Exploring the Anticancer Properties of Phytochemicals from *Jatropha gossypiifolia*

[1]Ajay Raj, [2]Prof. (Dr.) M.K. Gupta

[1]Student (M. Pharm 4th Semester), Career Point university Kota, Rajasthan

[2]Principal, School of Pharmacy, (Health and Allied Sciences) Career Point university Kota, Rajasthan

DOI: https://doi.org/10.51244/IJRSI.2025.12050005

Received: 12 May 2025; Accepted: 16 May 2025; Published: 27 May 2025

ABSTRACT

The present study aimed to isolate and evaluate bioactive compounds from Jatropha gossypiifolia through a series of extraction, fractionation, and chromatographic techniques. Various extraction methods followed by multiple liquid-liquid partitioning and compound isolation using preparative and column chromatography were employed. Phytochemical screening of the fractions revealed the presence of phytosterols, copper acetate, alkaloids, and proteins. A compound isolated from the chloroform fraction was further characterized using proton nuclear magnetic resonance (^1H NMR) spectroscopy. Antioxidant activity of the methanolic extract was assessed using the DPPH radical scavenging assay, which exhibited 37.50% scavenging activity, whereas the standard ascorbic acid showed 97.92%. Additionally, cytotoxicity was evaluated using the MTT assay on the MDA-MB-231 human breast cancer cell line, where the petroleum ether fraction demonstrated significant activity with 10 µg/mL concentration resulting in reduced cell viability. These findings indicate that Jatropha gossypiifolia possesses potent antioxidant and anticancer compounds, highlighting its potential in pharmaceutical applications.

Keywords: Jatropha gossypiifolia, MTT assay, Anti-cancer activity, Extraction, Fractionation

INTRODUCTION

Jatropha gossypiifolia (JG) is “bellyache bush” which belongs to Euphorbiaceous family and mainly found in Africa and America [1]. The plant is used as a traditional medicine in the treatment of bellyache, fever, and diabetes [2]. According to the Greek words JG can be explained as Jaros and trophy, which represent doctor and food, respectively [3]. According to the English name, this plant is known as "bellyache bush," "purge nut," and "red fig-nut flower" while the French names are "herb à mal de ventre" and "medicinier sauvage" [1]he vernacular name for JG are "baga" in Malinke and Dioula, "sataman" in Bambara, "lapalapa" in Yoruba, "Athalia" in Hindi, and "pinhao-roxo" in Brazilian colloquial [3]. In Cameroon, this plant is known as "maagami balmol" and "sambaali," which derived from two regional dialects that are spoken in the Sudano Sahelian region of the nation and are called Haoussa and Fufulde, respectively. The Americas and Africa's tropical and subtropical areas are home to a large population of this species. [4] It has a variety of uses in traditional medicine, including analgesic, anti-inflammatory [5], anti-haemorrhagic, homeostatic, and a remedy for snakebites. [6]According to phytopharmacological studies this plant possess several properties like anticoagulant properties [7], hypoglycemic and antidiabetic [8], antiulcer, antihypertensive, anti-inflammatory [5], analgesic, antipyretic properties [8], antimicrobial properties [9], antitumoral properties [10], antianemic properties [11], antifertility properties [12], hepatoprotective properties [13], and neuroprotective, anticancer and antibacterial.

Cancer is a life-threatening disease where cells grow uncontrollably and damage nearby tissues. Its incidence is increasing every year, leading to a rising number of diagnosed cases. In cancer, cells lose the ability to stop dividing, forming a group of abnormal cells. These are often called tumors, nodules, lumps, masses, or

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

lesions. Tumors can form in any part of the body and are sometimes found by accident during unrelated medical checkups.[14]

If the tumour stays in one place and does not spread, it is called a benign tumour. However, if it spreads to nearby tissues or other parts of the body, it is called a malignant tumour. This spreading process is known as metastasis. Benign tumors usually do not return after removal, but malignant tumors can reappear.[14]

Cancer has become one of the leading causes of death, especially in developed countries like the United States, accounting for about 25% of all deaths. According to 2020 data, cancer caused around 10 million deaths globally, which means one in every six people died due to cancer. Because of this, cancer is seen as a major challenge of modern society. Although many scientific studies are underway, there is still no perfect cure for cancer.[13]

