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Studies On Proximate And Phytochemical Constituents Of Euphorbia Hirta L. And Mucuna Pruriens Seed Var.

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ABSTRACT
Euphorbia hirta and Mucuna pruriens seed are known to be used traditionally in treatment of different ailments. Both plants are dominant in almost all parts of Nigeria. Proximate and phytochemical constituents of both plants were studied in other to discover their major constituents. Proximate and phytochemical analyses carried out followed standard procedures. Proximate constituents of carbohydrates, proteins and moisture were high in both plants. Qualitative phytochemical present in both plants were terpenoid, steroid, saponin, phenol, tannin, reducing sugar, alkaloid whereas phlobitannin was absent in both plants. In Euphorbia hirta alkaloids (68.17), phenols (61.95) were highest followed by terpenoid (45.74), flavonoids (45.68), tannin (45.06), , saponin (43.04) whereas that of steroids (38.84) and reducing sugar (26.84) were lower. In Mucuna pruriens steroid (57.92) and saponin (55.65) had the highest values followed by flavonoids (48.00), alkaloid (41.96), phenol (36.62), reducing sugar (33.76) whereas tannin (26.63) and terpenoid (17.63) had lowest value. Result revealed that both plants have good nutritional and phytochemical values important in medicinal plants.

I. INTRODUCTION

Euphorbia hirta and Mucuna prureins seed have been used over decades in treatment of several diseases such as asthma, diarrhea, dysentery, asthma, hay fever etc. Euphorbia contains about 1600 species, it is the largest genus of the family Euphorbia. It is characterized by the presence of white milky latex which could be toxic (Kumar *et al*., 2010). *Euphorbia hirta* , also referred to asthma weed is an important herb growing up to 40 cm tall, occupying open waste spaces, roadsides and gardens in the West African subregion (Oyeyemi *et al*., 2009). It prefers sunny to lightly shaded dry conditions, and is an early colonizer of bare ground. The leaves of *E. hirta* leaves are simple with dark green colour about 2-6cm long in size, transverse section of the leaf revealed the presence of stomata, upper and lower epidermis, vascular bundle. There is presence of starch granules, covering trichomes. (Ahmad *et al*., 2012). *Euphorbia hirta* was reported to be used traditionally for female disorders, respiratory ailments (cough, coryza, bronchitis, and asthma), worm infestations in children, dysentery, jaundice, pimples, gonorrhea, digestive problems, and tumors (Kumar *et al*., 2010). *Euphorbia hirta* was reported to possess anti-microbial, anti-diabetic, anti-cancer, anti-tumor, anti-plasmodial, anti-fertility, wound healing, anti-inflammatory, sedative, and diuretic properties (Ghosh 2019). It was also reported to contain alkanes, triterpenes, phytosterols, tannins, polyphenols, and flavanoids (Kumar *et al*., 2010). Studies have shown that *Euphorbia hirta* has antibacterial, antimolluscidal, antimalarial and anti-inflammatory properties (Wrong *et al*., 2013). Despite the good phytochemicals contained in *E. hirta*, it impacts negatively on reproductive potentials. Oyeyemi *et al*. (2009) reported that the fertilization capacity and livability of spermatozoa were negatively affected by it. This shows that E. hirta could be a source of good contraceptive if it could perform similar effects in female.

The *Mucuna prureins* is reffered to as velvet bean seeds. *Mucuna* and their accessions are herbaceous twining annual plants. They possess trifoliolate leaves (leaflets are broadly ovate, elliptic or rhomboid ovate and unequal at the base); flowers white to dark purple and hang in long clusters (pendulous racemes); pods are sigmoid, turgid and longitudinally ribbed, seeds ovoid (4-6 per pod) and black or white. *Mucuna* pods are covered with reddish-orange hairs, which readily dislodge and cause intense skin irritation and itch due to presence of a chemical called mucunain (Okafor, 2015). Previous research showed that it contains high level of protein (26-30%) and starch (34-40%), desirable amino acid, fatty acid and mineral composition with good nutritional properties (Pugalenthi and Vadivel,2007). It is easy to cultivate *Mucuna* under dry farming and low soil fertility conditions and exhibits many favourable agronomic characters with reliable yield (Pugalenthi and Vadivel 2006). It is easy to cultivate under dry farming and low soil fertility conditions and exhibits many favourable agronomic characters with reliable yield (Pugalenthi and Vadivel 2006). Many varieties and accessions of *Mucuna* are in great demand in food and pharmaceutical industries. *Mucuna* seeds as a rich source

of protein supplement , it also contains 3,4-dihydroxy-L-phenylalanine (L-DOPA), which provides symptomatic relief in Parkinson's disease (Okafor, 2015). Methanolic extract of *E. hirta* shows immunomodulatory activity, which has been proved using simple techniques like the macrophage activity testing, carbon clearance test and mast cell degranulation assay (Ramesh *and* Padmarathi 2010). The present study was carried out to determine the proximate and phytochemical constituents of *Euphorbia hirta* . and *Mucuna pruriens* seed var**.**

