# PHYTOCHEMICAL ANALYSIS OF TH CRUDE AND FRACTIONETED EXTRACT OF JATROPHA CUCAS LEAVES: A COMPARATIVE STUDY.

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## ABSTRACT

*An ornamental plant such as Jatropha curcas has been identified with the presence of variety of phytocompounds also known as plant secondary metabolites that are relevant in the treatment of variety of human illments. The aim of this study is to comparatively evaluate the extraction of phytocompounds of Jatropha curcas leaves from the crude (methanol) to the fractionated extracts (using Ethyl acetate, N-hexane and chloroform in their order of polarity. The data obtained was analyzed using analysis of variance. The phytochemical screening of the crude (methanol) extract and the fractionated extracts of Jatropha leaves was carried out using universal laboratory technique and the amount quantified. The quantitative phytochemical analysis revealed the presence of ten (10) compounds across the solvents of extraction. The result showed that ethylacetate(13. 28÷0.07b)has higher value of terpanoid, followed by N-hexane(12.3+0.07c). The least is the crude (7.86+0.05a), with a significant difference across the solvents at p>0.01. Tannin ranged from 0.03+0.14a to 0.05+0.14a with N-hexane (0.05+0.14a) having the higher value. Chloroform and crude (0.014+028 and 0.04+0.34a) had the same value, while the ethylacetate had the least 0.03+0.014a) with significant difference across the extraction solvent. Crude and N-hexane have the same mean values (7.87±0.92b and 7.87±0.07b) and higher than ethylacetate (7.83±0.31b) and (7.82±0.35a) of chloroform. For flavonoid, the crude extract has the high value of 7.68±0.3a and ethylacetate 3.34±0.28a with no significant difference. In a descending order crude>N-hexane>ethylacetate>chloroform. The crude (methanol) had 7.57±0.21d higher value of saponin. Phenol was high in crude extraction (1.85±0.07c) with a significant difference, while ethylacetate and N-hexane had no significant difference. The result of the triterpene and phytate, crude extract was in the lead with high value of 10.07±0.35b, 13.72±0.28c respectively with significant difference, while the N-hexane, and ethylacetate with the same value -0.30±0.79 had no significant difference. The steroid value was the same across all the solvents. Crude extract showed high value of (25.49±0.78c) glycoside, followed by ethylacetate (25.37±0.14b). The least is chloroform 24.97±0.43a) for glycoside with significant difference showed by only the crude extract and chloroform. The result obtained showed that the crude extract (methanol) had high yield in eight if the phytocompounds when compared to the others (fractionated). The same value of steroid was also revealed in all the extraction solvents. N-hexane showed high value for tannin compared to others. This implies that methanol (crude) is a better extraction solvent for phytochemical screening of Jatropha curcas leaf compared to the fractionated.*

**Key words**: Extraction, fractionated, Jatropha curcas, phytochemical and comparative

## INTRODUCTION

The increasing resistance to drug currently used for the treatment of infectious human ailments, the high cost of the synthetic drugs and treatment, shortage of drugs supply due to the increase in population have become a global concern to those in developed and developing country's. It has been estimated that about 80% of over 6 billion people globally, can not afford the drugs produced by the western pharmaceutical industry; this therefore has arouse the search for a safe, secured affordable and sustainable source of drug for the populace globally (Dillard & German, 2000).

Medicinal plant renaissance has become the most common approach to solve the obvious challenges post by the use of synthetic products (Toy, Thomas Matthew & Skaria, 1998). Medicinal plants are plants with the natural potentials embedded as the natural constituent of the plant capable of preventing, managing and cuing human

ailments; though made for the plants safety and security against predictors. . Phytocompounds found in medicinal plants have been proven responsible for the medicinal nature of the planta. The phytocompounds (secondary metabolites (are grouped into four major groups, the terpenes, phenolics,, nitrogen containing and surfur containing secondary metabolites. Their role in medicine ranged from antibacterial, antifungal antiinflammatory, antidiabetic, anticancer and antioxidant activity (Xu et al, 2017).

Different parts of medicinal plants (the leaves, seed, stem, back, roots etc) serves as sources of the phytocompounds (Igbinosa, Igbinosa, Aiyegoro, 2009), which can be obtained through different extraction methods. The extraction of phytocompounds from medicinal plants starts with the choice of the plant, it's identification, followed by the extraction methods employed; which depends on the nature of the plant (fresh or dry). The consideration if the length of extraction period, pH of the solvent, the type of solvent to be used, the particles size of orhe plant tissue, the temperature and solvent to sample ratio defines the method of extraction to be employed (Al-shahway, 2019). Fractionation method or serial exhaustive extraction methods involves the use of solvent of increasing polarity from a non-polar (hexane) to more polar solvent to ensure a wide polar range of compounds are extracted (Doughari, 2012). The choice of the solvent is another thing to be considered using specific extraction methods; methanol, ethanol and water have been reported in most of the literature vas the most commonly used solvent. Ethanol been more effective in the extraction of bioactive compounds; water solvent used for plant product with antimicrobial activity. Tanine and phenol are reported tobe extracted effectively in aqueous, acetone and methanol (Tonkens, 2005). Chloroform been reported as effective solvent in the extraction of non-polar bioactive compounds (Tonkens, 2005).

