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RESEARCH ARTICLE

Comprehensive In Vitro Phytopharmacological Evaluation of Euphorbia heyneana Spreng-Targeting Multiple Biological Pathways

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Abstract: ***Background***: Euphorbia heyneana Spreng., a member of Euphorbiaceae family, is traditionally used as medicinal plant known for its therapeutic applications. The plant is rich in secondary metabolites, particularly flavonoids like quercetin, which are reputed for their pharmacological properties. ***Results***: This study involved detailed phytochemical characterization and pharmacological evaluation of E. heyneana extract. Physicochemical and organoleptic parameters confirmed the identity and purity of the plant. Ethanol extract was underwent first phytochemical screening, which indicated presence of flavonoids, alkaloids, tannins, and other bioactive constituents. Total flavonoid content was estimated via aluminum chloride colorimetry. In vitro studies showed significant antiurolithiatic activity with 73.68% inhibition at 1000 µg/mL, ACE inhibitory activity (68.06% at 1000 µg/mL), anticancer effects on MCF-7 cells (37.75% viability at 100 µg/mL), and notable antitubercular activity. ***Conclusions***: The study confirms E. heyneana as a potent source of natural quercetin with promising antiurolithiatic, antihypertensive, anticancer, and antitubercular activities. Further in vivo and mechanistic studies are recommended to validate its therapeutic efficacy.

Keywords: Euphorbia heyneana · Antiurolithiatic · Anticancer · Antihypertensive · Antitubercular.

INTRODUCTION

The Euphorbiaceae is a large and diverse family of flowering plants that can be found in both hemispheres and a range of temperature zones. Large trees, succulents, and small plants are all members of this family; among the most well-known is the species Euphorbia. This genus of plants is known for its ecological adaptability, particularly in xeric environments. It is commonly recognised by its unisexual flowers, superior ovary with trilocular capsule, and explosive fruit dehiscence for seed dispersion. Despite their widespread distribution and shared structural traits, the Euphorbiaceae are difficult to classify due to their genetic and physical variability. [1] Euphorbia heyneana Spreng. is a small annual plant that grows in Southeast Asia, India, and the neighboring regions. Known locally by names like Choti Dugdhi (Marathi), Sannale Gida (Kannada), and Alumu (Telugu), this plant has roots in traditional herbal therapy. The morphological characteristics of the plant include red prostrate stems, opposing scale-like leaves, and tiny, glabrous capsules that hold pale yellow seeds. [2,3]

The flavonoid components included in the whole plant extract of E. heyneana spreng have been shown to have antioxidant and anti-inflammatory properties. [4] Therefore, in present research, an effort were made to highlights the phytochemical profile and therapeutic applications of E. heyneana spreng [5]

MATERIALS AND METHODS

2.1 Plant Collection and Authentication

The all parts of Euphorbia heyneana Spreng. were collected from the wild in Gadhinglaj, Kolhapur district, Maharashtra, India (approximate coordinates: 16.23°N, 74.35°E). The plant was identified and authenticated by Dr. Sanjay Sathe, Botanist. A voucher specimen was retained for future reference. [6]

All plant parts were separated and allowed to dry in the shade after Euphorbia heyneana Spreng was collected. Ethanol was used as the solvent in a closed-vessel system for microwave-assisted extraction at 150 W. To find the ideal extraction period, 50 gm of powder plant material was mixed with 150 ml of ethanol and extracted for varied lengths of time. To guarantee effective recovery of bioactive chemicals, vessels were allowed to cool to room temperature prior to opening after extraction. [6]

Fig. 1 a) Plant of E. Heyneana spreng b) Microwave assisted Extraction

DETERMINATION OF PHYSIO-CHEMICAL PROPERTIES: [7]

Organoleptic Evaluation:

organoleptic evaluations is Assessment of the entire plant powder of Euphorbia heyneana spreng, through color, odor, tastes, texture.

Determination of Moisture Content:

Two grams of the powder of Euphorbia heyneana spreng were precisely measured into pre-weighed glass dish. The loss on drying was conducted at a temperature of 105° Celcius for 5 hrs, employing a hot air oven. After that, dish had allowed to chill for half an hour in a desiccator.