Many medicinal plants have natural compounds that help in treating and managing cancer. Cassia is one such plant and belongs to the Fabaceae family. There are about 250 to 300 known species of Cassia found mostly in tropical and subtropical regions.[15]

MATERIAL AND METHODS

Preparation of extract and Fractionation

The whole plant was taken and kept for drying under shade. The dried plant was powered using grinder. Further, using methanol as a solvent, the grinded powdered (1.5 litre) material was extracted in Soxhlet apparatus using percolation method.[16] After obtaining 20 cycles, the methanol extract was collected and concentrated under reduced pressure. Further, using different solvents (according to the polarity) multiple liquid-liquid fractionations were done to obtain different fractions[17]

Multiple liquid-liquid fractionation

Jatropha gossypiifolia is a medicinal plant known for its rich phytochemical profile. To isolate its bioactive compounds, multiple liquid-liquid fractionation was performed. This technique uses immiscible solvents to separate compounds based on their polarity. Initially, the crude extract was dissolved in water and sequentially partitioned with solvents such as hexane, chloroform, ethyl acetate, and butanol. Each fraction was collected separately and concentrated. These fractions were then subjected to phytochemical screening. The goal was to identify compounds like alkaloids, flavonoids, terpenoids, and phenols. This method improves the purity of isolated compounds. The fractions can be further used for biological and pharmacological studies. This step is crucial for identifying potential therapeutic agents from the plant.[18]

Phytochemical test

Preliminary phytochemical screening of the various solvent extracts of Jatropha gossypiifolia was carried out using standard qualitative methods to detect the presence of major secondary metabolites. The tests were performed to identify alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and phenolic compounds. Alkaloids were detected using Mayer's and Wagner's reagents, while flavonoids were identified by the alkaline reagent and lead acetate tests. Tannins were tested using ferric chloride, and saponins were identified by the froth test. The presence of glycosides was confirmed by Keller-Kiliani and Legal's tests. Terpenoids were detected using the Salkowski test, while steroids were tested with Liebermann–Burchard's reagent. Phenolic compounds were confirmed using the ferric chloride and lead acetate tests. These assays provided a preliminary understanding of the chemical constituents present in each fraction, supporting further isolation and characterization of bioactive compounds.[19]

DPPH Assay

The antioxidant activity of various extracts of Jatropha gossypiifolia was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as described by Blois (1958) with slight modifications. A

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

0.1 mM solution of DPPH in methanol was freshly prepared and protected from light. Different concentrations of each plant extract (ranging from 10 µg/mL to 100 µg/mL) were mixed with 2.0 mL of the DPPH solution in a test tube and adjusted to a total volume of 4.0 mL with methanol.

The mixture was shaken vigorously and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV–Visible spectrophotometer against a blank containing methanol. Ascorbic acid was used as the standard antioxidant for comparison. The percentage of DPPH radical scavenging activity was calculated using the following formula:[20]

Scavenging activity (%) = {(A 0 − A 1) / A 0} × 100

Where A0A_0A0 is the absorbance of the control (DPPH solution without extract) and A1A_1A1 is the absorbance in the presence of the extract or standard. All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD).

MTT Assay

The cytotoxic activity of Jatropha gossypiifolia extracts was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay on the MDA-MB-231 human breast cancer cell line. Cells were seeded in 96-well plates at a density of 5×10^3 cells per well in 100 µL of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 hours to allow cell attachment.[21]

Following incubation, cells were treated with various concentrations of the plant extracts (ranging from 10 µg/mL to 100 µg/mL) and incubated for an additional 24 hours. After treatment, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hours at 37 °C. Subsequently, the medium was carefully removed, and the resulting formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm using a microplate reader.[22]

RESULT AND DISCUSSION

The whole plant of JG was powered using grinder and found to be 117.2201 gm. After percolation the extract was concentrated to obtain 2.935 gm. After obtaining the multiple liquid-liquid fractions the yield for petroleum ether, chloroform, ethyl acetate and methanol were 0.5477 gm, 0.9517 gm, 0.9319 gm and 0.1074, respectively. Fraction 9 (JGE-9) was collected from the methanolic extract which was yield to be 1.1 mg.

1. Weight of Eppendorf with extract and labelled sticker **JGE- Chloroform**1.2413
2. Weight of Eppendorf with extract and labelled sticker **JGE- Pet-ether** = 1.1054gm
3. Weight of Eppendorf with extract and labelled sticker **JGE- Methanol** =1.1445gm
4. Weight of Eppendorf with extract and labelled sticker **JGE- Ethyl acetate** = 1.0594 gm.