II. MATERIALS AND METHODS

1. Proximate analysis

a. Crude Protein Analysis

The analysis was conducted with the aid of micro kjedhal system in accordance with Association of Official Agricultural Chemist (2000) procedures. A small quantity of the sample (approximately 1 gram) was introduced to the digestion tube (kjeltec 2200 FOSS) and a catalyst (2 tablets of 5 g k_2S0_4 and 5 mg of Se) and 12ml of concentrated potassium tetraoxosulphate VI acid were added. The digestion was run for one hour at 420° C. Eighty millilitre, 40 ml of water and sodium hydroxide (NaOH) respectively were used in the distillation using 2200 FOSS distillation unit and the distillate was collected in 4 % Boric acid. Percentage nitrogen was calculated thus:

% N= (Titre- Blank) x 14.007 x 0.1 x 100

1000 x sample weight (mg) % CP = % N X6.25

b. Crude Fibre Analysis

The crude fiber of the sample was determined according to AOAC (2000). Two gram of the sample was defatted with petroleum ether and then boil under reflux for 30 minutes with 200 ml of a solution containing 1.25 g of H2S04 per 100ml of solution. The solution was then filtered through linen on a fluted funnel. It was then washed with boiling water until the washings were no longer acidic. The residue was then transferred to a beaker and boiled for 30 minutes with 200 ml of a solution containing 1.25 g of carbonate free NaOH per 100 ml. The final residue was then filtered through a thin but a closed pad of washed and ignited asbestos in a Gooch crucible and dried in an electric oven and weighed. It was then incinerated, cooled and weighed. The percentage crude fiber was calculated as:

% CF= Loss of weight after incineration x 100.

c. **Determination of Ash**

Crucibles were rinsed and dried in hot air oven (SM9053) maintained for 30 minutes at 105 °C. These were cooled in a desiccators and weighed, 2.5 g of the sample was burnt on a heater inside a fume cupboard to get rid of smoke. The sample were moved to preheated muffle furnace (SM9080) maintained at 550°C until such a time when a light grey ash was noticed. The crucibles were cooled in desiccators and weighed. The ash content was calculated as:

$$\frac{W3-W1}{W2-W1} X \frac{100}{1}$$

d. Determinatio of Crude Fat

The fat contents were determined using fat extractor with automated control unit. The equipment has six extraction units with each unit carrying a thimble which accommodates the samples to be analyzed within 75 minutes. Percentage of fat is the differences between weight of the pre-weighed cups and after extraction. One gram of the sample was weighed into the thimble and its mouth plugged with defatted cotton wool, after which it was inserted into the extraction unit, 80 ml of petroleum ether were dropped in each cup and maintained at 135 °C. Each cup was aligned with its corresponding thimble. The extraction and rinsing was done for 30 minutes each, after which the sample was aerated for 15 minutes and crude fat was calculated thus:

$$\text{Fat} = \frac{W2-W1}{Weight\ of\ the\ sample} X \frac{100}{1}$$

c. Determination of Moisture Content

The moisture content of the sample was determined in accordance with AOAC (2000), samples were introduced to an oven maintained at 105 °C for one to four hours until uniform weight was attained. The moisture value was obtained using the equation:

$$\frac{W2-W3}{W2-W1} X \frac{100}{1}$$

Where MC= moisture content, W1= weight of original sample and W2= weight of oven dry sample.

2. Phytochemical Screening

Chemical tests were carried out on the aqueous extract of *Euphorbia hirta* and on powdered specimen of *Mucuna pruriens* seed using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

a. Qualitative phytochemical analysis of *Euphorbia hirta* and *Mucuna pruriens* seed

Test for Tannins: Weighed 0.5 g of each sample was dissolved in 5 ml of distilled water, boiled gently and cooled, 1 ml of this solution was put in a test tube and 3 drops of ferric chloride solution was added. A deep greenish-black coloration indicates a positive test for tannis.

Test for Plobatannins: Weighed 0.5 g extract of each plant sample was boiled with 2 ml of 1 % aqueous hydrochloric acid for 10 minutes, a red precipitate confirms the presence of phlobatannin.

Test for Saponin: Measured 2 g of the sample was boiled in 20 ml of distilled water in a water bath and filtered. Ten milliliter of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observe the formation of emulsion, which indicates the presence of saponin.

