Abstract—Crushed stems of the plant were subjected to sequential extraction (maceration) by increasing polarity index of solvents (hexane, ethylacetate and methanol). The methanol extract (3.68%) gave the highest percentage yield followed by Ethylacetate (2.98%) and Hexane (2.24%) was the least in the sequential extraction. All the solvent extracts were then used for phytochemical screening and Thin Layer Chromatography (TLC). Six (6) phytochemicals which include Alkaloid, Tannin, Phenol, Cardiac-active glycoside, Xanthoprotein and Carbohydrate were found to be present in all the plant extracts, these phytochemicals are important and could have medicinal usefulness. Two phytochemicals which include; Flavonoid and Protein were absent in all the three extracts, out of the fourteen (14) phytochemical tests carried out. The TLC carried out showed that the Hex:EtAc (2:8 and 1:9) solvent system gave a better separation for Hexane and Etylacetate extracts, showing different green and yellow colours as viewed under UV, while none of the solvent systems used was found to be suitable for the separation of components in Methanol extracts.

Keywords— Phytochemicals, Thin Layer Chromatography (TLC), Solvent Systems, Senna occidentalis

I. INTRODUCTION

Plants are common sources of drugs especially in traditional medicine. It is a common practice in Nigeria and other parts of the world to use plants in the form of crude extracts to treat common infections and chronic conditions. According to WHO, over 70% of the world’s population rely on medicinal plants for primary health [1] (WHO, 2008).

Medicinal plants have been identified and used from pre-historic times. The early historic record of herbs (medicinal plants) is found from the Sumerian civilization, where the hundreds of medicinal plants were listed on clay tablets [2] (Kelly, 2009). A number of medicinal plants abound in Nigeria flora [3] (Gbile, 1986), which is the richest country in West Africa with regards to medicinal plant resources. The country exhibits a wide range of climate and topology which has a good bearing on its vegetation and forest composite [4]. Traditionally, the use of plants as source of herbal preparation for the treatment of various ailments is based on experience passed from generation to generation. The knowledge of medicinal plants by traditional healers is jealously guarded with utmost secrecy for economic reasons. Many traditional herbal practitioners hide the identity of plants used for the treatment of different ailments largely for the fear of patronage, should the patient learns to cure himself [5]. Thus, to mystify their trade, cultivation of medicinal plants is not encouraged, making collection to be from the wild. Herbal preparations are used in traditional medicines as drugs in various dosage forms; as crushed powder, decoctions, dried extracts, infusions and tinctures [6].

Plants contain many chemicals which when extracted serves biological functions; including fighting against disease pathogens (bacteria, fungi, and virus) and also serve as food nutrients [7]. Over 12,000 biologically active compounds are already known. This plant extracts work on the body exactly the same way as pharmaceutical drugs [8]. However, since a single plant may contain many substances and the effect of taking a plant medicine can be complex, proper isolation gives a more effective health benefit. Plants form the basis for conventional pharmaceutical drugs. Many parts of plants have been investigated in recent times and found to contain active substances that are medically useful, whereas many more are yet to be scientifically investigated [9].

Senna occidentalis, which is commonly called coffee senna is a small medicinal shrub, about 3ft tall, belonging to the fabaceae family. It is found in the tropical areas of America and naturalized in Australia, eastern Africa, southern and eastern USA [10]. It can be found in opened pastures and in fields cultivated with cereals such as soybean, corn, sorghum; thus during the harvest it is almost impossible to prevent this plant from mixing with the cultivated crop [11].
Plants belonging to the Fabaceae family have extensively been investigated because of their high medicinal and economic uses. The antibacterial and antimalarial activities of the leaves and root-bark of *Senna occidentalis* have been reported [12]. The plant is also known by some vernacular names such as Septic weed, Coffee senna, Coffee weed, Mogdad coffee, Negro coffee, Senna coffee, Stephanie coffee, Stinking coffee or Styptic weed. The plant is locally called Bana Chakunda in Odisha, India. [13]. The specie was formally placed in the genus *Cassia* and thus was formally called *Cassia occidentalis* [14]. The plant is reported to be poisonous to cattle’s when consumed. The plant contains anthraquinones, the roots contains emodin and the seeds contains chrysarobin (1,8-dihydroxy-3-methyl-9-anthrone) and N-methylmorphine [15]. The leaves of *Senna occidentalis* Linn from data revealed in a research “Nutritional and Anti-nutritional Analyses of *Senna occidentalis* Linn” [16] showed that it contains protein, carbohydrate, fiber, lipids, vitamins, moisture, caloric value and low levels of anti-nutrients of the leaves whose levels could be reduced on processing before consumption. Thus conclusion was made; that the plant can contribute significantly to the nutrient requirements of man and may ameliorate some nutrition related illnesses.

