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# Phytochemical Analysis of Mucuna Pruriens Root By Thin Layer Chromatography (Tlc)

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**ABSTRACT**
The early phytochemical analysis of Mucuna pruriens root has revealed the presence of alkaloids, flavonoids, phenolic compounds, steroids, saponin, tannins, quinones, and terpenoid in the plant root. The main aim of this study was to carry out phytochemical analysis of extracts of Mucuna pruriens root by thin layer chromatography. The chromatographic (TLC)analysis was carried out using different solvent systems for the five extracts of Mucuna pruriens root which are petroleum ether, ethyleacetate, methanol, ethanol and water extracts. In solvent system 1; no spot was detected for petroleum ether extract,2 spots were detected for ethylacetate extract,3 spots were found for methanolic extract, 2 spots were found for ethanolic extract and 2 spots were found for water extract, in solvent system 2; 1 spot was detected for petroleum ether extract,3 spots were detected for ethylacetate extract,4 spots were found for methanolic extract, 3 spots was found for ethanolic extract and 2 spots were found for water extract, in solvent system 3; 2 spots was detected for petroleum ether extract,1 spot was detected for ethylacetate extract, 3 spots were found for methanolic extract, 2 spots were found for ethanolic extract and 2 spots were found for water extract, in solvent system 4; 2spots was detected for petroleum ether extract, 2 spots were detected for ethylacetate extract,3 spots were found for methanolic extract, 1 spot were found for ethanolic extract and 3 spots were found for water extract, and in the solvent system 5; 1 spot was detected for petroleum ether extract,3 spots were detected for ethylacetate extract,2 spots were found for methanolic extract, no spot were found for ethanolic extract and 2 spots were found for water extract. The presence of different spots in the various extract is in line with qualitative analysis which indicated that the root extracts of Mucuna pruriens root constituted different phytochemical compounds with different $R_f$ values. The presence of a number of bioactive components in Mucuna pruriens root extracts supports the idea that it can be used as a therapy, and isolating and purifying specific phytochemical elements may lead to the development of new medicines and methods to treat diseases.

**Keywords:**Mucuna pruriens root**,**Phytochemical, Thin Layer Chromatography**(TLC)**

## I. INTRODUCTION

Before the advent of modern medicine, traditional medicine has been the mainstay of many African countries like Nigeria. Many Nigerians, particularly those in the rural areas, depend on traditional medicine extracted from medicinal plants for their health-related problems. The use of medicinal plants is crucial for maintaining the health of both individuals and entire societies.Mucunapruriens is a very useful plant which has a long historical usage in herbal (traditional) Ayurvedia Indian medicine for treating different form of ailment and diseases (Kavitha & Thangamani, 2014). The plant has been generally and traditionallyused as powerful aphrosidiac in Ayurveda medicine, and has been also used to treat nervous disorder and arthritis (Taylor, (2003). The plant is very active against snake venom and indeed, its beans are used in traditional medicine to prevent the toxic effects of snake bite (Paul, Detta, Aninda, Ghosh & Halderi, 2011).All parts of Mucuna pruriens possess important medicinal usages. Mucunapruriensroot which is the focus of this study isuseful in treating diseases of the nervous system, such as facialparalgsis and hemiplegra. In most community, a strong in fusion of the root sweetened with honey is given in cases of cholera morbus(Kumar & Saha, 2013).The root is also useful; for delirium in, fevers and, when powered and made into a paste, is applied for dropsy, it is use as blood purifier, for asthma, to relieve dysmenorrheal, in catarrh (Rahaman, 2012). It is also being used in fever, gout, renal stones, and cataract and to relieve rheumatism (Kumar, Muthu, Smith & Manavalan, 2010).

The medicinal value of Mucuna pruriens lies presences of various phytochemical component that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. Plant

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compounds known as phytochemicals, which are not edible, have curative or disease-preventive effects. The body needs them to maintain life. The fact that plants create these substances to defend themselves is widely known, but new research has revealed that they can also shield people from disease (Saikarthik, Ilango, Kumar & Vijayaraghavan, 2017). The three most important bioactive substances found in plants are alkaloids, flavonoids, and phenolic compounds (Sudhira, Venkateswara, & Kamakshamma, 2015). These chemical component are usually identified by using different qualitative test like Dragendorff, Wagners, Alkaline test, Lead acetate test, Frothingtest, Sulphuric acid test, Quinones test, Ferric chloride test and Terpenoids test. Besides theses qualitative analysis, the presence of different bioactive components in medicinal plant could also be confirmed using thin layer chromatography.