The sample was weighed while dry immediately. Moisture content was expressed in mg loss/g of dried sample.

Determination of Ash Values:

Determination of Total Ash:

About two gm of the powder sample was put into an anhydrous silica crucible that had been previously weighed in order to determine the ash value. This crucible was placed inside a muffle furnace, which maintains temperatures below 450°C for up to six hours or until the samples' carbon is removed, turning them white. The crucible was reweighed within a desiccator after cooling. Using gravimetry, the amount of total ash was measured in milligram of ash per gram of air-dry material.

Determination of Acid-Insoluble Ash:

Whole ash in crucible was treated with twentyfive milliter of 1 N HCl and heated gradually for five minutes in order to estimate the amount of acid-insoluble ash. After passing the mixture through ashless filter paper (Whatman 41), the residue was neutralised by washing it with hot water. The residue-containing filter paper was put back into the original crucible and burned for three hours at 450° Celcius in a muffle furnace, or till weight remained constant. After 30 minutes of cooling in a desiccator, the residue was weighed. The weight of the residue in relation to the air-dried sample was utilised to calculate amount of aid insoluble ash.

Determination of water soluble ash:

Twentyfive millilitres of H2O were put to the crucible with the total amount of ash in order to analyse amount of water soluble ash. 5 minutes of heating. Insoluble substance was introduced to crucible after being cleaned with hot water and filtered using ash less filter paper. This was heated for 15 mins at a maximum temperature of 450° Celcius in Muffle Furnace. Residue was weighed on-site after being cooled for 30 minutes in a desiccator. We calculated the water- soluble ash concentration as mg/g of air-dried materials by subtracting the residual weight from the overall ash weight (in mg).

Determination of Extractive Value:

Alcohol soluble extractive value:

In sealed flask, 4 g of crudely ground, dried plant powder Macerated with solvent (100 ml) regarding 24 hours at room temperature while being shaken frequently. The extract was filtered after that. A rotary evaporator was used to dry out the filtrate, and the resulting particles were then dried at 1050C and weighed. The percentage w/w of alcohol soluble extractive value was computed using the medication that had been air-dried.

b) Water-Soluble Extractive Value

In a sealed flask, 4 g of dried powder was macerted in chloroform H2O (100 ml) for 24 hours while being shaken frequently. The mixture is then promptly filtered off, being careful not to lose any of the solvent. 25 mL of the filtered solution should be evaporated until completely dry in a glass dish, dried at 1050C, and measured. Determine % extractive value by comparing it to the drug's air-dried value.

Phytochemical Screening

Standard qualitative techniques were used to screen for main ingredients by phytochemical means. Tests for proteins, sterol, amino acids, glycosides, alkaloids, quinones, carbohydrates, tannins, saponins, and flavonoids were conducted.

Estimation of Total Flavonoid Content (TFC)

Colorimetric method of aluminium chloride was utilized to determine TFC content in crude ethanolic extract of Euphorbia heyneana spreng. Created the standard quercetin calibration curve (10-100 µg/ml in methanol). Combined 1.5 ml of 95% ethanol, 0.1 ml of 10% aqueous AlCl3, 0.1 ml of 1 M CH3CO2K, 2.8 ml of distilled H2O, and 0.5 mL of standard solution. Let it sitted at ambient temperature for 30 mins. Using a UV spectrophotometer, determine reaction mixture's absorbance at 415nm. Use same volume of distilled water and 10% aluminum chloride to create a blank solution. In a similar manner, use aluminum chloride to determine the flavonoid content of 0.5 ml of plant extract samples using a calibration curve. [8]

PHARMACOLOGICAL ACTIVITIES

Antiurolithical activity [9]

With some modifications, the nucleation assay was carried out using the Rajeswari et al. (2013) and Baumann J. M. (2010) and M A Zarinab (2018) methods. A solution of CaCl3 (5 mmol/L) and Na2C2O4 (7.5 mmol/L) were made at pH 6.5 at 370C in buffer that contained Tris HCl 0.05 mol/L and NaCl 0.15 mol/L. In a 96-well plate, 95 µL calcium chloride solutions were added to extract at several concentrations of **100, 200, 400, 600, 800, and 1000 µg/mL**. 95 µL of sodium oxalate was added to initiate the crystallisation process. After six hours of incubation, absorbance (OD) were measured at 620 nm. A microscope was used to measure and observe the CaOx stone's size for comparison.