Similarly, in the Review on Nutritional Potential of the plant carried out by [17]. An attempt was made to collect all possible ethno botanical and nutritional potential of *S. occidentalis* with reference to its food and medicinal applications. A baseline survey was conducted between 2011-2015, and information about *S. occidentalis* was collected through semi-structured interviews and discussion with the local healers, elderly and experienced people. Additionally, all available literature on *S. occidentalis* was reviewed and studied through an online search engine Scopus and Google Scholar. Literature collection was done from 1965 to 2015 and all the information were compiled and presented. The research suggests a huge nutritional potential of this plant, it suggests that raw seeds might have some toxicological side effects, but after proper processing, identification and removal of the harmful properties of seeds, they may be utilized to prepare a good, nourishing coffee. The research suggests that *S. occidentalis* should be further exploited in the future as a source of useful phytochemicals and nutritional compounds for the nutritional industry.

A study carried out on the Similarly, in the study phytochemical, antimicrobial and antioxidant activities of the hexane, ethylacetate and methanol crude extracts of *Senna occidentalis* leaves extracts revealed the presence of tannins, alkaloids, reducing sugar, phenols, anthraquinones, resins, saponins and glycosides. The antimicrobial screening was carried out using the following organisms; *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizopus stolonifer*. The results obtained showed that *Senna occidentalis* leaf extracts have interesting pharmacological active compounds with great radial scavenging and antimicrobial effects and as such could be used in ethnomedicine for treatment of some infections and ailments [18].

Studies carried study on the in-vitro antioxidant activity of the plant. Their study was based on the measurement of the scavenging ability test substances towards the stable DPPH radical. The research concluded that the plant has antioxidant property [19].

The phytochemical screening, determination of bioactive constituent of *Senna occidentalis* methanolic leaf extract using Gas chromatography-mass spectrometer (GC-MS) was carried out [20]. The phytochemical study revealed the presence of tannins, alkaloids, glycoside, flavonoids, steroids, saponins, anthraquinones and phlobatannins while cardiac glycoside was not detected. GC-MS chromatogram showed nine peaks. A total of 31 compounds were identified when the mass spectra of the constituents was taken. The first compounds identified with less retention time (15.929s) were n-hexadecanoic acid, octadecanoic acid and pentadecanoic acid, while decanoic acid, decyl ester, ether, octadecyl vinyl, oleic acid, hexyl ester, stearic acid, octadecyl ester and decyl fluoride took the longest retention time (20.600s) for identification. Thus, the research concluded that the presence of these compounds in the plant extract may at least be responsible for one of the pharmacological properties of *S. occidentalis* and thus could be of considerable interest to the development of new drugs. As seen from the literatures reviewed above, more research has been done on the leaves, roots and seed, with little attention to the stem of *Senna occidentalis*.

II. MATERIALS AND METHODS

A. PLANT MATERIAL

*Senna occidentalis* (L.) stem bark was harvested from a river bank at Tudun wada area of Jos, Jos North L.G.A, Plateau State.
The plant collected was authenticated at the Department of Plant Science and Biotechnology, University of Jos. The sample was then chopped into small sizes and dried at room temperature for four (4) weeks. The dry sample was crushed into small sizes using mortar and pestle. The dry crushed sample was stored in a clean dry polyethene bag until needed for further analyses.

**B. PREPARATION OF PLANT EXTRACT**

The dried sample (50g/200ml) was extracted successively with n-hexane, ethylacetate and methanol using cold extraction method (maceration). The solvent was left in contact with the plant for 72 hours (3 days) in each case. The extract was decanted and allowed to stand, after which it was filtered leaving the extract with little amount of the solvent. The extract was transferred into a pre-weighed beaker and placed on a water bath at 40°C until a plastic form of the extract is obtained (soft extract). All the crude extracts were then weighed to determine the percentages yield of extraction. The percentage yield of extraction was calculated using the following equation:

\[
\% \text{ Yield} = \frac{\text{Weight of the dried concentrated crude extract}}{\text{Weight of the dried crushed plant sample used}} \times 100\%
\]

**C. PHYTOCHEMICAL SCREENING**

The phytochemical test for various phytochemicals present in the extract was carried out using standard methods as described below [21].

1. **Test for Alkaloid**
   0.2g of the molten extract was mixed with little amount of HCl and then wagners reagent. Formation of a white precipitate indicates the presence of alkaloid.