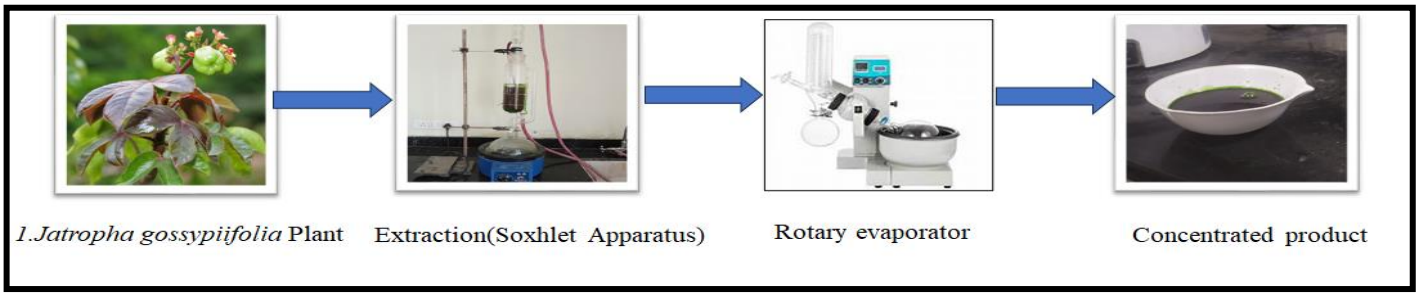

Figure 01: extraction of JG plant

Phytochemical test

Phytochemical screening of various solvent extracts of Jatropha gossypiifolia was carried out to identify the presence of major bioactive compounds. The following qualitative tests were performed:

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

Table 01: Phytochemical test

S. No	Constituents	Phytochemical test	Results
1	Glycosides	Killer killani test Ferric chloride test	+ -
2	Alkaloids	Hager's test Wagner's test	+ +
3	Carbohydrates	Fehling test	-
4	Saponins	Foam test	+
5	Tannins	Gelatine test	-
6	Proteins	Xanthoproteic test Ninhydrin test	- -
7	Diterpenes	Copper acetate test	+
8	Phenolic	Ferric chloride test	+

- **Alkaloids**: The presence of alkaloids was confirmed using Wagner's and Mayer's reagent, which produced a positive result in the chloroform and methanolic extracts.
- **Flavonoids**: The alkaline reagent and lead acetate tests revealed the presence of flavonoids in the methanolic and ethyl acetate extracts.
- **Tannins**: Ferric chloride testing indicated the presence of tannins in the aqueous and ethyl acetate extracts.
- **Saponins**: The froth test showed positive results for saponins in the ethyl acetate and aqueous extracts.
- **Phytosterols**: The presence of phytosterols was detected using the copper acetate test in both the methanolic and chloroform fractions.
- **Proteins**: Biuret and ninhydrin tests revealed the presence of proteins in the aqueous and methanolic extracts.

DPPH Assay

The antioxidant activity of various Jatropha gossypiifolia extracts was evaluated using the DPPH radical scavenging assay. The methanolic extract exhibited a scavenging activity of 37.50% at a concentration of 100 µg/mL, while the standard antioxidant, ascorbic acid, demonstrated a significantly higher scavenging activity of 97.92% at the same concentration. The chloroform extract showed the least antioxidant activity, with a scavenging percentage below 10%. These results indicate that the methanolic extract of Jatropha gossypiifolia possesses moderate antioxidant potential, though less than that of the standard ascorbic acid. The observed activity in the methanolic extract supports further investigations into its bioactive compounds for potential antioxidant and therapeutic applications.

Table 02. Percentage scavenging activity of different fractions at different concentrations and Bar graph of % RSA (radical oxygen activity) of different fractions.