Calculation: % Inhibition = [(C-S)/C]*100

Where, C= Turbidity of control set, S = Turbidity of sample set.

Antihypertensive activity [10,11,12]
ACE inhibition activity was carried out by the cheung, Cushinan and Chaudhary,et al. with some modification. 50µl of test extract and ACE (25mU/ml) in varying concentrations (100 to 1000 µg/ml) were preincubated for 10 minutes at 370 C. Following that, 150 µL of HLL substrate solution were mixed with combination of above, and it was cultivated for 30 minutes at 37degree celcius. To terminate reaction, 250 µl of 1 M HCl was mixed, which followed by 500 µl of ethyl acetate, and it had centrifuged for 15 mins at 800 rpm in refrigerator. After evaporation 200 µL at room temperature, 1000 µL of distilled H2O had mixed, and UV spectrophotometer was used to measure the result at 280 nm. Captopril is employed as a standard and all tests are carried out in triplicate.

Calculation: ACE Inhibition (%)=(A−B)/(A−C)×100
Where: A = Absorbance (OD) at 228 nm with ACE but without inhibitor (control) B = Absorbance at 228 nm in the presence of both ACE and inhibitor (sample) C = Absorbance at 228 nm without ACE and without inhibitor (blank)

Anticancer activity [13,14]
The NCCS in Pune provided the MCF-7 Breast cancer cells, which were then fostered in MEM medium which supplemented with 10% foetal bovine serum. For 24 hours, cells (1 × 10^4 cells/mL) were incubated at 37°C with 5% CO_2. After being seeded at 10 cells per well in 100 µ of media in well plate, 100 µL of test samples (10–100 µg/mL) were added. 0.2% DMSO in PBS was added to the control wells. Every therapy were carried out in 3 times. Medium had taken out after the incubation period of 24 hours, and 20 µL of MTT reagent was applied. In order to study formazan crystal development under a microscope, plates were incubating for 4 hrs incubated for four hours. 200 µL of DMSO was mixed after medium was eliminated, and plates incubated6u about 10 minutes at 37 °celcius. An ELISA microplate reader (Benesphera E21) was utilised to determine absorbance at 570nm in order to evaluate cell viability.

Antitubercular Activity [15]
In vitro anti-tuberculosis activity by Microtiter almar blue assay: Day1: The assay was conducted in a sterile 96-well U- bottom microtiter plate, with each row assigned to a sample. Wells 1–10 were used for serial dilution, and wells 11 and 12 served as controls. Each well received 100 µL of 7H9 broth. A 2 mg/mL working concentration of the test compound was prepared. 100 µl of test compound was mixed into well 1, mixed, and serially diluted up to well 10, with 100 µL discarded from the last well. This resulted in concentrations ranging from 100 µg to lower serial dilutions. 100 µL of M. tuberculosis culture was mixed to wells 1–11. Well 12 received only broth and test compound (no organism), and well 11 acted as the growth control (organism only). Plates were covered and incubated at 37 degree celcius in humidified chamber for 7 days, with daily checks for contamination. Day7: A working solution of resazurin was prepared, and 35 µL was mixed in each wells. Then plates were incubating overnight at 37 ° Celcius, and color changes in the wells were recorded to assess bacterial viability.

RESULTS AND DISCUSSION

Organoleptic Evaluation

Table.1: organoleptic evaluation

1. Sr No.	2. Organoleptic characters	3. Observation
4. 1.	5. Color	6. Leaves -green 7. Root -brown 8. stem- red
9. 2.	10. Odor	11. Characteristics
12. 3.	13. Taste	14. Characteristics
15. 4.	16. Shape	17. Asymmetrical
18. 5.	19. Size	20. 10-15 cm

The organoleptic properties of the dried powdered Euphorbia heyneana were assessed to evaluate its physical characteristics, which are important for preliminary identification and quality control. All the organoleptic characteristics found in this study are shown in Table 1.