2. **Test for Flavonoid**
   0.2g of the molten extract was mixed with 1ml of 2% ammonium chloride and the exposed to light. Yellow precipitate indicates the presence of Flavonoid.

3. **Test for Anthraquinone**
   A few drops magnesium acetate was added to 0.2g of the molten extract. Pink colour formation indicates the presence of anthraquinone.

4. **Test for Quinone**
   0.2g of the molten extract was treated with few drops of conc. H2SO4 or aqueous NaOH solution. Red colour formation indicates the presence of quinone.

5. **Test for Phenols**
   0.2g of molten extract was mixed with ferric chloride solution. A green or dirty green precipitate indicates the presence of phenol.

6. **Test for Phlobatannins**
   0.2g of the molten extract was mixed 2% HCl solution. Appearance of red precipitate indicates the presence of phlobatannin.

7. **Test for Tannins**
   0.2g of the molten extract was mixed with few drops ferric chloride solution. A blue-black, green or blue-green precipitate indicates the presence of tannin.

8. **Test for Saponins**
   0.2g of extract was shaken 5ml of distilled water in a test tube. Frothing which persists on warming indicates the presence of saponin.

9. **Test for Xanthoproteins**
   0.2g of extract was mixed with few drops of conc. HNO3 and then few drops of ammonia. A red precipitate indicates the presence of xanthoproteins.

10. **Test for Steroids (Salkowski’s test)**
    0.2g of the extract was mixed with 3ml of chloroform and 2ml of conc. H2SO4. A red colour appearance indicates the presence of steroid.

11. **Test for Cardiac-active glycoside (Keller-killani test)**
    0.2g of the extracts dissolved in 2ml of glacial acetic acid containing one few drops of ferric chloride solution followed by the addition of few drops of concentrated sulphuric acid. A brown ring at the interface confirmed the presence of cardiac-active glycoside.

12. **Test for Carbohydrate**
    0.2g of extract was mixed with few drops of concentrated sulphuric acid then heat. Black colouration indicates the presence of carbohydrate.

13. **Test for Protein**
    0.2g of extract was mixed with few drops of Biuret reagent. A pink colouration indicates the presence of protein.

14. **Test for Fixed oil (Spot test)**
    A small quantity of extract was pressed between two filter papers. Appearance of grease spot will indicate the presence of fixed oil.

**D. TIN LAYER CHROMATOGRAPHY (TLC)**

Each extract was dissolved in respective solvent. 7 x 5cm pre-coated silica gel 60 F254 TLC plate was cut with ordinary household scissors. Plate markings were made with soft pencil (1cm from bottom of plate). Glass capillaries were used to spot the sample for TLC at distance of 1.5 cm at 3 tracks. The solvent system used were Hexane: Etylacetate (1:9, 2:8, 3:7, 4:6, 5:5, 8:2), Hexane: Etylacetate: Methanol (3:2:5, 4:4:2). The developed TLC plates were sprayed with 10% H2SO4 solution and heated on a hot-plate to allow colour development. The
Retention factor (Rf) value was determined by using the following formula:

\[ R_f = \frac{\text{Distance travelled by the compound (cm)}}{\text{Distance travelled by the solvent (cm)}} \]

III. RESULTS

The Results obtained are presented in the following tables below.

Table 1: Weight and Percentage Yield of Extract

<table>
<thead>
<tr>
<th>Extract</th>
<th>Colour of extract</th>
<th>Wt. of sample (g)</th>
<th>Wt. of extract (g)</th>
<th>Percentage yield(%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE</td>
<td>Dark green</td>
<td>500</td>
<td>11.20</td>
<td>2.24%</td>
</tr>
<tr>
<td>EE</td>
<td>Dark green</td>
<td>500</td>
<td>14.60</td>
<td>2.92%</td>
</tr>
<tr>
<td>ME</td>
<td>Brown</td>
<td>500</td>
<td>18.40</td>
<td>3.68%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td>44.20</td>
<td>8.84%</td>
</tr>
</tbody>
</table>

Key: HE: Hexane Extract, EE: Ethylacetate Extract, ME: Methanol Extract

Table 2: Phytochemicals screening of *Senna occidentalis* stem

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>HCE</th>
<th>ECE</th>
<th>MCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid (Wagners test)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoid (NH4Cl test)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinine</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponin (Frothing test)</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatannin</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Steroid &amp; terpenes</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac-active glycoside</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xanthoprotein</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fixed oil (Spot test)</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>