However, the choice of the solvents of extraction must be screened of the following properties, low toxicity, wase of extraction at low heat, promotion of rapid physiologic absorption of the extract, preservation and inability to cause the extract to dissociate (Meadows, 2002). The complexity of the extraction methods depending on the plant material necessitate our choice of extraction of the crude extract and fractionation of the extract to compare their effectiveness in extraction of bioactive compounds of the *Jatropha curcas* leave extract.

## MATERIALS AND METHOD

### Plant collection and authentication

The plant was gotten from the surrounding of federal polytechnic Ilaro and it was authenticated at the University of Ibadan by botanist Dr Nodza George with the Authentication number 10067.

### Plant preparation and extraction

J. curcas leaf was gathered and cleaned with distilled water before being dried at room temperature and powdered form. 500g of the powdery of the *jatropha curcas* leaf was soaked in

2.5ml of methanolic for 72hours.it was then filtered using muslin cloth,after which the filtrate gotten will be concenrated using a rotatory evaporator.

### Fractionation method

During fractionation, specific solvents were introduced in the order of increasing polarity:n hexane,ethyl acetate, and chloroform.
Separation funnel method: 0.01g of crude extract was dissolved in 50ml of water. After that, the mixture was placed into the Separating funnel, stirred, and allowed to settle. The dissolved crude

extract was then mixed with 50ml of n-hexane in the Separating funnel. Before opening the bottom of the separating funnel to remove the aqucous layer, the content was allowed to settle.Rocked and separated once more. The ethyl acetate and chloroform fractions received the same treatment.Because the crude extract was first dissolved in water, the residual aqueous fraction (RAF) is the amount that remains after fractionation. Khatua, Ghosh, and Acharya (2017

### Quantitative Phytochemical Estimation

### Determination of Total Phenolic Content

The Folin–Ciocalteu reagent is the method used to determine total phenolic content in plant extracts. The results were measured in terms of mg of gallic acid equivalents (GAE) per gram of extract using gallic acid as the standard. The standard calibration curve (Figure II) was prepared by dissolving gallic acid in methanol at concentrations of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/mL The plant extract samples were prepared in methanol at concentrations of 0.1 mg/mL and 1 mg/mL. The assay procedure involved mixing 0.5 mL of each sample with 2.5 mL of a 10-fold diluted

Folin–Ciocalteu reagent followed by adding 2 mL of 7.5% sodium carbonate solution. The test tubes were sealed with parafilm and left at room temperature for 30 minutes. The absorbance was measured using a spectrophotometer at a wavelength of 760 nm after incubation.

**Determination of tannins**

Tannin in the example was estimated utilizing the Van-Weight and Robinson 1981 method.] In a 500 ml carafe, 50 ml of refined water was added to 500 mg of material and upset for 60 minutes.It was sifted into a 50 ml volumetric jar, and 5 ml of the filtrate was pipetted into a test tube prior to being joined with 2 ml (multiple times weakened) of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. In something like 10 minutes, the absorbance of the example at 605 nm was estimated utilizing a spectrophotometer.

**Determination of the Total Flavonoid**

The aluminum chloride approach was utilized to decide flavonoid fixations. In this strategy,quercetin was utilized as a kind of perspective, and flavonoid levels were determined as quercetin reciprocals. For this goal, the quercetin adjustment bend (Figure II) was created. 1ml of standard or concentrate arrangement (20, 40,60,80,100 mg/1) was blended in with 4ml of distil.

water in a 10ml volumetric cup. 0.3ml of 5% NaNO2 was added to the cup. Following 5 minutes,0.3ml 10%AlC13 was added to the blend. 2ml of 1M NaOH was added at the sixth moment,trailed by 10ml of distils water. The absorbance at 510nm was estimated with an UV-Noticeable spectrophotometer.

**Determination of Saponin content**

The material received heating in 20 ml of 1NHCI for 4 hours with 0.5 g of the material. The solution was filtered after cooling down before adding 50 ml of oil ether to the filtrate which was then dried. The remaining solution received 5 ml of CH3CO2 ethanol. The test tubes received 0.4 ml each. The solution contained six milliliters of ferrous sulfate reagent combined with two milliliters of concentrated H2SO4. The solution became well mixed after 10 minutes before the absorbance at 490 nm reached stability. Oloyed (2005) used standard saponin to create the standard curve.

**Determination total alkaloid content**

The research of Singh et al. (2004) used 1,10-phenanthroline to develop a method for determining the total alkaloid content in materials. The test powder required 100mg to be extracted in 10ml of 80% ethanol. The mixture underwent centrifugation at 5000rpm for 10 minutes. The solution above the precipitate was used to determine the total alkaloids. The solution received 1ml of plant extract followed by 1ml of 0.025M FeC13 in 0.5M HCl and 1ml of 0.05M 1, 10-phenanthroline in ethanol. The mixture underwent heating at 70 2o C for 30 minutes in a major trouble shower. The absorbance of the red complex was measured at 510nm against the reagent clear solution. The alkaloid concentrations were determined through a quinine standard curve (0.1mg/ml,for example 10mg dissolved in 10ml ethanol and diluted to 0.1mg/ml). Singh et al. (2004). The readings were expressed in milligrams per gram of dry weight.