Test For Flavonoids: Measured 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration was observed in each of the extract indicating the presence of flavonoid.

Test for Steriods: Measured 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H_2SO_4. The colour changed from violet to blue in *M. pruriens* and in *E.hirta* green indicating the presence of steroids.

Test for Terpenoids (Salkowski test): Weighed 5 ml of each extract were mixed in 2 ml of chloroform and concentrated H_2SO_4. (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoid.

Test for Alkaloid: Weighed 5 mg sample of the extract dissolved in 3 ml of acidified ethanol was warmed slightly and then filtered. Few drops of Mayer's reagent and 1 ml of Dragendroffs reagent were added to 1 ml of the filtrate and turbidity was observed indicating the presence of alkaloid.

Phenol Determination: For the extraction of the phenolic component, the sample was boiled with 50 ml of ether for 15 minutes, 5 ml of extraction was pipetted into a 50 ml flask, and 10 ml of distilled water was added, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added to the samples and were left for 30 minutes for colour development. Yellowish-red colouration indicates the presence of phenol.

2. Quantitative phytochemical analysis of *Euphorbia hirta* and *Mucuna pruriens* seed

Estimation of Total phenolic compound: Weighed 0.5 g sample of extract was dissolved in 50 ml of water, 0.5 ml was added to 0.1 ml of Folin-Ciocalteu reagent (0.5N) mix and incubated at room temperature for 15 minutes. 2.5 ml sodium carbonate solution (7.5% w/v) was added and further incubated for 30 minutes at room temperature. The absorbance of the solution was measured at 760 nm. The concentration of total phenol was expressed as gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

Total Flavoniod Content Estimation: Weighed 1 ml of sample solution (100 µg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10 % Aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water. The resulting mixture was incubated at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415 nm.

Reducing power Assay: Various concentrations of the extracts (20 to 100 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50 °C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 16 µg/ml) was used as standard.

Estimation of Total Saponins Content: Estimation of total saponins content was determined by the method described by Makkaret al.,(2007). Based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5 % ethanol) was added. Then 2.5 ml of 72 % sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60 ^{o} C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from a standard.

Estimation of tannins content: Tannins content was estimated by the method of Siddhuraj and Manian. A total of 500 uL of the extracts were taken in test tube separately and treated with 100 mg of polyvinyl polypyrrolidone and 500µL of distilled water. This solution was incubated at 4 ^{o}C for 4 h. Then the sample was centrifuged at 5000 r/min for 5 min and 20µL of the supernatant was taken. This supernatant has only simple phenolics free of tannins (the tannins would have been precipitated along with the polyvinyl polypyrrolidone). The phenolics content of the supernatant was measured at 725 nm and expressed as the content of free phenolics on a dry matter basis. From the below results, the tannins content of the extract was calculated as follows:

Tannins (mg GAE/g extract) = Total

Phenolics (mg GAE/g extract) = Free

Phenolics (mg GAE/g extract)

Determination of Alkaloids: A total of 200 ml of 20 % acetic acid was added to the samples and covered to stand for 4 h. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample, concentrated ammonium hydroxide was added drop-wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed the percentage of total alkaloid content was calculated as: percentage of total alkaloids (%) = weight of residue x 100/Weight of sample taken.

III. RESULTS

1. Proximate constituents

The percentage proximate analysis of *E. hirta* indicates that the plant contains high level of carbohydrate (69.37 %), moisture content (11.08 %), ash (10.06 %), protein (7.94 %), fibre (0.78 %) and the least was fat (0.77 %) (Table 1).

Table 1: Proximate constituents of *Euphorbia hirta*

S/N	Parameters	Mean % values
1.	Moisture	11.08
2.	Ash	10.06
3.	Fat	0.77
4.	Protein	7.94
5.	Fibre	0.78
6.	Carbohydrate	69.37

M. prurines seed revealed the following proximate constituents in increasing order: carbohydrate (71.07 %), protein (14.55 %), moisture (7.87 %), ash (3.67 %), fat (2.50 %) and fibre (0.34 %) (Table 2).

Table 2: Proximate constituents of *Mucuna pruriens* seed

S/N	Parameters	Mean % values
1.	Moisture	7.87
2.	Ash	3.67
3.	Fat	2.50
4.	Protein	14.55
5.	Fibre	0.34
6.	Carbohydrate	71.07

2. Phytochemical constituents

Both *Euphorbia hirta and Mucuna prurines seed had* similar qualitative phytochemical constituents (Table 3 and 5). The quantitative phytochemical constituents of *E. hirta revealed higher constituents* of alkaloid (68.17 mg), phenol (61.95 mg),terpenoid (45.74 mg), flavonoid (45.68 mg), tannin (45.06 mg), saponin (43.04 mg), steroid (38.84 mg) and reducing sugar (26.85 mg) (Table 4). Whereas the quantitative phytochemical constituents of *Mucuna prurines* seed indicated higher constituents of steroid (57.92 mg), saponin (55.65 mg), flavonoid (48.00 mg), alkaloid (41.96 mg), phenol (36.62 mg), reducing sugar (33.76 mg), tannin (26.63 mg) and terpenoid (17.63 mg) (Table 6).