Table 3: TLC Analysis of Etylacetate Crude Extract

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Number of spots</th>
<th>Rf Value</th>
<th>Colour viewed under UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etylacetate: Methanol- 4:4:2</td>
<td>2</td>
<td>0.40</td>
<td>Light yellow Light yellow</td>
</tr>
<tr>
<td>Etylacetate: Methanol- 3:2:5</td>
<td>1</td>
<td>0.89</td>
<td>Light yellow</td>
</tr>
</tbody>
</table>

Table 2: TLC Analysis of Methanol Crude Extract

<table>
<thead>
<tr>
<th>Solvent system</th>
<th>Number of spots</th>
<th>Rf Value</th>
<th>Colour viewed under UV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol- 4:6</td>
<td>1</td>
<td>0.13</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Methanol- 3:2:5</td>
<td>1</td>
<td>0.90</td>
<td>Yellowish green</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
IV. DISCUSSION

1. Percentage Yield

The solvents used in the present study were selected based on their different polarity ranges. In chemistry, polar substances would dissolve in polar solvents while non-polar substances will dissolve in non-polar solvents. Hexane, ethylacetate and methanol (in order of increasing polarity, respectively) were selected to enable the extraction and separation of a wide range of components that are present in the samples. Powdered samples were first soaked in hexane to extract out the non-polar compounds, while methanol was used last, after ethylacetate, to extract out the polar compounds which constitutes the bulk compounds present in the sample. Methanol is classified as high polarity solvent that can be used to extract sugar, amino acids and glycosides from the samples [22].

The ability of a solvent to extract the bioactive compounds from plants is determined by calculating the percentage yield of extraction. The percentage yield of plant extract is mainly dependent on the solvent used in the extraction. Different polarity index of solvents give different percentage yields and extract different phytochemical compounds. The percentage of crude extract yield as shown in Table 1 was based on the weight of dried and crushed plant materials. The percentage yield of crude methanol extract (3.68%) is the highest among the three solvent extracts, followed by ethylacetate extract (2.92%), whereas hexane extract showed the lowest percentage yield (2.24%).

The result is in agreement with earlier research conducted [23], which reported that polar solvents usually have higher extraction yield compared to non-polar solvents. This is because the polar compounds such as polyphenols are highly extracted in polar solvent compared with non-polar solvents [24]. This indicates that the plant contained more polar compounds as these compounds will be dissolved in similar polarity of solvents which apply to the “like dissolves like” principle. The low percentage yield of extracts by the plant material which was collected late March, agrees with a research conducted at different quarters of the year (i.e at different seasons), which showed that low percentage yield was obtained at the first quarter season. However, high percentage yield of plant extraction does not indicate that the solvent extract will perform high antibacterial activity [25].

2. Phytochemical Screening

The preliminary phytochemical screening revealed the presence of twelve (12) out of the fourteen (14) phytochemicals tested in the different solvent extracts, with flavonoid and protein absent in all the extracts. The hexane extract shows the presence of eight (8) phytochemicals which include; alkaloid, tannin, phenol, saponin, cardiac-active glycoside, xanthoprotein, carbohydrate and fixed oil. The ethylacetate extract also shows the presence of eight (8) phytochemicals which include; alkaloid, tannin, phenol, anthraquinone, steroid, cardiac-active glycoside, xanthoprotein and carbohydrate, while the methanol
extract has the highest number of phytochemicals present, being ten (10) which include; alkaloid, tannin, phenol, saponin, phlobatannin, steroid, cardiac-active glycoside, xanthoprotein, carbohydrate and fixed oil. All the solvent extracts showed the presence of Alkaloid, tannin, phenol, cardiac-active glycoside, xanthoprotein and carbohydrate. Alkaloid, Tannin, Phenol, Cardiac-active glycoside, Xanthoprotein and Carbohydrate were found to be present in all the solvent extracts, while Flavonoid and Protein were absent in all the solvent extract. Phytochemicals such as flavonoids and tannins are known to scavenge free radicals and build up immunity respectively, thus taking care of the stimulation of free radicals and oxidative stress as a result of high glucose levels. The root of *Senna occidentalis* is a rich source of antioxidants [26]. The presence of tannins may be the reason why *Senna occidentalis* is used in the treatment of constipation and intestinal worms in India. Tannins could also be the probable reason why the plant is used for treatment of poisonous bites. Tannins also have the potential to remedy complex divalent ions such as zinc, iron and copper in soils resulting in their unavailability [27].

