



EVALUATION OF THE PHYSICO-CHEMICAL AND UV-VISIBLE SPECTROPHOTOMETER CHARACTERISTICS OF *PSEUDARTHRIA HOOKERI* LEAF

¹Turinawe Blaise, ^{1,2,3}Albert Nyanchoka Onchweri, ¹Jacqueline Njeri Muchiri, ^{4,5}Arthur Saniotis,

⁶ Maniga Josephat Nyabayo, ¹Tenywa Mercy Gladys

1. School of Pharmacy, Kampala International University- Western Campus, Uganda
2. Department of Pharmaceutical Analysis and Pharmacognosy, School of Pharmacy, Kampala International University-Western Campus, Uganda
3. Department of Pharmacy, DDT College of Medicine. Gaborone, Botswana.
4. Bachelor of Doctor Assistance Department. DDT College of Medicine. Gaborone, Botswana.
5. School of Biomedicine, The University of Adelaide. Adelaide, Australia.
6. Department of Microbiology, School of Medicine, Kampala International University-Tanzania Campus, Tanzania

ABSTRACT

Despite the development of modern medicine, the use of herbal medicines has been widespread since antiquity. Natural product adulteration is the process of partially or entirely replacing a crude medication with another material that is either free of or less effective as a medicinal agent. According to the WHO, up to 25% of herbal medications used in developing nations are either fake or of low quality, and this number is increasing. Despite the WHO's published quality assurance criteria, many African nations still have inadequate regulatory frameworks for traditional medicines. The most important step in identifying a crude medication and evaluating its quality and purity is pharmacognostic evaluation, which is performed via organoleptic, macro and microscopic, powder, physicochemical, phytochemical, and fluorescence examination. The measured pharmacognostic characteristics also aid in identifying and accessing the quality and purity of leaf extracts from *Pseudarthria hookeri*. This study employed an experimental approach, quantitative in nature and research was carried at KIU-WC laboratories. Ethanol solvent extracted the highest number of active constituents from *Pseudarthria hookeri* (7.63 ± 0.0376), followed by water (6.02 ± 0.0811), acetone (4.58 ± 0.0493), Methanol (3.96 ± 0.0706) and lastly chloroform (1.79 ± 0.0493). The total ash content, acid insoluble ash content and water-soluble ash content of *Pseudarthria hookeri* were, in percentage (5.33 ± 0.1764), (1.07 ± 0.0667) and (3.4667 ± 0.0667). The different peaks of the absorbance spectra indicate different constituents in *Pseudarthria hookeri* leaves at corresponding wave lengths. For complete standardization of *Pseudarthria hookeri*, more evaluation parameters that remain unknown should be researched on.