**Determination cyanogenic glycoside**

Onwuka and Olopade (2005) used the basic picrate procedure to determine cyanogenic glycoside.Gauging the ground test (5.0g), it was broken down in 50 cm3 of unadulterated water. Prior to separating,the cyanide extraction was permitted to settle for the time being (InuIwa et al., 2011).

**Preparation of cyanide standard curve**

At different focuses, KCN arrangements containing 0.1 to 1.0 mg/ml cyanide were made. 4 ml of soluble picrate arrangement (1g picrate and 5g Na2 Co3 in 200 cm3 refined water) was added to 1ml of test filtrate and standard cyanide arrangement in test tubes and hatched in a water shower for 15 minutes.Following variety improvement, absorbance at 490 nm was estimated in contrast with a clear containing just 1 mL unadulterated water and 4 cm3 soluble picrate arrangement.The cyanide content was determined by extrapolating the cyanide standard bend.

**Determination of Steroid**

1ml of steroid arrangement test extract was transferred to 10ml volumetric cups. The first additions included sulphuric acid (4N, 2ml) and iron (111) chloride(0.5% w/v, 2ml) followed by potassium hexacyanoferate (111) solution (0.5% w/v, 0.5ml). The mixture was heated at 70 20C in a water bath for 30 minutes with occasional shaking before it was diluted to the mark with distilled water. The

absorbance was measured at 780nm against a reagent blank.

**Determination of terpernoids**

5ml of the plant remove was very much blended in with two ml of chloroform and three ml of 30% sulphuric corrosive (H2SO4) prior to hatching for 5 minutes in a water shower at 700c with methanol as a clear.

## RESULT AND DISCUSSION

**Table 1: Quantitative Phytochemical Analysis of the crude extract and the fractionated extract of *Jatropha* curcas leaf**

| PHYTOCHEMICALS | CRUDE EXTRACT | ETHYL ACETATE | N-HEXANE | CHOLOROFORM |
|---|---|---|---|---|
| TERPENOID | $7.86 \pm 0.05a$ | $13.28 \pm 0.07d$ | $12.3 \pm 0.07c$ | $8.24 \pm 0.10b$ |
| TANIN | $0.04 \pm 0.35a$ | $0.03 \pm 0.14a$ | $0.05 \pm 0.14a$ | $0.04 \pm 0.28a$ |
| ALKALOID | $7.87 \pm 0.92b$ | $7.83 \pm 0.31b$ | $7.87 \pm 0.07b$ | $7.82 \pm 0.35a$ |
| FLAVONOID | $7.68 \pm 0.36a$ | $3.34 \pm 0.28a$ | $3.75 \pm 0.28b$ | $4.11 \pm 0.28c$ |
| SAPONIN | $7.57 \pm 0.21d$ | $1.32 \pm 0.57c$ | $4.55 \pm 0.92a$ | $-3.98 \pm 0.28b$ |
| PHENOL | $1.85 \pm 0.07c$ | $1.82 \pm 0.49b$ | $1.82 \pm 0.49b$ | $1.06 \pm 0.49a$ |
| TRITERPENE | $10.07 \pm 0.35b$ | $-9.95 \pm 0.35c$ | $9.95 \pm 0.35c$ | $-10.34 \pm 0.71a$ |
| STEROID | $0.14 \pm 0.00a$ | $0.14 \pm 0.21a$ | $0.14 \pm 0.21a$ | $0.14 \pm 0.14a$ |
| PHYTATE | $13.22 \pm 0.28c$ | $-0.30 \pm 0.79a$ | $-0.30 \pm 0.07a$ | $1.38 \pm 0.28b$ |
| GLYCOSIDE | $25.49 \pm 0.78c$ | $25.37 \pm 0.14b$ | $25.4 \pm 0.14b$ | $24.97 \pm 0.43a$ |

