Isolation of Phenolic Compound and Antibacterial Activity of Phenolic Extract of Patah Tulang (Euphorbia Tirucalli. L)

Muhammad Furqan
Department of Chemistry
Faculty of Mathematics and Natural Science
Universitas Sumatera Utara

Sovia Lenny
Department of Chemistry
Faculty of Mathematics and Natural Science
Universitas Sumatera Utara

Lamek Marpaung
Department of Chemistry
Faculty of Mathematics and Natural Science
Universitas Sumatera Utara

Abstract

In this study, the phenolic compound was isolated and characterized, and their biological activity was assayed. The phenolic compound was isolated and characterized from the methanol extract using chromatographic techniques. The antimicrobial activity of the phenolic crude extract compound was assessed using disk diffusion method. Phytochemical investigation on the methanol crude extract of E. tirucalli resulted in the isolation of the phenolic compound structure approached to derivative of gallic acid. Their structures were deduced on the basis of UV-Vis Spectroscopy, FT-IR, ^{1}H NMR, ^{13}C NMR. The crude extract antimicrobial phenolic compound showed significant antibacterial activities against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus polymyxa in the disk diffusion assay. Inhibition zones were 9–15 mm. The maximum inhibition was shown at concentration 100 ppm against E. coli. The results showed that the isolated crude antimicrobial phenolic compound have significant antimicrobial activity, which can be used as antibiotics.

Keywords: E. Tirucalli, Screening, Antibacterial Activity, Phenolic Isolation, Spectroscopy

I. INTRODUCTION

With the tropical climate, Indonesia is a second country with the largest biodiversity in the world. There are about 6.000 species of flowering plants in Indonesia that can be applied as traditional medicine.. The use of plants as medicines has prompted experts to find the new chemical content of some plants that are useful in the manufacture of modern medicine.

Euphorbia L, (Euphorbiaceae) is the third largest genus of flowering plants, after Astragalus (Fabaceae) and Psychotria (Rubiaceae)[1]. Euphorbia types can be distinguished easily by the viscosity of the latex and the nature of the particular inflorescences (cyathia)[2]. This plant is known for its use as an ornamental plant that generally grows in the yard of the house such as: E. milii Des Moul., E. tirucalli L., E. lactea Roxb[3] and latex in this plant has an economic value in some species such as E. antisyphilitica Zucc. and E. intisy Drake[4]. Active natural ingredients in the latex of Euphorbia species have contributed a great deal in the field of treatment wherever they are found[5].

E. tirucalli has been used widely as a traditional medicine in east asia East Asia, India, Africa, and South America have been used to treat several diseases, such as syphilis, asthma, cancer, stomach pain, parasites in the intestines, skin diseases, and leprosy[6]. Chromatography and spectroscopy analysis of bark extract of E. tirucalli contain phenolic and terpene compound[7].

The ferulic acid content of E. tirucalli extract has potential activity as antibacterial to several bacterial species, such as S. epidermidis, E. faecalis and P. Aeruginosa. In other research, the phenolic content and antioxidant properties of E. tirucalli has been determined with the value of is 7.73 to 30.54 mg / 100 g of gallic acid equivalents and 12.15 mg / mL-16.59 mg / mL (DPPH assay), also extract of E. tirucalli showed potential activity as antibacterial for Staphylococcus and MiaPaCa-2 inhibitors in pancreatic cancer cells[8], [9].

The high phenolic content of E. tirucalli and supported with its potential activity as antibacterial agent, the further investigation is needed to determine the phenolic compound that presence in methanolic extract and its antibacterial activity. The study is conducted with several steps, such as phytochemical screening, extraction-isolation, characterization (UV-Vis, FTIR, 1H-NMR and 13C-NMR) and bioactivity determination.

