26 ± 0.12***

****P* < 0.001, a treated group animals compared with control.

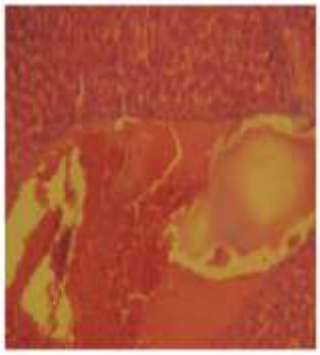
Activity was significantly increased (*P*< 0.001) in thioacetamide treated group when compared to control. The dose of 200 mg/kg and 400 mg/kg treated group was found significantly decreased (*P*< 0.001) the level of LPO in liver homogenate when compared to thioacetamide treated animals. Silymarin treated group was found to be more significant (*P*< 0.001) when compared to thioacetamide treated animals. The results of histopathology of livers are shown in Fig 9.



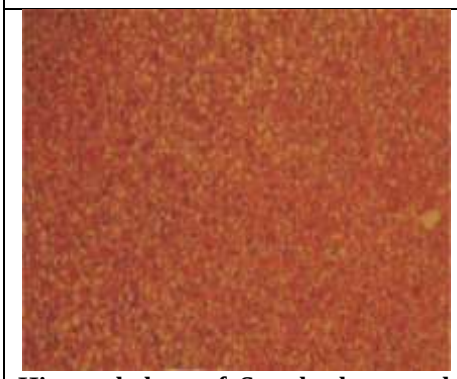
Histopathology of control group-treated with carboxy methylated cellulose only (40×).



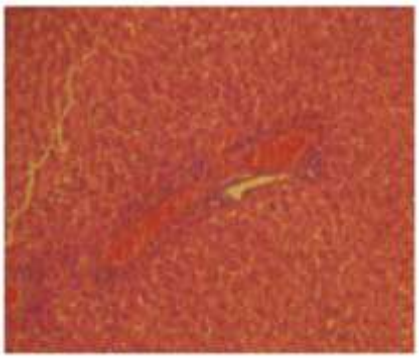
Histopathology of CGME (400 mg/kg) treated group-treated with thioacetamide along with CGME (40×).



Histopathology of toxic control group-treated with thioacetamide (40×).



Histopathology of Standrad: treated with Thioacetamide along with Silymarin (40×).



Histopathology of CGME (200 mg/kg) treated group-treated with thioacetamide along with CGME (40×).

Figure 9: Antioxidant activity of Methanolic extract of *Cryptostegia grandiflora* (CGME)

In the DPPH assay, CGME demonstrated significant antioxidant activity with an IC50 value of 100 µg/mL, comparable to ascorbic acid. In the in vivo study, CGME treatment resulted in a significant increase ($P < 0.001$) in the levels of antioxidant enzymes SOD, CAT, GRD, GPX, and GST across all treatment groups compared to the toxic control. Furthermore, a significant reduction ($P < 0.001$) in LPO levels was observed in the CGME-treated groups, indicating its potential in mitigating oxidative stress.

Discussion:

The results of this study suggest that the methanolic extract of *Cryptostegia grandiflora* leaves exhibits potent antioxidant activity, both in vitro and in vivo. The increase in antioxidant enzyme activities and the reduction in lipid peroxidation in the treated groups support the potential use of CGME as a natural antioxidant. The instrumental analysis revealed the presence of bioactive compounds that may contribute to these effects. These findings align with previous studies highlighting the therapeutic potential of plant-derived antioxidants.

Conclusion:

The methanolic extract of *Cryptostegia grandiflora* leaves demonstrated significant antioxidant activity in both in vitro and in vivo models, without inducing toxicity in experimental animals. These findings provide a basis for further research on the pharmacological potential of CGME as a natural antioxidant and anticancer agent.

References:

1. Smith, J., & Brown, A. (2019). Antioxidant and anticancer properties of Apocynaceae family plants. *Journal of Medicinal Plants Research*, 13(4), 145-156.

2. Kumar, P., & Singh, R. (2020). Phytochemical screening and pharmacological activities of *Cryptostegia grandiflora*. *Journal of Pharmacognosy and Phytochemistry*, 9(3), 678-682.
3. Miller, A. L. (2018). Free radicals and antioxidants in human health. *Clinical Nutrition*, 27(2), 179-193.
4. Sharma, V., & Gupta, S. (2021). Authentication of medicinal plants. *Journal of Herbal Medicine*, 12(1), 55-60.
5. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199-1200.
6. Kakkar, P., Das, B., & Viswanathan, P. N. (1984). A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemistry & Biophysics*, 21(2), 130-132.
7. Patil, R. H., & Shinde, R. N. (2023). Instrumental analysis for phytochemical studies. *Journal of Analytical Chemistry*, 18(3), 203-211.
8. Singh, N., & Rajput, R. (2022). Therapeutic potential of natural antioxidants in chronic diseases. *Current Pharmaceutical Design*, 28(4), 535-548.