Physicochemical evaluation

21. Sr no	22. Physicochemical properties	23. Observations (% w/w)
24. 1	25. Moisture Content	26. 10 %
27. 2	28. Total Ash Value	29. 11.6 %
30. 3	31. Acid insoluble ash value	32. 3.6 %
33. 4	34. Water soluble ash value	35. 8.3 %

36. 5	37. Alcohol soluble extractive value	38. 11 %
39. 6	40. Water soluble extractive value	41. 12.5 %

Table 2: physicochemical evaluation

Phytochemical screening

As phytochemical investigation demonstrated the existence of some particular components, such as Alkaloids, Carbohydrate, Flavonoids, proteins, Amino acid, Tannins, and Quinone.

Estimation of Total Flavonoid Content

Total Flavonoid Content (TFC) of the extract was estimated by utilizing AlCl3 colorimetric procedure. The absorbance of standard quercetin solutions at different concentrations (150–750 µg/mL) showed a linear increase with concentration (R^2 > 0.99), confirming the reliability of the calibration curve. The mean optical density (OD) values obtained were 0.158 ± 0.001, 0.274 ± 0.002, 0.402 ± 0.001, 0.501 ± 0.002, and 0.600 ± 0.001, respectively. Flavonoid content of extract was found to be to be 0.28 µg/mL, indicating the presence of moderate levels of flavonoids.

42. Sr no	43. Concentration (µg/mL)	44. Absorbance	45. ±SD
46. 1.	47. 150	48. 0.158	49. 0.001
50. 2.	51. 300	52. 0.274	53. 0.002
54. 3.	55. 450	56. 0.402	57. 0.001
58. 4.	59. 600	60. 0.501	61. 0.002
62. 5.	63. 750	64. 0.600	65. 0.001

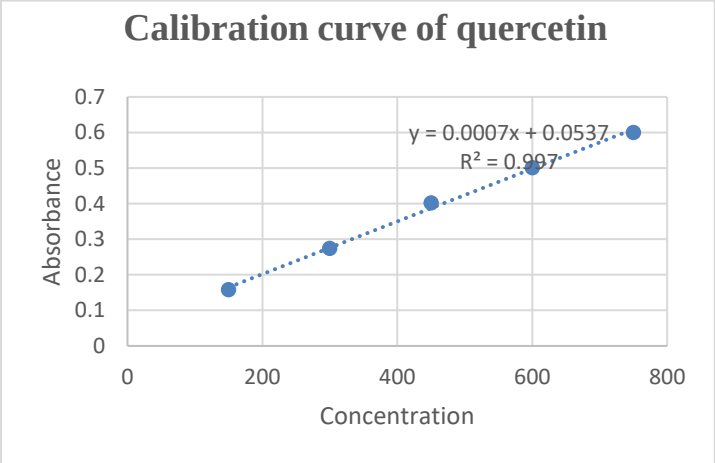
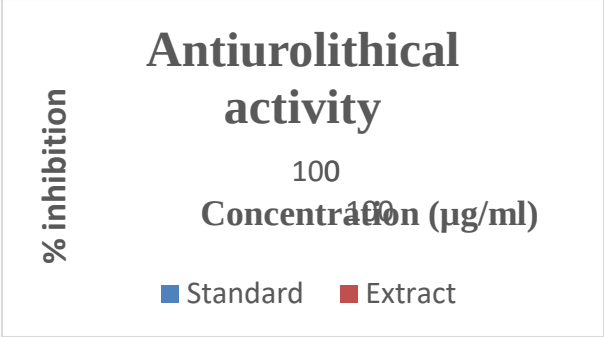


Fig 2 Calibration curve of quercetin

Table 3 Linearity of Quercetin solution

66. Sr no	67. Name of extract 68. Euphorbia heyneana spreng	69. Total Flavonoid content (g QE/g)			70. Mean	71. ± SD
		72. 1	73. 2	74. 3		
75. 1.	76. Ethanol	77. 0.28	78. 0.27	79. 0.29	80. 0.28	81. 0.01