Cardiac glycoside acts on the heart muscles and increase renal flow (diuresis). Saponins are often referred to as "natural detergent" because of their foamy nature and they have anti-carcinogenic properties, immune modulation activities and regulation of cell proliferation as well as health benefits such as inhibition of the growth of cancer cell and cholesterol lowering activity [28]. This can also explain why the plant is used as an antioxidant. Occurrence of steroidal saponins from various studies indicates their importance and the interest in pharmacy due to their relationship with such compounds such as sex hormones especially in development of the female contraceptive pills [29]. Also, the presence of saponins may be the reason why the decoction of the roots of *S. occidentalis* are widely used in Africa in the treatment of women’s problems including improving female fertility, and in treating sterility and dysmenorrhea since steroidal structures could serve as potent starting material in the synthesis of these hormones. Steroids have been reported in clinical studies as anti-inflammatory and analgesic agents [30], they are also used as treatment for congestive heart failure [31]. Thus, the reason why *S. occidentalis* is used as anti-inflammatory and analgesic agents. Phlobatannins are known to exhibit diuretic property [32].

Alkaloids, the most revered of all the phytochemicals, are said to be pharmacologically active and their actions are felt in the autonomic nervous system, blood vessels, promotion of diuresis, respiratory system, gastrointestinal tract, uterus, malignant diseases, infections and malaria [21] (Trease and Evans, 1989). Alkaloids are anti-plasmodic, analgesic and also have bactericidal effects. Alkaloids present in the plant extract have toxicity against cells of foreign organisms, such as enzymes in microbial cells [33]. The presence of phenolic compound in the plant proves that they have anti-microbial and anti-fungal effect. Also, plants that contain phenols could be used as anti-inflammatory, immune enhancers and hormone modulators. Phenols are also known to have the ability to block specific enzymes that cause inflammation and to prevent disease [34]. Phenolic compounds are well known potential phytotoxins and exist as free forms, esters or as glycoside when combined with sugars. Such compounds contribute to the bitter taste. There are also enough documented data, which suggest that there is a positive correlation between total phenolic content and antimicrobial activity [35].

3. Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) is used to provide insight into the chemical composition present in the plant extracts. TLC works on the principles that like forces attract and unlike forces repel. It will retain the polar compounds on the TLC plate (stationary phase) that is coated with a polar adsorbent, silica gel. The sample is then moved upward via the mobile phase. The mobile phase moves through the TLC plate by capillary action and assisted with gravity force [36].

Rf value is used to characterize the different phytochemical compounds that are present in the extract. The formula used to calculate the Rf value is mentioned earlier in the methodology. The phytochemical compound will be obtained by different Rf values, due to their polarity. The polar compounds will have strong interaction with the stationary phase on TLC and travel shorter path. Meanwhile, the non-polar compounds have weaker interaction with stationary phase and travel longer path [37]. Thus, the lower the Rf value, the higher the polarity of phytochemical compounds. This information is important by choosing appropriate and suitable solvent system to further isolate pure compounds from the plant extract by other advance chromatographic techniques [38]. The comparison of Rf value of a known standard to an unknown molecule would allow for partial characterization of the compound.

The plates below show the developed chromatogram which resulted from the various solvent extracts and the Rf values of the components separated:
All the solvent systems showed at least a spot for hexane extract. The hexane extract was found to contain polar, semi-polar and non-polar compounds. The hexane extract showed a better separation with 2:8 (Hex:EtAc) solvent system, having the highest and lowest Rf value as 0.86 and 0.08 respectively, with more of the non-polar compounds. The ethyl acetate extract contains polar, semi-polar and non-polar, showing more of the non-polar compounds. Results as seen above showed that the solvent system used is not suitable for separating polar compound as expected in the methanol extract.

V. CONCLUSION

This research showed that the methanol extract of *Senna occidentalis* gave the highest percentage yield of extraction. This indicated that the stem extract of *Senna occidentalis* contained more polar compounds than non-polar compounds. The results from the phytochemical screening test revealed that the stem bark of *Senna occidentalis* contains interesting phytochemicals such as Tannins (antioxidant, antiviral), Cardiac glycosides (anti-hypertensive), Alkaloids (anti-malarial) and Steroids (anti-inflammatory, analgesic) which are bioactive compounds with important pharmacological properties. Thus, the plant (*Senna occidentalis*) could be explored and used as a starting material for drug synthesis and discovery and also to remedy infections that might arise in the body if these phytochemicals are ascertained on further isolation and purification to be medicinal biomolecules. Results from the TLC showed that the best solvent system that can be used for the separation of compounds from hexane and etylacetate extracts is 2:8 (Hex:EtAc). The TLC also shows that the plant contains compounds that can be isolated from it. Hence, anticancer, antiviral, anti-hypertensive and other important drugs could be developed from this Plant.

VI. ACKNOWLEDGEMENT

Professor (Mrs) E.A. Adelakun and Dr. B.M. Wufem for their critical review, knowledgeable and valuable advice.

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