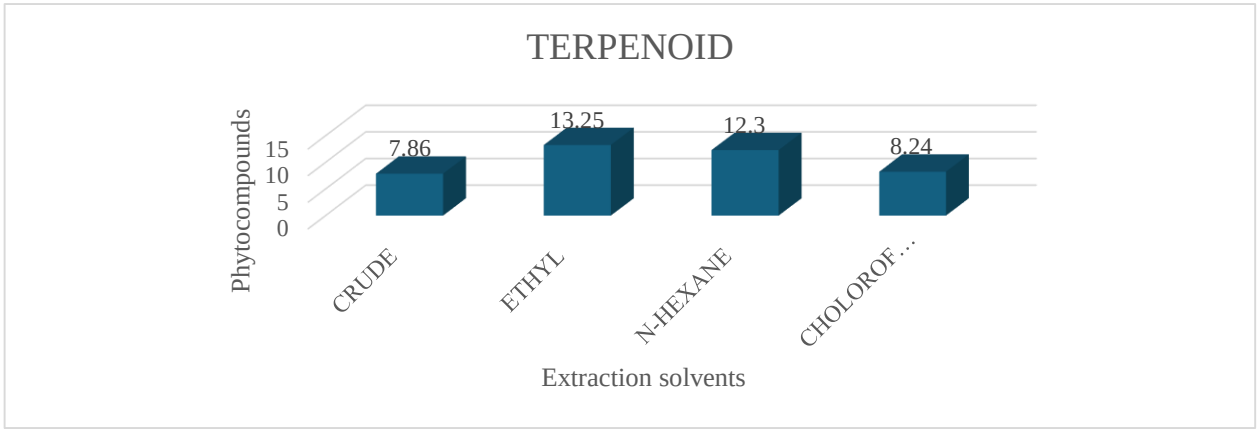


**Figure 1**:Quantitative terpenoid values of crude extracts and the fractionated extract of *Jatropha* curcas leaf**.**
This result showed that ethyl acetate and N-Hehane had the higher value compared to the crude axtract.

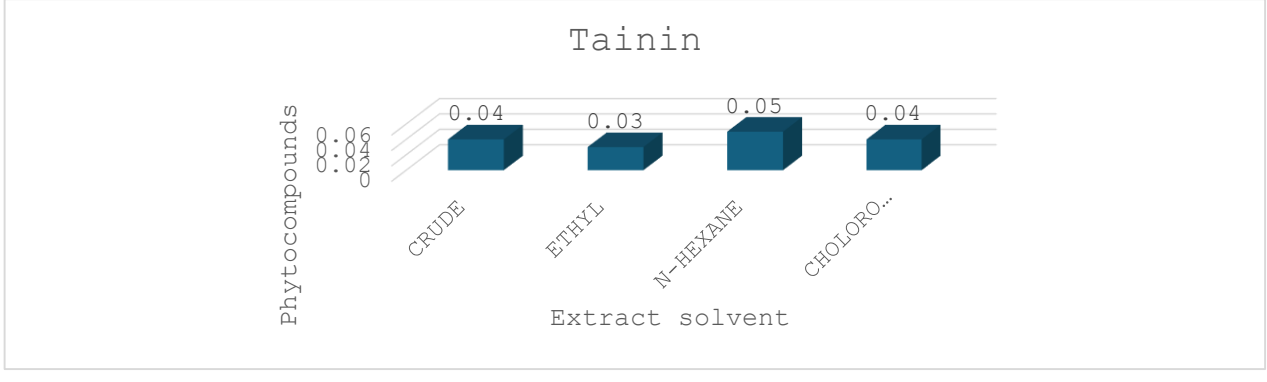


**Figure 2**:Quantitative tannin values of crude extracts and the fraction extract of *Jatropha* curcas leaf**.**
The content of the N-hexane extraction had higher value, followed by the crude extraction. The least been the ethyl acetate.

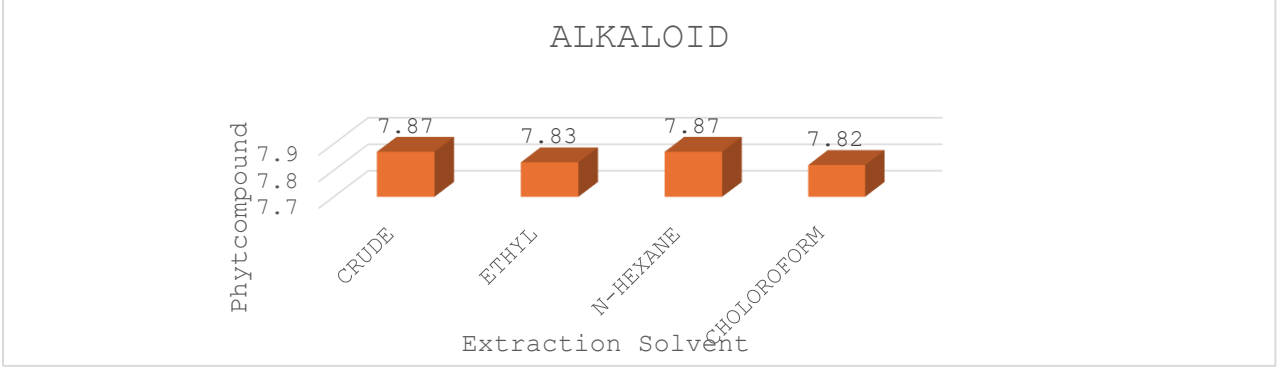

**Figure 3**:Quantitative Alkaloid values of crude extracts and the fraction extract of *Jatropha* curcas leaf**.** The descending order of values of the extraction is crude>n-hexane>ethyl acetate?chloroform