Thin layer chromatography(TLC), is a chromatographic method used in separating non-volatile mixtures. TLC, like other chromatographic methods, is based on the separation principle. Thin layer chromatography uses a thin glass plate coated with either aluminum oxide or silica gel as the solid phase (Alebiosu & Yusuf, 2015). The mobile phase is a solvent chosen according to the properties of the components in the mixture. The distribution of a chemical between a liquid mobile phase that is moving over the solid phase and a liquid fixed phase that is put on a glass or plastic plate forms the basis of TLC. A starting point slightly above the bottom of the TLC plate is covered with a small amount of a compound or combination. (Cai, 2014).

In thin layer chromatography, the stationary phase is a thin glass plate coated with either silica gel or aluminium oxide. A solvent is selected for the mobile phase based on the characteristics of the mixture's constituent parts. The distribution of a chemical between a liquid mobile phase that is moving over the solid phase and a liquid fixed phase that is put on a glass or plastic plate forms the basis of TLC.At the beginning point just above the TLC plate's bottom, a small quantity of a substance or mixture is applied. After that, the plate is developed in the developing chamber, which features a small pool of solvent beneath the level where the sample was applied (Rohom, Aher & Dengale, 2022). The solvent is drawn up through the particles on the plate through the capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate (Kumar, Jyotirmayee, & Sarangi, 2013). The physical characteristics of each individual molecule and, consequently, its molecular structure, particularly functional groups, determine whether the compound advances along the plate or lags behind. It adheres to the "Like Dissolves Like" solubility rule ((Archana & Anubha, 2011). The compound will remain in the mobile phase for a longer period of time, the more physically similar its properties are to those of the mobile phase. The mobile phase will carry the most soluble compounds the furthest up the TLC plate. The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind(Singhal, singhal, & Agarwal, 2009). All the constituents of the mixture move more quickly when the solvent system's polarity rises (and vice versa with lowering the polarity). This means that the best separation is provided by the appropriate solvent solution. The best system will separate the desired component from the rest of the mixture by a difference in TLC Rf values of 0.25 to 0.35, and it will also separate the component from its nearest neighbour by at least 0.20(Course Hero, 2022).

The earlier phytochemical investigation and antioxidant evaluation conducted by Okposo, Etinagbedia, Orogu and Okuda (2018), revealed the presence of alkaloids, flavonoid, phenolic compounds, steroids, saponin, tannins, quinones and terpenoid,The $IC_{50}$ value for the methanolic and ethanolic extracts was found to be 154µg/ml and165µg/mlrespectively was found to be effective. The present study was done to compare the phytochemical compounds present in the petroleum ether, ethyleacetate, methanol, ethanol and water extracts of Mucuna pruriens root by thin layer chromatography to validate its therapeutic effects.

## II. MATERIAL AND METHODS

**Sample Collection andPreparation of the Extract.**

The Mucuna pruriens root was harvested from a garden in a residential area in Abraka. According to Okposo, Etinagbedia, Orogu, and Okuda (2018), a botanist from the Department of Plant Biology and Biotechnology at the University of Benin has already taxonomically identified and authenticated the plant for use. The pulverised plant root was extracted using distilled water (maceration), petroleum ether, ethylacetate, methanol, and ethanol, in that order, in a soxhlet extractor. Using a rotary evaporator, each extract was concentrated, then dried, weighed with an

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OHAUS electric weighing balance (0.001–190g), and the percentage yield for each solvent was computed.

**Phytochemical Analysis**

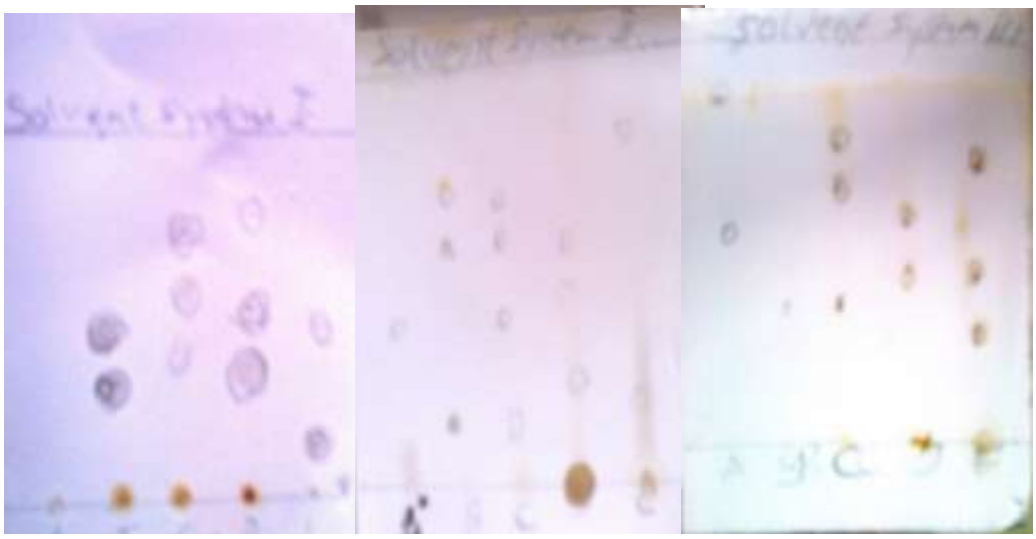
Okposo, Etinagbedia, Orogu, and Okuda (2018) have used a standard procedure to identify bioactive chemical constituents such as alkaloids, flavonoids, phenolic compounds, saponins, steroids, quinones, tannins, and terpenoids in Mucuna pruriens roots. Hence, this study mainly focuses on the phytochemical analysis of the petroleum ether, ethyleacetate, methanol, ethanol, and water extracts of Mucuna pruriens root by thin layer chromatography to validate its therapeutic effects.