Table 3: Qualitative phytochemical constituents (mg) of E*uphorbia hirta*

Terpenoid	Steroid	Saponin	Phenol	Tanin	Reducing sugar	Alkaloid	Phlobatannin
+	+	+	+	+	+	+	-

Table 4: Quantitative phytochemical constituents (mg) of *Euphorbia hirta*

Phenol	Flavonoids	Alkaloid	Steroid	Reducing sugar	Tannin	Saponin	Terpenoid
61.95	45.68	68.17	38.84	26.85	45.06	43.04	45.74

Table 5: Qualitative phytochemical constituents of *Mucuna pruriens* seed

Terpenoid	Steroid	Saponin	Phenol	Tanin	Reducing sugar	Alkaloid	Phlobatannin
+	+	+	+	+	+	+	-

Table 6: Quantitative phytochemical constituents (mg) of *Mucuna pruriens* seed

Phenol	Flavonoids	Alkaloid	Steroid	Reducing sugar	Tannin	Saponin	Terpenoid
36.62	48.0	41.96	57.92	33.76	26.63	55.65	17.63

IV. DISCUSSION

The proximate analysis revealed that carbohydrates and protein were the major constituent of *E. hirta*. The result is comparable to earlier report of *Ghosh et al.,* (2019), who found similar report. Stability of carbohydrates contribute to the calorific value of the plant. The high values (percentage) of carbohydrates and proteins shows that the plant is a good nutritional source.

Carbohydrate, crude protein and moisture were major constituents of *Mucuna pruriens* seed. Protein and carbohydrate constituents are comparable to the earlier report of Vadivel and Janardhanan (2000). The crude carbohydrate in seeds is advantageous as carbohydrate add to the calorific value as well as an anti-marasmus in infant nutrition (*Vadivel and Janardhanan* 2000). The moisture, ash, fat and fibre contents were reported lower in *Mucuna Poggei* in an earlier report of Oko *et al.,* (2012). Our result revealed that terpenoid, steroid, saponin, phenol, tannin, reducing sugar and alkaloid were all present in both plants whereas phlobatannin was absent in both. The presence of alkaloid and phenol showed that both plants could possess anti-inflammatory and pharmacological activities (*Norayanan,et al.,* 2011; *Shih et al.,*2010).

The qualitative screening has shown that *Euphorbia hirta* and *Mucuna pruriens* are potential sources of therapeutical, pharmaceutical and nutritional bioactive compounds, which could the reason for their usage in treatment of ailments such as asthma, hay fever, conjunctivitis, diarrhea and dysentery.

The considerable values of alkaloid, phenol, terpenoid, flavonoid, tannin, saponin, steroid and reducing sugar of *Euphorbia hirta* clearly indicated their anti-inflammentory, anti-asthmatic, immunostimulant, anticancer, pharmacological and anti-diabetic activities. The above results agrees with the earlier report of *Sudhakar et al.,* (2006) though with slight variations in values.

The higher values of phenol and alkaloid in *Mucuna pruriens* seed clearly indicated that it has good pharmacological activities. Its reducing sugar has been shown to possess antidiabetic activities (Kusuma *et al*., 2016).This study has proved that *Euphorbia hirta* and *Mucuna pruriens* seeds are good source of carbohydrate, protein, pharmacological and anti-inflammatory activities.

V. CONCLUSION

The study revealed that *Euphorbia hirta* and *Mucuna pruriens seed* contain good nutritional and phytochemical constituents. Both plants are good source of energy and proteins. The high contents of alkaloid, phenol and reducing sugar showed that both possess pharmacological, anti-inflammatory and antidiabetic activities. The most dominant proximate constituents were carbohydrates, proteins and moistures while fibre and fat were lower. In qualitative phytochemical analysis, all the parameters analysed were present except phlobatannin. Quantitative phytochemical analysis revealed higher constituents of alkaloid and phenol in *Euphorbia hirta whereas higher values of* steroid and saponin were found in *Mucuna pruriens* seed. *Euphorbia hirta* could possess more pharmacological and anti-inflammatory properties than *Mucuna pruriens* seed, though *Mucuna pruriens* has higher nutritional values.

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