II. MATERIALS AND METHODS

A. General

All the solvents and reagents used in this study were of analytical grade and were used without further purification. TLC was performed using silica gel 60 F254 (E. Merck). Dimethyl sulfoxide (DMSO, purity 99%) was obtained from Sigma. The remaining mentioned chemicals and solvents used in this experiment were obtained from Sigma–Aldrich. Uv-Vis spectroscopy Varian Cary 100 Conc was used to measure λ_{max}, FT-IR Shimidzu Prestige (Japan) was used to determine the presence of functional group of phenolic compound. ^{1}H NMR and ^{13}C NMR spectra were recorded on an Agilent (500 MHz and 125 MHz) instrument in CD_3OD (purity 99.99%) with TMS (99.999%) as an internal standard (chemical shifts in δ ppm).

B. Microorganism

Bacterial strains, Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), and Bacillus polymixa. The collected organisms were subcultures in nutrient agar plates and kept at 4 ^{o}C until needed for use.

C. Plant Materials

The samples was collected from Gampong Teungoh village, district of Langsa during the month of Juni 2017. The taxonomic identification of the plan was conducted at the Herbarium Madanense, University of Sumatera Utara.

D. Preparation of Crude Extract

The collected samples was washed thoroughly with water and cut into small pieces. The wet sample of E. tirucalli (2000 g) were extracted for 24 h with methanol (5 L). The methanol was evaporated from the methanol crude extract using a rotary evaporator at 60°C under reduced pressure to get the crude extract. The methanol free crude extract (70 g) was suspended in ethyl acetate to remove tannin from extract. The ethyl acetate was evaporated using a rotary evaporator at 60 $^{\circ}$C. The crude extract (10 g) was fractionated with n-hexane and methanol. The phenolic compound was assumed to be present in the methanol fraction (4 g).

E. Antibacterial activity

The antimicrobial activity of phenolic crude extract from the E. tirucalli was estimated using the agar disk diffusion method against two Gram positive (S. aureus and Bacillus polumixa) and two Gram-negative bacteria (E. coli and P. aeruginosa) cultured at different concentration. Two concentrations, 10 and 100 ppm were prepared with dimethyl sulfoxide (DMSO) in this experiment. Sterile filter paper disks of 6 mm in diameter were impregnated with each pure compound of E.tirucalli and placed on the inoculated agar. The plates were incubated micro aerobically at 37 ^{o}C for 24 h. The antibacterial activity was measured by the diameter of the zone of inhibition against the tested bacteria.

F. Isolation of Pure Compound

Gravitational elution of methanol extract in silica gel column with chloroform: methanol in increasing amounts of methanol gave two fractions; fractions A and B. Fraction A was continued for further purification using chloroform: ethyl acetate (80:20 and 60:40, respectively) in preparative TLC and giving yellowish pictorial paste (4(A):2).

III. RESULT AND DISCUSSION

A. Phytochemicals Screening

In present study, phytochemical tests confirmed the occurrence of phenolic, terpenoids/steroid, alkaloids and saponins in the methanol extracts. The results demonstrated that E. tirucalli is dominated with phenolic and terepenoid/steroid compounds (Table 1). The phytochemical analysis result in this work is similar with the other species of Euphorbia[10]–[12].

Table – 1
Screening test results methanol extracts patah tulang plants (e. tirucalli)

No.	*Group*	*Reactor*	*Result*
1.	*Phenolic*	*Phenolic (methanol extract) $FeCl_3$ 5%*	+
2.	*Terpenoids / steroid*	*$CeSO_4$ 1% in H_2SO_4 with TLC plate*	+
3.	*Alkaloids*	*Bouchardat*	-
		Meyer	-
		Dragendorf	-
4	*Saponin*	*Distilled water*	-

B. Antibacterial Activity Test

The antimicrobial effect of methanol E. tirucalli extracts was investigated through disk diffusion agar (DDA) method. The results (Table 2 & 3) presented that extracts had the most detrimental effect against Eschericia coli and Pseudomonas aeruginosa at

concentration 100 ppm. The smallest inhibition zone diameter against different methanol E. tirucalli concentrations belonged to Gram-positive bacteria. Inhibition zone was observed at all concentrations for all Gram-positive and Gram-negative bacteria.
The antibacterial activity possessed by the total phenolic extract tend to be strongly active/ moderate with a range of values between 9 to 15 mm. The antibacterial activity of plant extracts are grouped based on the diameter of inhibition on an agar medium into three categories: strongly active (> 11mm), moderately active (> 6mm - <11mm) and inactive (<6mm)[13].