**Thin-layer Chromatographic (TLC) Studies**

The chromatographic studies were carried according to the method used by Rajendra and Estari (2013) with slight modification. In the method, TLC was carried out on all the extracts by means of pre-coated silica gel 60F254, 7X6 cm (Merck) by using one way ascending technique.

$$R_f = \frac{\text{Distance moved by the solute}}{\text{Compound Distanced moved by the solvent (solvent front)}}$$

The silica gel plates were cut with scissors and marked with pencil about 1cm from the bottom of the plate. Each sample was faintly dissolved in their respective solvent and uniformly applied on the plates and allowed to dry. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro liter by means of capillary at space of 1 cm at 5 tracks (Rajendra & Estari,2013).The plates were developed in a chromatographic tank using the different solvent systems. Petroleumether: Acetic acid (9:1) solvent system 1,In solvent system(2)petroleum ether: ethylacetate: Acetic acid (5:4:1), in solvent system (3)petroleum ether: ethylacetate: Acetic acid (4:4:2), in solvent system (4)petroleum ether: ethylacetate: Acetic acid (3:6:1), in solvent system (5)petroleum ether: ethyl acetate: Acetic acid (2: 7: 1). The plates were immediately transfer into iodine chamber and visualized under normal day light. The retention factor Rf for each visualized spot was calculated for each root extract using the following formula;



Solvent System I Solvent System II Solvent System III

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Solvent System IV Solvent System V

**Figure 1: Photographs of thin layer chromatographic studies**

## III. RESULTS AND DISCUSSION

**Percentage Yield of Extracts**

Table 1 displays the yield of several crude extracts from the 40g of crushed Mucuna pruriens root that were extracted using various solvents. For the petroleum ether, ethylacetate, methanol, ethanol, and water extracts, 40g of the sample yielded 1.10gm, 7.9gm, 10.2gm, 4.0gm, and 4.2gm of material, respectively. Thepercentage yield was determined by dividing the amount of each dried extract by40g of pulverized Mucuna pruriens root.

Table 1: The Percentage Yield of Dried Extracts of Mucuna pruriens root

| S/No. | Solvent | Colour of extract | Amount of dried extract(in gm) | % Yield of dried extract |
|---|---|---|---|---|
| 1 | Petroleum ether | Pale Yellow | 1.10 | 2.75 |
| 2 | Ethyl acetate | Brown | 7.9 | 19.75 |
| 3 | Methanol | Brown | 10.2 | 25.5 |
| 4 | Ethanol | Reddish brown | 4.0 | 10.0 |
| 5 | Water | Brown | 4.2 | 10.5 |

Table 1 indicates that the percentage yield of extracts were found to be in the following order: root methanolic extract> root Ethylacetate extract>root ethanolic extract >root water extract> root petroleum ether extract. This result suggested that methanol was a better solvent for the extraction of Mucuna pruriens root. This was in line with Mujeeb, Bagri Aeri and Khan (2010) who earlier reported that methanol is a better solvent for extracting medicinal plant. In another study by Martin-Puzon and Rivera (2015) showed that methanol gave a higher yield compared to ethanol and chloroform. This was also in line with Okposo, Etinagbedia, Orogu, and Okuda (2018)who found hjigher percentage yield when methanol was used for extraction of Mucuna pruriens root.

**Phytochemical constituents of extracts of Mucuna prurien Roots**

The earlier phytochemical investigation conducted by Okposo, Etinagbedia, Orogu and Okuda (2018), confirmed the presence of common constituents such as in petroleum ether extracts contains steroids, quinones and terpenoid, ethylacetate extract contains alkaloids, flavonoid, phenolic compounds, steroids, tannins, quinones and terpenoid, methanol extract contains alkaloids, flavonoid, phenolic compounds, steroids, tannins, and terpenoid, ethanol extract contains alkaloids, flavonoid, phenolic compounds, steroids, saponin, tannins, quinones and terpenoid, and the water extract contains alkaloids,flavonoid, phenolic compounds, steroids, and tannins.