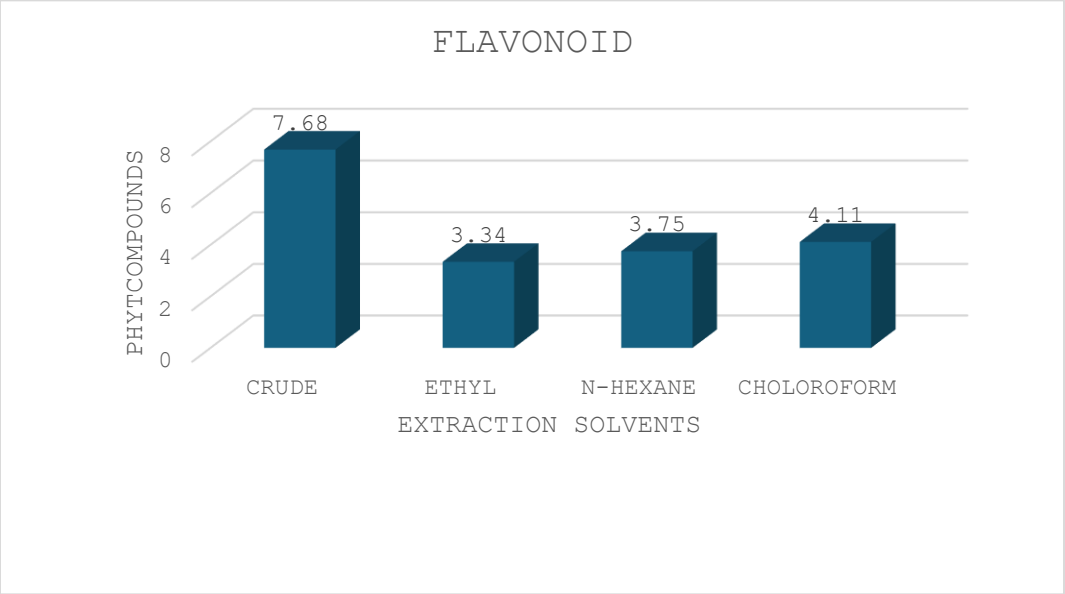


**Figure 4**:Quantitative flavonoid values of crude extracts and the fraction extract of *Jatropha* curcas leaf**.** The flavonoid content of the crude is far higher than the fractionated extraction, the chloroform followed.