Conc.(µl)	Abs. of control	Percentage scavenging activity (%)				
		Chloroform	Ethyl acetate	Methanol	Pet. Ether	Ascorbic acid
20	0.1959	24.20	14.29	28.20	11.20	96.83
40	0.1959	26.30	17.91	32.50	15.23	97.49
60	0.1959	27.31	19.39	34.52	17.52	97.52
80	0.1959	31.52	22.39	35.60	18.72	97.80
100	0.1959	35.60	24.40	37.50	21.62	97.92

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

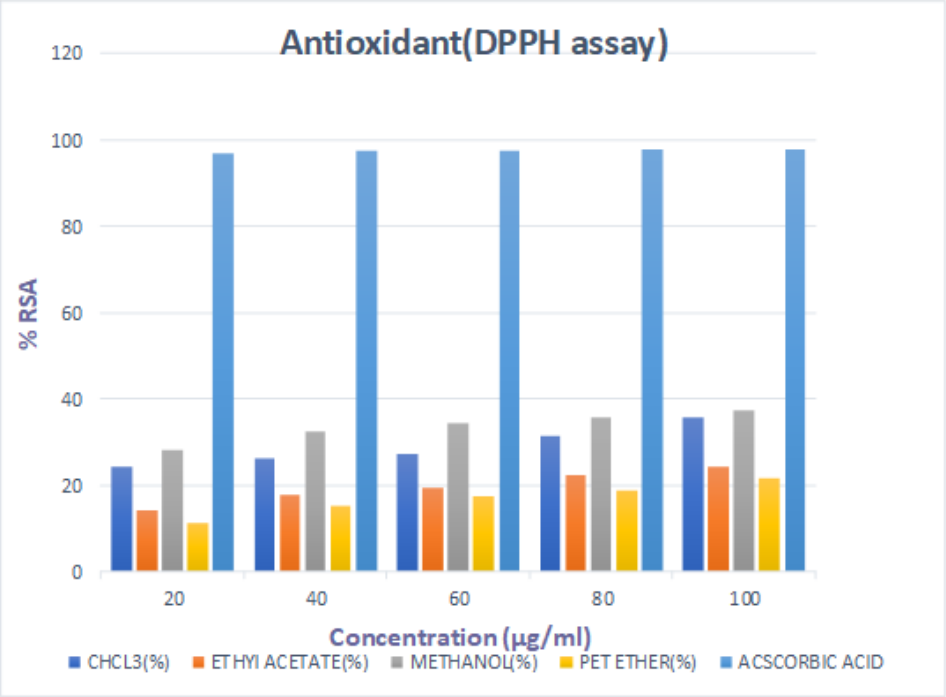

Figure 02: Percentage scavenging activity of different fractions at different concentrations and Bar graph of % RSA (radical oxygen activity) of different fractions.

MTT Assay

The cytotoxic activity of various solvent fractions of Jatropha gossypiifolia was evaluated against the MDA-MB-231 human breast cancer cell line using the MTT assay. Among the tested fractions, the petroleum ether extract exhibited the most significant cytotoxic effect. At a concentration of 10 µg/mL, the petroleum ether fraction reduced cell viability, indicating a notable inhibitory effect on cancer cell proliferation. The percentage of viable cells in this treatment group was considerably lower compared to the control, demonstrating the extract's potential anticancer activity. The methanolic and chloroform extracts also showed moderate cytotoxic effects, whereas the aqueous fraction exhibited minimal activity. These findings suggest that non-polar fractions, particularly petroleum ether, may contain potent bioactive compounds responsible for the observed cytotoxicity.

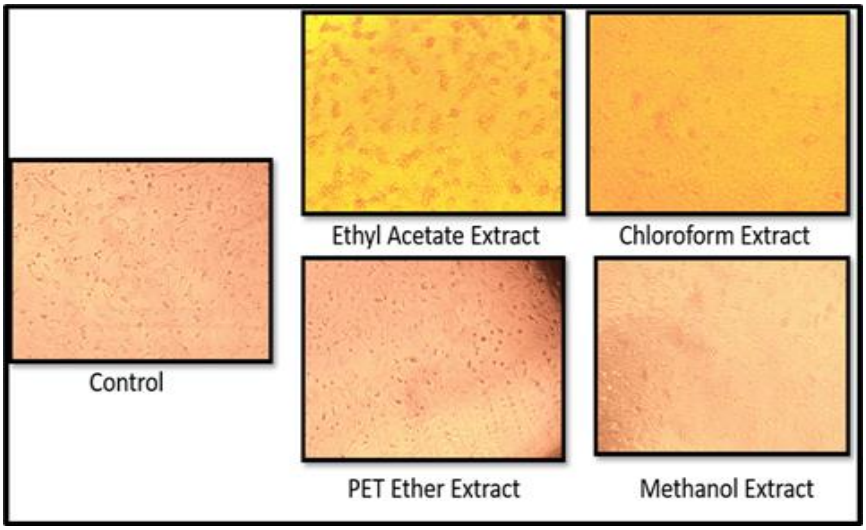

Figure 03: The figure showing MD-MB231 cells after treatment with the fractions of J. gossypiifolia and Bar graph showing the graph of effect on cell viability of cancer cell after treatments with the fractions of J. gossypiifolia