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**Result of Thin Layer Chromatographic (TLC) Analysis.**

TLC analysis of all the extracts of Mucuna pruriens using different solvents systems indicated the presence of different spots as shown in Table 1.

**Table 1: $R_f$values of TLC solvent systems for different extract of Mucuna pruriens root**

| S/No | Name of Extract | Solvent system 1 | | Solvent system 2 | | Solvent system 3 | | Solvent system 4 | | Solvent system 5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No. of spots found | Calc. Rf value | No. of spots found | Calc. Rf value | No. of spots found | Calc. Rf value | No. of spots found | Calc. Rf value | No.of spots found | Calc. Rf value |
| 1 | Pet.ether | Nil | Nil | 1 | 0.38 | 2 | 0.53<br>0.72 | 2 | 0.33 | 1 | 0.81 |
| 2 | Ethyl acetate | 2 | 0.34<br>0.45 | 3 | 0.13<br>0.57<br>0.70 | 1 | 0.35 | 2 | 0.36<br>0.87 | 3 | 0.35<br>0.51<br>0.88 |
| 3 | Methanol | 3 | 0.41<br>0.57<br>0.73 | 4 | 0.15<br>0.40<br>0.58<br>0.68 | 3 | 0.35<br>0.67<br>0.77 | 3 | 0.31<br>0.53<br>0.71 | 2 | 0.47<br>0.96 |
| 4 | Ethanol | 2 | 0.36<br>0.52<br>0.77 | 3 | 0.26<br>0.45<br>0.57 | 2 | 0.42<br>0.58 | 1 | 0.36 | nil | Nil |
| 5 | Water | 2 | 0.14<br>0.45 | 2 | 0.19<br>0.83 | 3 | 0.29<br>0.46<br>0.71 | 2 | 0.20<br>0.78 | 2 | 0.38<br>0.84 |

The aim of this study was to carry out thephytochemical analysis of mucuna pruriens root by thin layer chromatography. The chromatographic (TLC)analysis was carried using different solvent systems for the five extracts of Mucuna pruriens root. In solvent system 1; no spot was detected for petroleum ether extract,2 spots were detected for ethylacetate extract,3 spots were found for methanolic extract, 2 spots were found for ethanolic extract and 2 spots were found for water extract,in solvent system 2; 1spot was detected for petroleum ether extract,3 spots were detected for ethylacetate extract,4 spots were found for methanolic extract, 3 spots was found for ethanolic extract and 2 spots were found for water extract,in solvent system 3; 2 spots was detected for petroleum ether extract,1 spot was detected for ethylacetate extract,3 spots were found for methanolic extract, 2 spots were found for ethanolic extract and 2 spots were found for water extract, in solvent system 4; 2spots was detected for petroleum ether extract,2 spots were detected for ethylacetate extract,3 spots were found for methanolic extract, 1 spot were found for ethanolic extract and 3 spots were found for water extract, and in the solvent system 5; 1 spot was detected for petroleum ether extract,3 spots were detected for ethylacetate extract,2 spots were found for methanolic extract, no spot were found for ethanolic extract and 2 spots were found for water extract. The presence of different spots in the various extract is in line with qualitative analysis which indicated that the root extracts of Mucuna pruriens root constituted different phytochemical compounds with different $R_f$ values (Table2)(Rizwan &Sreemoy ,2013). Different phytochemicals produce various $R_{f\ values}$ in various solvent systems (Sanjay & Bhagyashri, 2013). This variance in $R_f$ values of the phytochemicals offers a crucial clue in determining their polarity and aids in the choice of the best solvent system for column chromatography's purification of compounds. To separate pure compounds from plant extracts, a mixture of solvents with varying degrees of

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polarity can be used. The best solvent system for a certain plant extract can only be chosen by comparing the Rf values of the components in different solvent systems. The polarity of the compound is also implied by the different Rf values of the compound. This information will help choose the best solvent solution for separating the parts of these plant extracts in the future (Rajendra & Estari, 2013; Nigam, Kulkarni, Parsai, & Chourey, 2018).

## IV. CONCLUSION

The search for medicinal herbs to treat various illnesses is an ongoing endeavour. There are numerous native plants with various bioactive substances that can be used as medicines. One such plant, specifically the plant's root, has promising medical significance is Mucuna pruriens. The fact that MP seed extract contains a variety of bioactive components supports the validity of its therapeutic applications, and further isolation and purification of specific phytochemical elements may open the door to the creation of novel medications and therapeutic approaches.

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