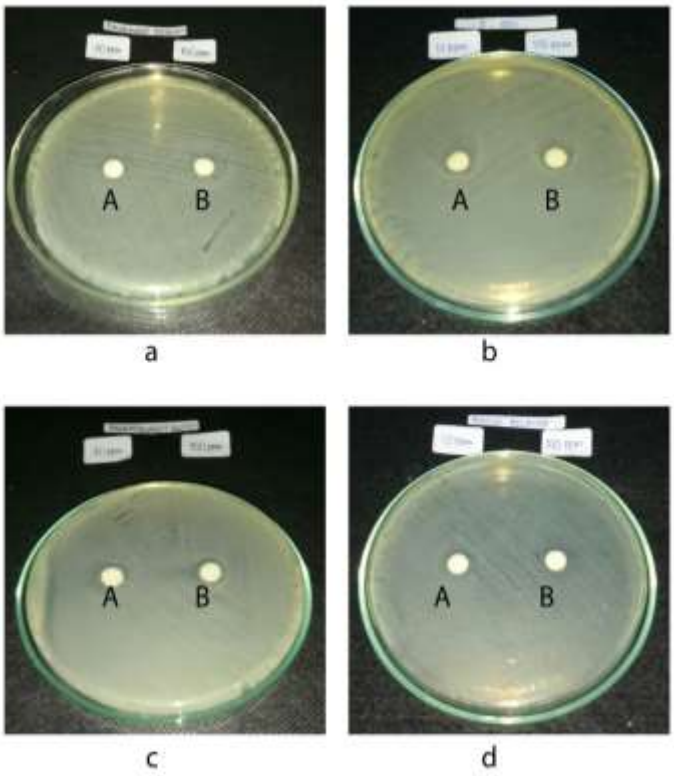

Fig. 1: Antibacterial measurement of crude phenolic extract of E. tirucalli against P. Aeruginosa (a), E. coli (b), S. aureus (c) and B. polymixa (d) with extract concentration 10 (A) and 100 (B) ppm

Table – 2
Results of measurement of antibacterial activity zone of inhibition (ZOI) of crude phenolic extract

No.	*Concentration (ppm)*	*Inhibition zone diameter (mm)*			
		E. coli	*P. aeruginosa*	*S. aureus*	*Bacillus polymixa*
1.	*10*	*12*	*10*	*10*	*9*
2.	*100*	*15*	*14*	*11*	*10*

Table - 3
Results of measurement inhibition index (MII) of crude phenolic extract antibacterials

No.	*Concentration (ppm)*	*Antibacterial Index*			
		E. coli	*P. aeruginosa*	*S. aureus*	*Bacillus polymixa*
1.	*10*	*1*	*0.67*	*0.67*	*0.67*
2.	*100*	*1.5*	*1.34*	*0.84*	*0.5*

C. *Analysis of UV-Vis and FT-IR*

UV-Vis spectrum of the phenolic compound that diluted in methanol is shown in Figure 2.
There are two peak that was observed, 219 and 272 nm, in Figure 2. Those maximum absorptions described the presence of $n\rightarrow\sigma^*$ and $n\rightarrow\pi^*$ transition, respectively. The trasition of $n\rightarrow\sigma^*$ is ascribed to the appearance of C-O group in the phenolic compound and C=O group for $n\rightarrow\pi^*$ transition.