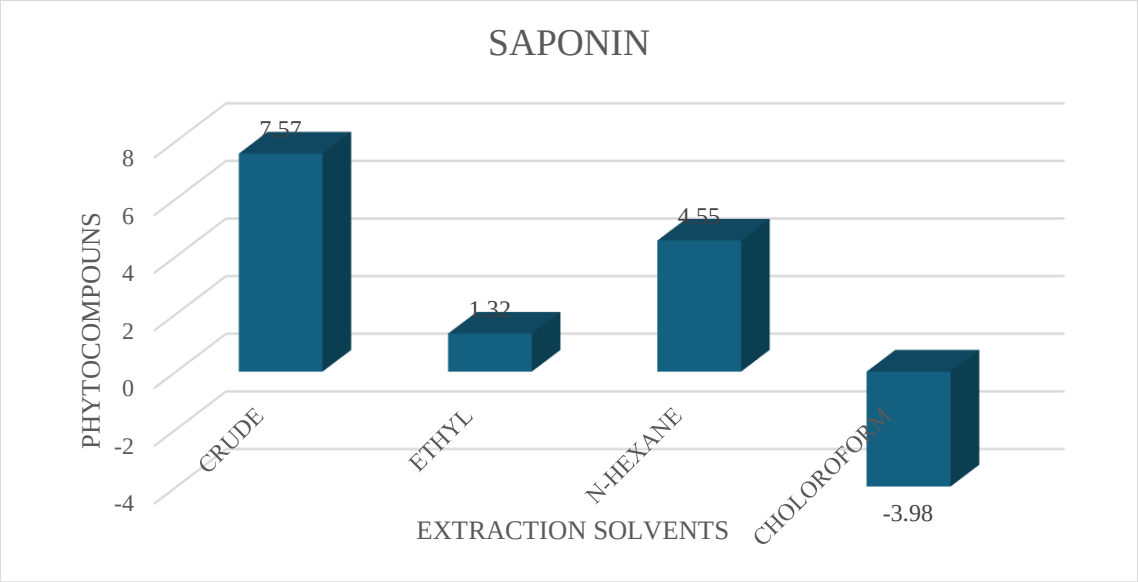


**Figure 5**:Quantitative saponin values of crude extracts and the fraction extract of *Jatropha* curcas leaf**.**

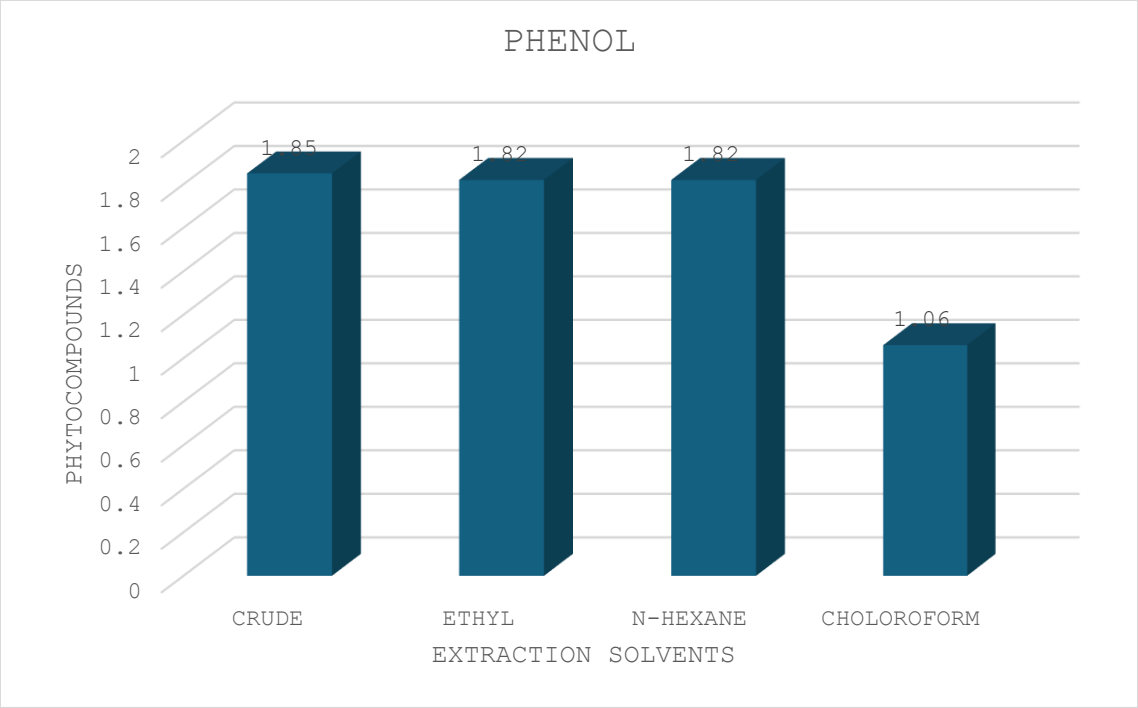


**Figure 6**:Quantitative phenol values of crude extracts and the fraction extract of *Jatropha* curcas leaf**.**

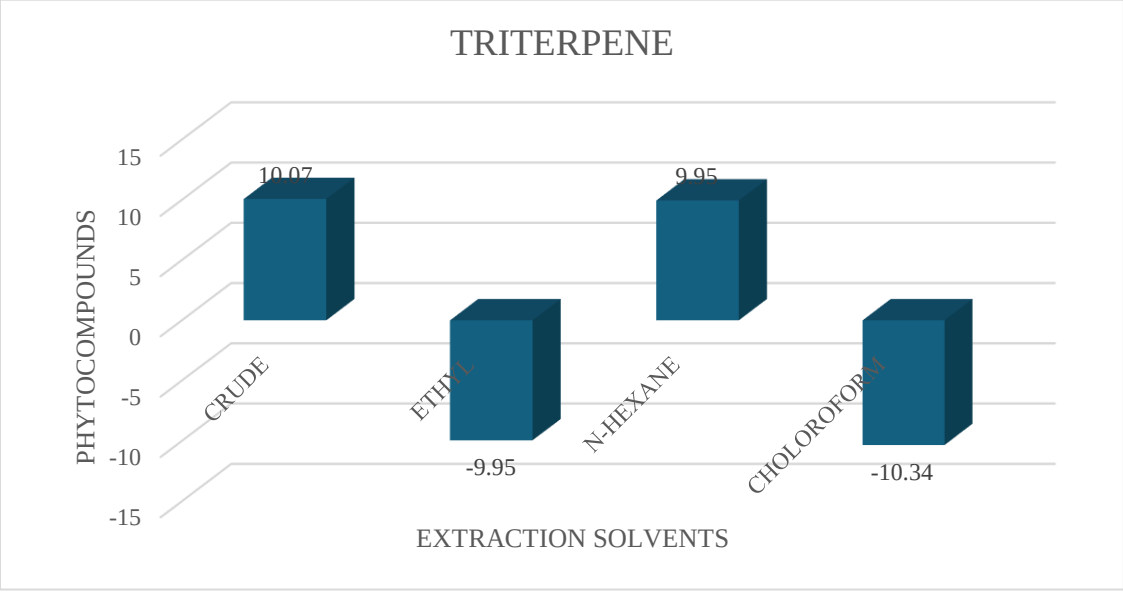


**Figure 7**:Quantitative tritepene values of crude extracts and the fraction extract of *Jatropha* curcas leaf**.**

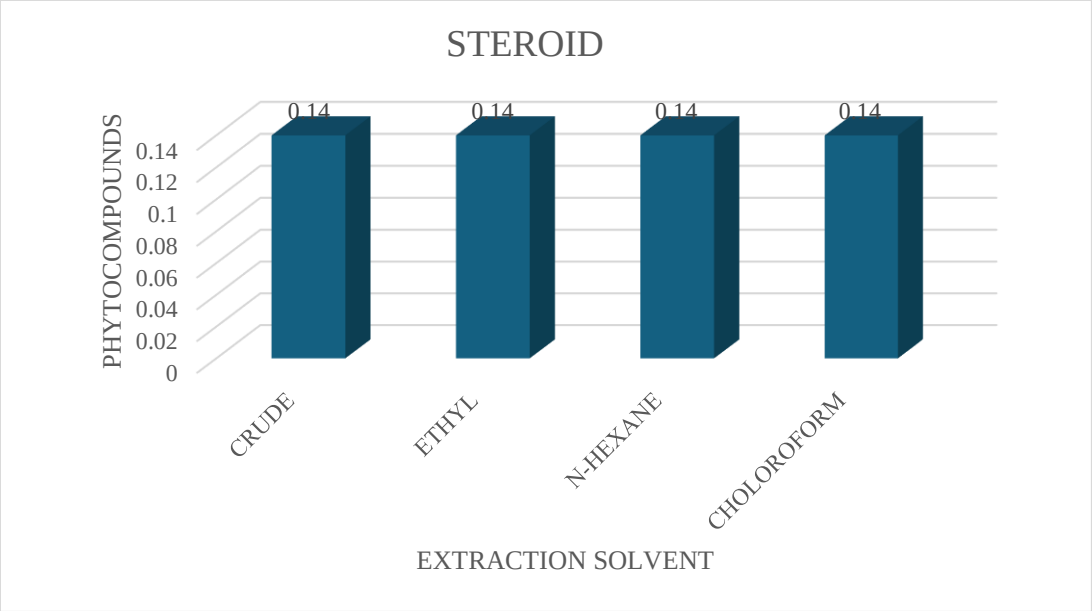


**Figure 8**:Quantitative steroid values of crude extracts and the fraction extract of *Jatropha* curcas leaf**.**

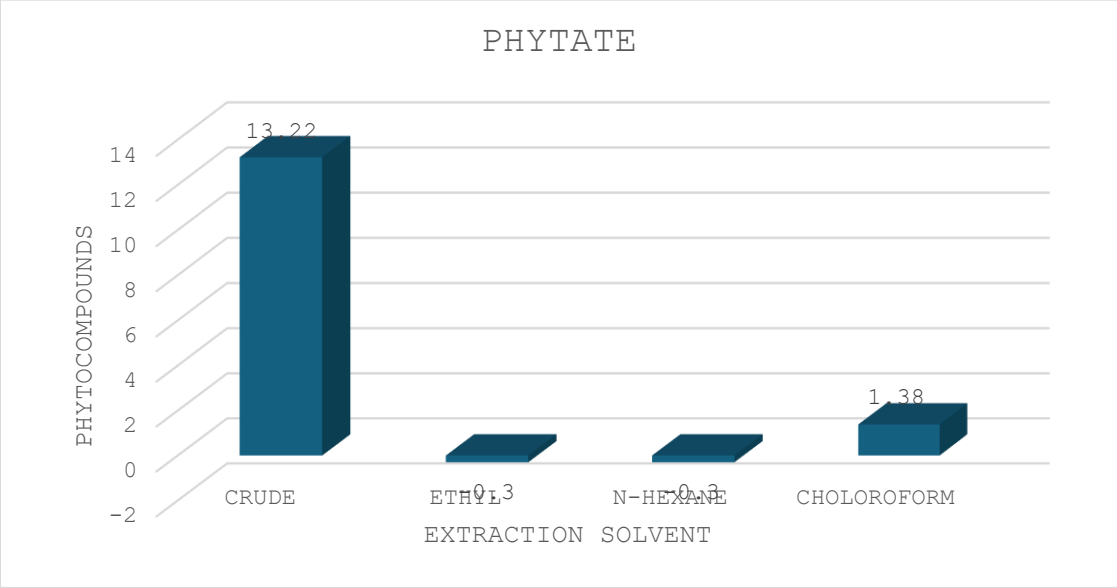


**Figure 9**:Quantitative phytate values of crude extracts and the fraction extract of *Jatropha* curcas leaf**.**

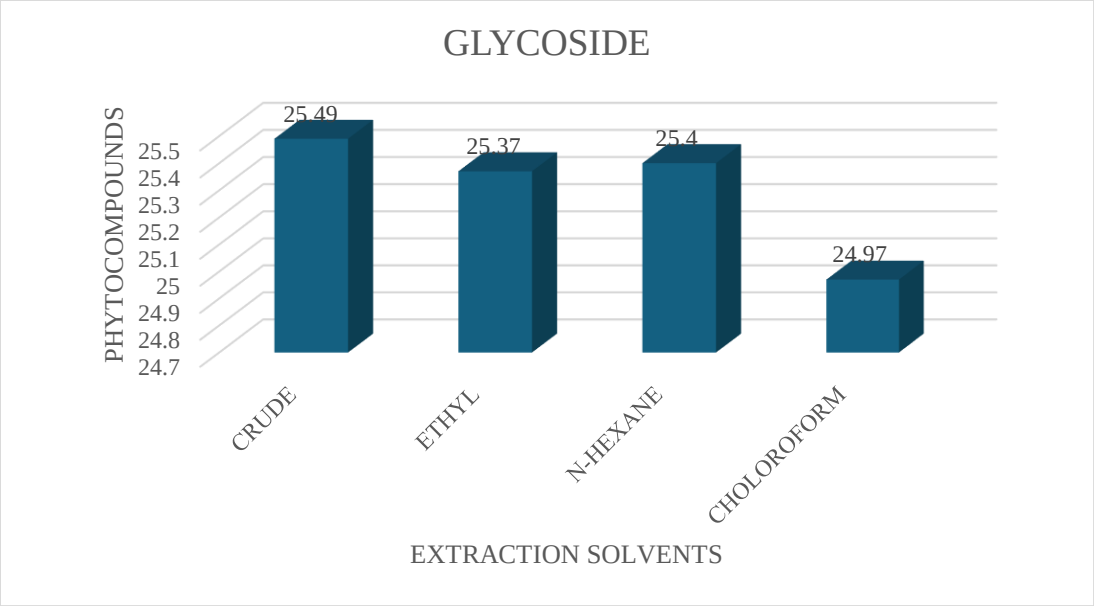


**Figure 10**:Quantitative glycoside values of crude extracts and the fraction extract of *Jatropha* curcas leaf**.**

**Discussion**

The Phytochemicals screening of different Phytochemicals constitutes between the crude extract and the fractionated extract shows that the crude extract has a higher concentration of the constituents than the fractionated extract, The terpenoid result for ethylacetate extract revealed that there was a higher quantity of terpenoid in ethylacetate extract, which was 13.28±0.07,followed by N-hexane, which was 12.3±0.07, followed by chloroform,which was 7.86±0.05,and followed by methanol, which has a lower quantity of terpernoid, which was 7.86±0.05. Terpernoids are used as an antioxidant agent and have an important role in the treatment of inflammatory disorders and cancer. Tanin results for both crude extract and fractionated extract showed that there was no significant difference in the number of phytochemical parameters