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue V May 2025

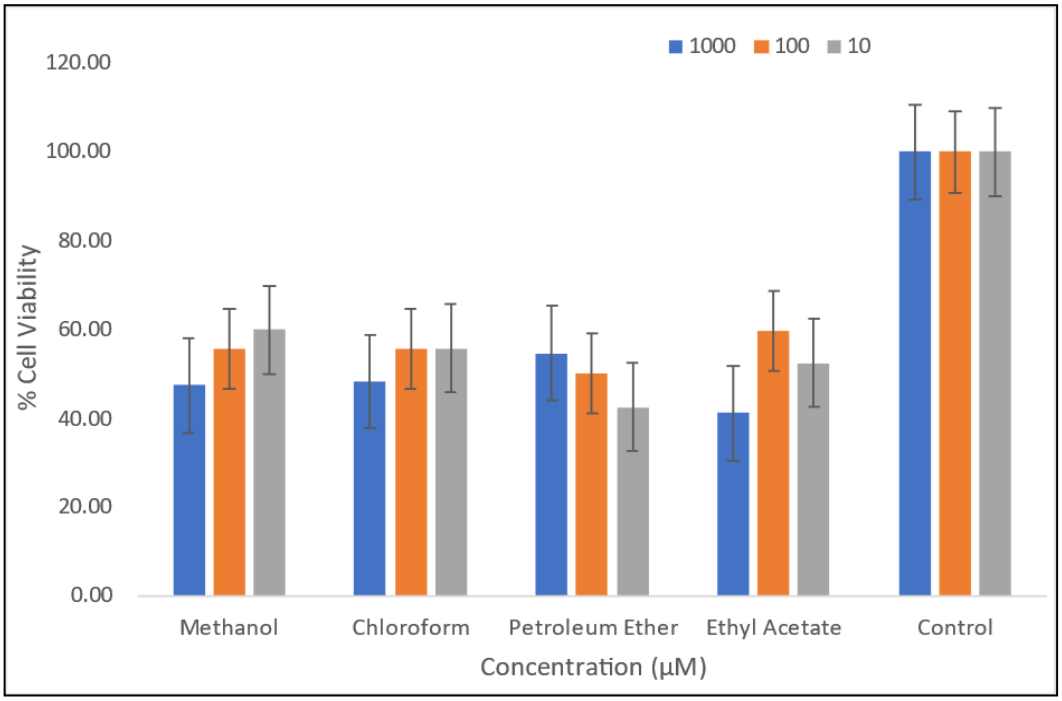

Figure 04. The figure showing MD-MB231 cells after treatment with the fractions of J. gossypiifolia and Bar graph showing the graph of effect on cell viability of cancer cell after treatments with the fractions of J. gossypiifolia.

CONCLUSION

Various extraction techniques, followed by multiple liquid-liquid fractionation and compound isolation using preparative and column chromatography, were employed to isolate bioactive constituents from Jatropha gossypiifolia. Phytochemical screening of the different fractions revealed the presence of phytosterols, copper acetate, alkaloids, and proteins. A specific compound was successfully isolated from the chloroform fraction through column chromatography and subsequently characterized using proton nuclear magnetic resonance spectroscopy. Antioxidant activity of the methanolic extract was evaluated using the DPPH radical scavenging assay, which showed a percentage scavenging activity of 37.50%, in comparison to the standard ascorbic acid, which demonstrated 97.92% activity. Furthermore, cytotoxic potential was assessed via the MTT assay on the MDA-MB-231 human breast cancer cell line, where the petroleum ether fraction showed notable activity, with cell viability at 10 µg/mL indicating its potential anticancer efficacy. These results suggest that Jatropha gossypiifolia contains promising bioactive compounds with antioxidant and anticancer properties.