The other data, FT-IR spectrum of the phenolic compound (4(A):2) is shown in Figure 3. Fig. 3 showed several band that indicated the presence of phenolic compound, such as at 3454 cm^{-1} ascribed for the appearance of –OH group. The other peak is observed at 1697 and 1244 that indicated the present of ester group (C=O and C-O). The confirmation of phenolic compound is declared by the presence of C=C aromatic band at 1581, 1508 and 765 cm^{-1}. The results of UV-Vis spectroscopy and FT-IR of phenolic compound (4(A):2) is shown similar result to the gallic acid spectra[14].

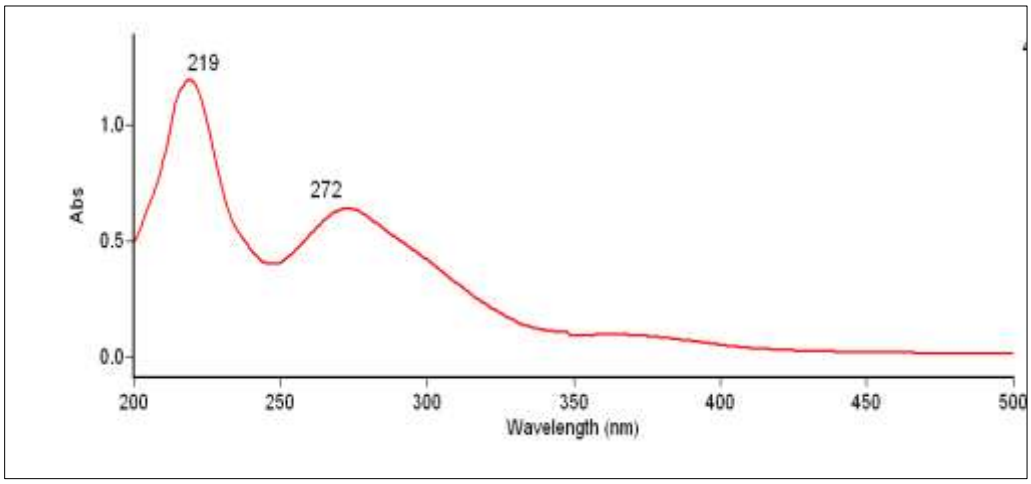

Fig. 2: The UV-Vis spectrum of phenolic compound

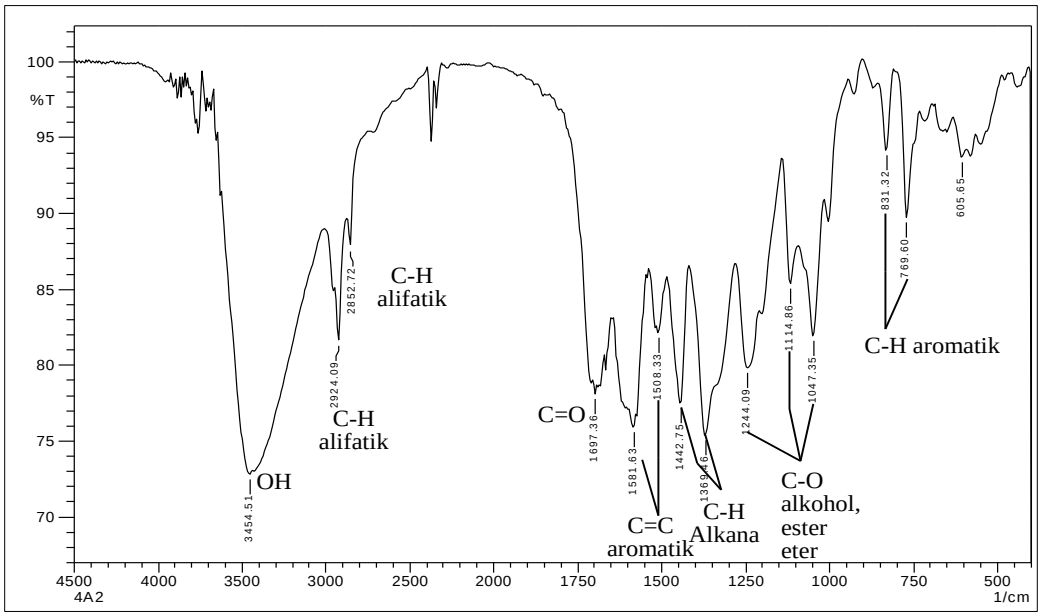

Fig. 3: The FT-IR spectrum results for phenolic compound

D. Analysis of ^{1}H-NMR and ^{13}C-NMR

The ^{1}H-NMR spectrum of the phenolic compound that diluted in methanol is shown in Figure 4. In the ^{1}H-NMR spectrum, all proton signal showed one singlet. The first signal at δ 7.04 ppm indicated the presence of two aromatic proton (H-2 and H-6). The second signal at δ 3.34 ppm indicated the presence of two proton at H-7. The last signal is appeared at δ 3.81 ppm ascribed the presence proton of methoxy group (H-9). To support the elucidation structure of phenolic compound (4(A):2) of E. tirucalli, the analysis of ^{13}C-NMR was also conducted (Figure 5).

The presence of aromatic ring in the phenolic compound was supported with the presence of several signal of ^{13}C-NMR at δ 110 (C2 and C6), δ 121 (C1) and δ 146 ppm (C3, C4, C5). The present of methoxy proton in ^{1}H-NMR is support with the presence of methoxy carbon signal at 52 (C8). In ^{13}C-NMR, also showed the presence of carbonyl group in the phenolic compound at δ 169 ppm (C7). The presence of carbonyl and methoxy signal in the ^{13}C-NMR leads to the ester group according to the structural identification of derivative of gallic acid, methyl gallate[15].