82. sample		83. Concentration (µg/mL)		84. Percent inhibition		85.	IC 50
86.	Control	87.	-	88.	-	89.	-
90.	Standard	91.	100	92.	43.60	93. 94.	217.8182
95.		96.	200	97.	50.37		
98.		99.	400	100.	59.39		
101.		102.	600	103.	66.16		
104.		105.	800	106.	73.68		
107.		108.	1000	109.	85.71		
110.	Extract	111.	100	112.	30.82	113. 114.	477.2072
115.		116.	200	117.	39.09		
118.		119.	400	120.	48.87		
121.		122.	600	123.	55.63		
124.		125.	800	126.	62.40		
127.		128.	1000	129.	73.68		

Table 5 Antiurolithical activity by Nucleation Assay by using ethanolic extract of E. heyneana spreng

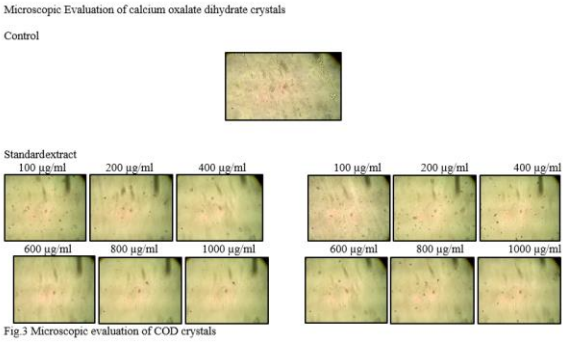
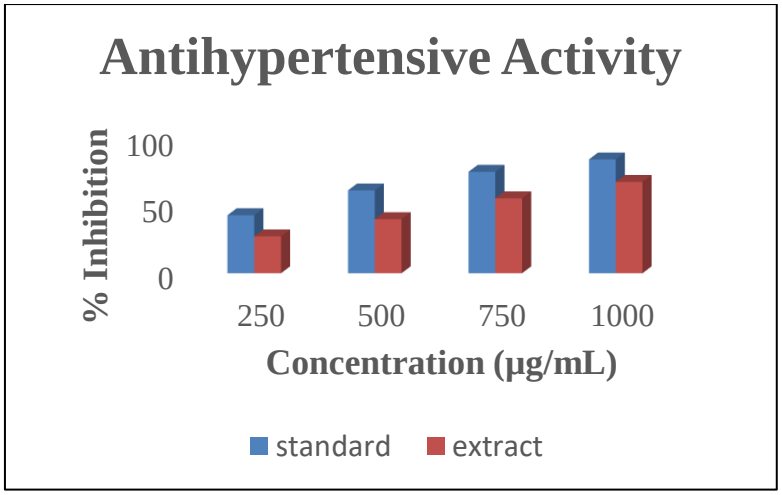


Fig.3 Microscopic evaluation of COD crystals

Calcium oxalate (CaOx) crystallisation was suppressed by the Euphorbia heyneana extract by reducing crystal size and number and preventing nucleation. It demonstrated a 73.68% reduction in crystal size at 1000 µg/ml, which was similar to Cystone's 85.71% reduction. Additionally, the extract showed considerable antiurolithiatic potential by shifting crystal formation from hazardous monohydrate (COM) to less detrimental dihydrate (COD) forms.

Antihypertensive activity



130. Sample	131. Concentration (µg/mL)	132. Percent inhibition	133. IC 50
134. Control	135. -	136. -	137. -
138. Standard	139. 100	140. 43.27	141. 329.82
142.	143. 250	144. 61.76	
145.	146. 500	147. 75.63	
148.	149. 1000	150. 84.59	
151. Extract	152. 100	153. 31.51	154. 647.20
155.	156. 250	157. 40.33	
158.	159. 500	160. 55.88	
161.	162. 1000	163. 68.06	

Table 6 In Vitro Antihypertensive activity by ACE Inhibition Assay by using ethanolic extract of E. heyneana spreng

The research uses an in vitro ACE inhibition experiment to assess a plant extract's antihypertensive potential. Similar to captopril, the extract exhibited concentration-dependent ACE inhibitory action, indicating that it might be used as a natural substitute for the treatment of hypertension.