collected for the extract. Tanin is a polyphenolic compound with astringent, diuretic, anti-inflammatory, anti-septic, and antioxidant effects. They are also utilized to treat gastric and duodenal cancers (Saxena et al., 2013). The crude extract has the highest amount of alkaloids (7.87±0.92), followed by N-hexane (7.87±0.31), ethylacetate (7.83±0.31), and cholorform (7.82±0.35). Although alkaloids are well known for their toxicity, not all of them are harmful; in fact, some of them are considered supplement detractors because of their effect on the sensory system (Elekofhinti 2015). The flavanoids for crude extract result revealed that there was a higher quantity of flavanoids in crude extract, which was 7.860.36, followed by cholorform, with 4.11±0.28, followed by N-hexane, with 3.75±0.28, and ethylacetate, which has a lower quantity of Flavanoids with 3.34±0.28. Flavonoids are polyphenolic compounds that have antioxidative, hepatoprotective, anti-inflammatory, and anti-cancer properties (Kumar & Pandey 2013). The result of saponin for crude extract revealed that there was a higher quantity of saponin in crude extract with 7.57±0.21, followed by N-hexane with 4.55±0.29, followed by ethylacetate with 1.32±0.57, chloroform had the lowest quantity of saponin -3.98±0.28. Saponin is a phytochemical with underlying variations and organic exercise Saxena et al., 2013). It has been shown to have antidiabetic, cell reinforcement, and antiobesity properties. The phenol result for crude extract revealed that there was a higher