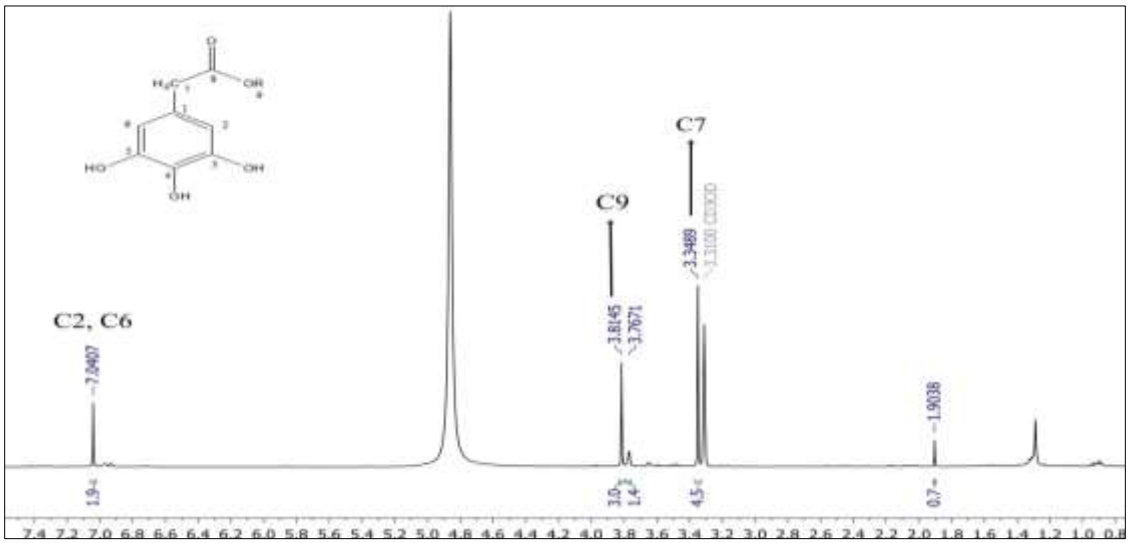

Fig. 4: ^{1}H NMR spectrum of the isolated phenolic compounds

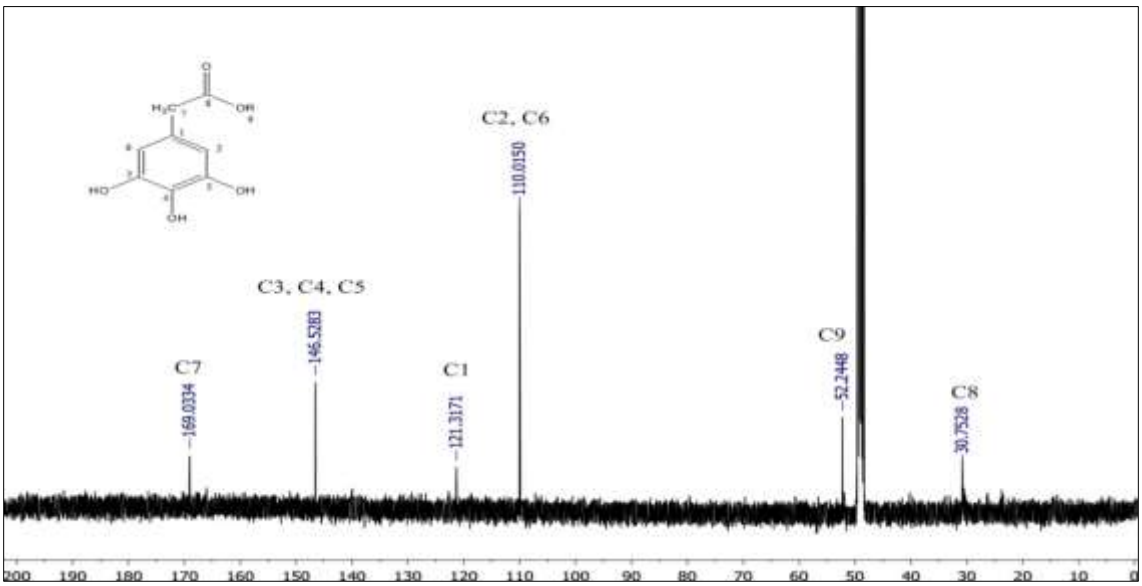

Fig. 5: ^{13}C NMR spectrum of the phenolic compound

According to the result of elucidation structure of phenolic compound of E. tirucalli from UV-Vis, FT-IR, ^{1}H-NMR and ^{13}C-NMR data and the comparation result to Xiaofeng et al (2005), the phenolic compound structure approached to derivative of gallic acid structure with ester group (Figure 6).

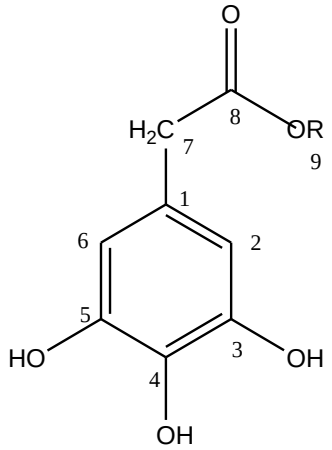

Fig. 6: Proposed structure of gallic acid derivative structure

IV. CONCLUSION

E. tirucalli was dominated with phenolic compound due to the phytochemical analysis. The phenolic crude extract of E. tirucalli showed a considerable antibacterial effect againts S. aureus (MII=0.84), E. col (MII=1.5), P. aeruginosa (MII=1.34), and B. Polymixa (MII=0.5). The isolated phenolic compound (4(A):2) from E. tirucalli has yellow color (yellowish pictorial paste) and its structure approached to the gallic acid derivative with ester group that obtained from the analysis of UV-Vis, FT-IR, ^{1}H-NMR and ^{13}C-NMR data.