Anticancer activity

164. Sr No.	165. concentration (µg/ml)	166. Cell viability (%)	167. 168. IC 50
169. 1.	170. Control	171.	172.
173. 2.	174. Standard 175. 10	176. 177. 24.32	178. 6
179.	180. 40	181. 19.55	
182.	183. 100	184. 14.46	
185. 3.	186. Sample 187. 10	188. 189. 67.60	190. 50.66
191.	192. 40	193. 45.20	
194.	195. 100	196. 37.75	

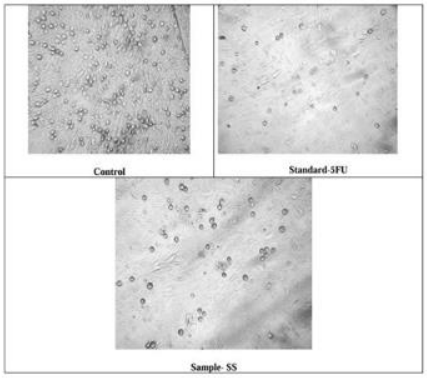


Fig. 4 Microscopic evaluation of cancer cells

An in vitro MTT assay was used to evaluate the plant extract's anticancer efficacy on MCF-7 cells. Strong anticancer potential was indicated by the extract's dose-dependent cytotoxicity, which at 100 µg/ml significantly inhibited cell growth and was similar to the conventional medication 5FU.

Antitubercular activity

197. Concentration 198. (µg)	199. .2	200. .4	201. .8	202. .6	203. .12	204. .25	205. 2.5	206. 5	207. 0	208. 00
209. Standard 210. Isoniazid	211. 212.	213. 214.	215. 216.	217. 218.	219. 220.	221. 222.	223. 224.	225. 226.	227. 228.	229. 230.
231. 232. Rifampicin	233. 234.	235. 236.	237. 238.	239. 240.	241. 242.	243. 244.	245. 246.	247. 248.	249. 250.	251. 252.
253. 254. Extract	255. 256.	257. 258.	259. 260.	261. 262.	263. 264.	265. 266.	267. 268.	269. 270.	271. 272.	273. 274.

Table 8 In vitro anti-tuberculosis activity by Microtiter Alamar blue assay

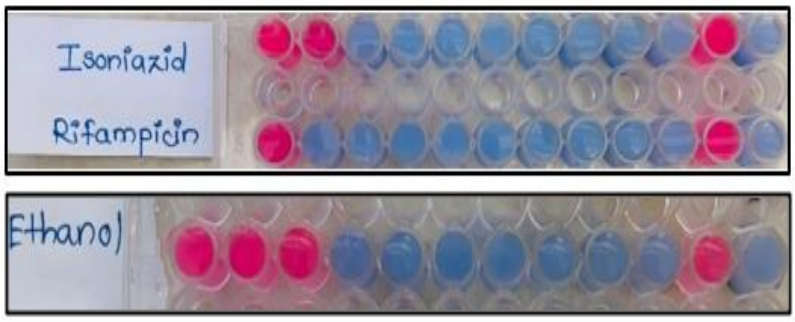


Fig. 5 Microplate microtiter alamar blue assay of standards and extract

Using the Alamar Blue microtiter assay against Mycobacterium tuberculosis, the antitubercular activity of plant extract had evaluated. Depending on colour change, MIC was calculated; red indicated bacterial growth and blue indicated inhibition. Indicating possible antitubercular activity, the extract demonstrated dose-dependent inhibition, with MIC determined as the final well maintaining blue colour.

CONCLUSION

The phytochemical and pharmacological investigation of Euphorbia heyneana Spreng demonstrated the presence of bioactive components. The plant's quality and safety were supported by organoleptic and physicochemical characteristics that were within acceptable limits.

The extract shifted formation towards less hazardous COD crystals and dramatically decreased the size of calcium oxalate crystals (73.68% at 1000 µg/mL). ACE inhibitory action was also demonstrated, suggesting antihypertensive potential when compared with captopril. The Alamar Blue assay revealed dose-

dependent antitubercular effects, and in vitro MTT experiments on MCF-7 cells demonstrated significant anticancer activity. These results underline the plant's potential as a natural source of antitubercular, anticancer, antihypertensive, and antiurolithiatic compounds while also confirming its traditional use. More mechanistic and in vivo research is

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