REFERENCES

1. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG. and Identification of Bioactive Compounds from Plant Extracts. Published online 2017. doi:10.3390/plants6040042
2. Asep S, Hening H, P SG. Anticancer Activity of Jatrophone an Isolated Compound from jatropha gossypifolia Plant Against Hepatocellular Cancer Cell HEP G2 1886. 2017;10(2):667-673.
3. Awobajo FO, Adegoke O a, Odugbemi TO. P Hytochemical and a Ntimicrobial S Creening of H Ybanthus. 2009;1(4):159-160.
4. Falodun A, Sheng-Xiang Q, Parkinson G, Gibbons S. Isolation and characterization of a new anticancer diterpenoid from Jatropha gossypifolia. Pharm Chem J. 2012;45(10):636-639. doi:10.1007/s11094-012-0693-4
5. Félix-silva J, Giordani RB, Silva-jr AA, Zucolotto SM, Fernandes-pedrosa MDF. A Review of Traditional Uses , Phytochemistry , Pharmacology , and Toxicology of This Medicinal Plant. Evidence-Based Complement Altern Med. 2014;2014:1-32.

6. Félix-Silva J, Gomes JAS, Xavier-Santos JB, et al. Inhibition of local effects induced by Bothrops erythromelas snake venom: Assessment of the effectiveness of Brazilian polyvalent bothropic antivenom and aqueous leaf extract of Jatropha gossypiifolia. Toxicon. 2017;125:74-83. doi:10.1016/j.toxicon.2016.11.260
7. Félix-Silva J, Souza T, Camara RB arro. G, et al. In vitro anticoagulant and antioxidant activities of Jatropha gossypiifolia L. (Euphorbiaceae) leaves aiming therapeutical applications. BMC Complement Altern Med. 2014;14:405. doi:10.1186/1472-6882-14-405
8. Gupta A, Naraniwal M, Kothari V. MODERN EXTRACTION METHODS FOR PREPARATION OF BIOACTIVE PLANT EXTRACTS. 2012;1(1):8-26.
9. Honoré DD, Bayoï JR, Darman RD. Journal of Natural Products and Resources gossypifolia L . Leaf Extracts Aiming Antimicrobial and Therapeutical Applications. 2022;8(1):284-288.
10. Jain S, Choudhary GP, Jain DK. Antioxidant and Hepatoprotective Potential of Ethanolic Leaves Extract of Jatropha gossypifolia. Int J Plant Sci Ecol. 2015;1(5):190-195.
11. Jain S, Choudhary GP, Jain DK. Pharmacological evaluation and antifertility activity of Jatropha gossypifolia in rats. Biomed Res Int. 2013;2013:2-7. doi:10.1155/2013/125980
12. Li W, Tang YQ, Sang J, Fan RZ, Tang GH, Yin S. Jatrofolianes A and B: Two Highly Modified Lathyrane Diterpenoids from Jatropha gossypiifolia. Org Lett. 2020;22(1):106-109. doi:10.1021/acs.orglett.9b04029
13. Mulpuri S, Carels N, Bahadur B. Jatropha, Challenges for a New Energy Crop: Volume 3: A Sustainable Multipurpose Crop.; 2019. doi:10.1007/978-981-13-3104-6
14. Nagaharika Y, kalyani V, Rasheed S, Ramadosskarthikeyan. Anti-inflammatory activity of leaves of Jatropha gossypifolia L. by hrbc membrane stabilization method. J Acute Dis. 2013;2(2):156-158. doi:10.1016/s2221-6189(13)60118-3
15. Nurwidayati A. The phytochemical screening and thin layer chromatography results of Jatropha gossypiifolia seeds. Chem Compd J gossypiifolia seeds. 2012;3(2):27.
16. Ohiagu FO, Chikezie PC, Chikezie CM, Enyoh CE. Anticancer activity of Nigerian medicinal plants : a review. 2021;6.
17. Omoregie ES, Sisodiab BS. In vitro Antiplasmodial activity and Cytotoxicity of leaf extracts from Jatropha tanjorensis. Pharmacologyonline. 2011;2(1):656-673.
18. Techniques M. No Title.
19. Wu Q, Patocka J, Nepovimova E, Kuca K. Jatropha gossypiifolia L. and its biologically active metabolites: A mini review. J Ethnopharmacol. 2019;234:197-203. doi:10.1016/j.jep.2019.01.022