REFERENCES

[1] R. Govaerts, R.,frodin, D.G., "World checklist and bibliography of Euphorbiaceae (with Pandaceae)," R. Bot. Gard., vol. 2, no. Kew, 2000.
[2] J. W. Horn et al., "Phylogenetics and the evolution of major structural characters in the giant genus Euphorbia L. (Euphorbiaceae)," Mol. Phylogenet. Evol., vol. 63, no. 2, pp. 305–326, 2012.
[3] A. M. Rizk, "The chemical constituents and economic plants of the Euphorbiaceae," Bot. J. Linn. Soc., vol. 94, no. 1–2, pp. 293–326, 1987.
[4] R. E. Schultes, "Members of Euphorbiaceae in primitive and advanced societies," Bot. J. Linn. Soc., vol. 94, no. 1–2, pp. 79–95, 1987.
[5] J. H. Andrea Vasas, Dóra Rédei, Dezső Csupor, Joseph Molnár, "Diterpenes from European Euphorbia Species Serving as Prototypes for Natural-Product-Based Drug Discovery," European J. Org. Chem., vol. 5115–5130, pp. 5115–5130, 2012.
[6] S. M. . R. Cataluna. P, "The traditional use of the latex from Euphorbia tirucalli Linnaeus (Euphorbiaceae) in the treatment of cancer in South Brazil," Acta Horticulturae, vol. 501. pp. 289–295, 1999.
[7] Q. V. Vuong et al., "Optimization of ultrasound-assisted extraction conditions for euphol from the medicinal plant, Euphorbia tirucalli, using response surface methodology," Ind. Crops Prod., vol. 63, pp. 197–202, 2015.
[8] J. R. S. A. L. Keline Medeiros de Araújo, Alessandro de Lima, Jurandy do N. Silva, Larissa L. Rodrigues, Adriany G. N. Amorim, Patrick V. Quelemes, Raimunda C. dos Santos, Jefferson A. Rocha, Éryka O. de Andrades, "Identification of Phenolic Compounds and Evaluation of Antioxidant and Antimicrobial Properties of Euphorbia Tirucalli L.," antioxidants, vol. 3, no. February, pp. 159–175, 2014.
[9] B. Munro, Q. V Vuong, A. C. Chalmers, C. D. Goldsmith, M. C. Bowyer, and C. J. Scarlett, "Phytochemical, Antioxidant and Anti-Cancer Properties of Euphorbia tirucalli Methanolic and Aqueous Extracts," pp. 647–661, 2015.
[10] B. Ashok, S. D. Bhat, V. Shukla, and B. Ravishankar, "Preliminary Phytochemical Evaluation of Euphorbia Fusiformis Buch-Ham," Pharmacogn. J., vol. 3, no. 25, pp. 44–47, 2011.
[11] Q. Shi, Y. wei Sun, and D. Meng, "Phytochemical and cytotoxic studies on the roots of Euphorbia fischeriana," Bioorganic Med. Chem. Lett., vol. 27, no. 2, pp. 266–270, 2017.
[12] V. Sharma and P. Janmeda, "Extraction, isolation and identification of flavonoid from Euphorbia neriifolia leaves," Arab. J. Chem., vol. 10, no. 4, pp. 509–514, 2017.
[13] M. Elgayyar, F. A. Draughon, D. A. Golden, and J. R. Mount, "Antimicrobial Activity of Essential Oils from Plants against Selected Pathogenic and Saprophytic Microorganisms," J. Food Prot., vol. 64, no. 7, pp. 1019–1024, 2001.
[14] A. Chanwitheesuk, A. Teerawutgulrag, J. D. Kilburn, and N. Rakariyatham, "Antimicrobial gallic acid from Caesalpinia mimosoides Lamk.," Food Chem., vol. 100, no. 3, pp. 1044–1048, 2007.
[15] X. Ma, L. Wu, Y. Ito, and W. Tian, "Application of preparative high-speed counter-current chromatography for separation of methyl gallate from Acer truncatum Bunge," J. Chromatogr. A, vol. 1076, no. 1–2, pp. 212–215, 2005.