quantity of phenol in crude extract, which was,1.85±0.07,while ethylacetate and N-hexane had no significant difference, followed by chloroform, which had a lower quantity of phenol, 1.06±0.49. Phenol has antibacterial, anticonvulsant, antioxidant, and antiproliferative properties. The triterpene result for crude extract revealed that there was a higher quantity of triterpene in crude extract, which was 10.07±0.35,followed by N-hexane, which was9.95±0.35,and both ethylacetate and chloroform were present in lesser amounts. Triterpene is thought to be a protective molecule against microorganisms and herbivores. There is no significant difference in triterpene results for crude extract and fractionated extract. The phytate result for crude extract revealed that there was a higher quantity of phytate in crude extract, which was 13.22±0.28, followed by chloroform, which was1.38±0.28,,and both ethylacetate and N-hexane were present in lesser amounts. Phytate is known to suppress polyphenol oxidase, which causes oxidative browning in fruits and vegetables, and to diminish iron-catalyzed oxidative processes (Pandey & Tripathi, 2014). The result of glycoside for crude extract showed high quantity of glycoside, which was 25.49±0.78, followed by ethylacetate and chloroform, which had no significant difference, and N-hexane, which was present in a lesser number of 24.97±0.43. Glycosides is known for its anticancer, antifungal, and anti-inflammatory effects..

**Conclusion**

This study revealed the supremecy of the rude methanol extract over the fractionated extracts, this was observed in virtually all the phytocompoind where the crude had higher values, excpt for terpernoid which showed ethyacetate having higher value than the crude and others, N-hexane having higher taninin than the crude and others and the crude and the N-hexane having equal values. This implies that methanol (crude) is a better extraction solvent for phytochemical screening of *Jatropha curcas* leaf compared to the fractionated

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