Introduction

Despite the development of modern medicine, the use of herbal medicines (HM) have been widespread since antiquity (Alkhamaiseh & Aljofan, 2020). They can be characterized as intricate mixes of organic molecules that can originate from any unprocessed or processed component of a plant, such as the leaves, stems, flowers, roots, and seeds (Bent, 2008). The World Health Organization (WHO) estimates that both in developing and wealthy nations, 80% of the world's population uses plants in primary healthcare (Tugume & Nyakoojo, 2019). Before the coronavirus disease (COVID-19) epidemic, previous surveys in Uganda claim that more than 60% of the population relied on traditional medicine. This trend is perhaps increasing due to the second wave of COVID-19's high mortality rate. (Musoke et al., 2021), with convenience, availability and accessibility, affordability, and cultural norms being the key drivers behind the use of HM (Nsibirwa et al., 2020). However, the demand for herbal medicine rise is directly proportionate to their effectiveness, which increases their demand. Crude medicinal plants and their parts are frequently adulterated or substituted in commerce, which may cause a loss of their efficacy and tendency to toxicity (Joharchi & Amiri, 2012). This is in addition to some morphological similarities of plant parts, improper identification by consumers and herbal plant sellers, plus a lack of a standard identification system. Natural product adulteration is the process of partially or entirely replacing a crude medication with another material that is either free of or less effective as a medicinal agent (Ahmed & Hasan, 2015) Adulterations fall into one of two categories: intentional adulteration, which is primarily promoted by traders who are unwilling to pay premium prices for herbs of exceptional quality and instead buy less expensive goods. The second category is unintentional adulteration, which takes place without the knowledge of manufacturers or suppliers (Professor et al., 2017). By deceptively boosting the weight or quantity of herbal ingredients, replacements and foreign substances are typically added to herbal medications in order to enhance revenues (J. Zhang et al., 2012). There have been more instances of intentional adulteration with other species to reduce expenses due to the increased pricing of some commercial natural goods on the market (Wang et al., 2019). Some manufacturers, wholesalers, distributors, and retailers intentionally tamper with products across the entire supply chain, from collection or harvesting to distribution to production (Xu et al., 2019). Furthermore, there is currently a lack of scientific proof of the safety and efficacy of herbal products among the wide range of treatments referred to as complementary and alternative medicine (CAM). In a study on adulterated traditional-herbal medicines and its safety indicators in Malaysia, it was found that from 2008 to 2014, various offenses led to the seizure of 59,440 traditional herbal medicine (THM) goods. Eleven percent (6452/58,440) of THM goods that were confiscated had full information that could be used for analysis (Rashrash et al., 2017). It has been shown that many HB contain synthetic prescription pharmaceuticals as chemical adulterants. When the National drug Authority (NDA) recently tested natural herbal products in the Kampala area, it discovered that 55% of the tested samples were adulterated. As a result, adulteration combined with the complexity of herbs and extracts poses serious quality concerns, raising the necessity for suitable analytical methodologies for their identification and standardization through pharmacognostic evaluation (Muyumba et al., 2021). The first and most important step in identifying a crude medication and evaluating its quality and purity is pharmacognostic evaluation, which is conducted through organoleptic, macro and microscopic, powder, physicochemical, phytochemical, and fluorescence examination (Jain & Shukla, 2011). Since there is no current literature on these parameters, this study will focus on determining the physico-chemical and fluorescence characteristics of the leaf deriving from *Pseudarthria hookeri*, a medicinal plant mostly utilized in Western Uganda.

Material and Methods

Study design

This study employed an experimental approach that was quantitative in nature. Research was conducted at Kampala International University-Western campus (KIU-WC) laboratories, and was in compliance with the Good Laboratory Practice (GLP) set by the laboratory management.

Plant collection and identification

With assistance from herbal medicine collectors and traditional healers, fresh *Pseudarthria hookeri* leaves were procured in January 2022 on one early morning from Rukungiri Municipality, Western Division, Northern A Ward. The plant sample was then identified and verified by a taxonomist from Mbarara University of Science and Technology (MUST) **Specimen preparation, storage and drying**

The collected leaves were cleaned by washing with running tap water, stored and dried under the shade to avoid direct sunshine that could degrade the leaf compounds. The leaves were placed on a dry cemented surface in an isolated room and turned often on a daily basis to prevent fungal attack until completely dried. The dried leaves were then ground into powder which was stored in a brown bottle to prevent deterioration till further procedures and analysis. **Equipment, Chemicals and reagents**

The equipment and reagents that assisted this research was of scientific grade and sourced from Merck KGaA, laboratories (German).

Fluorescent determination, loss on drying, ash and extractive contents

All the ash and extractive contents, loss on drying and fluorescence determination were carried out by methods specified in the quality determination procedures for medicinal plants by the WHO (Updated Version of 1998). Final values for ash, extraction, loss on drying and fluorescence was expressed as a mean of three determinants \pm the SEM. The Standard deviation was also calculated.

Total ash content

An empty silica vessel was accurately weighed using an analytical balance, its weight was recorded as weight 1. 4 g. 2 g of the ground air-dried powder was accurately weighed using an analytical balance and introduced into silica vessel. The silica vessel was then incinerated by increasing the heat to 500–600 °C in a muffled furnace until the powder attained a white color. The incinerated powder was cooled in a desiccating instrument for 30 minutes, after which its weight was taken and recorded as weight 3.

$$\frac{(W3-W1) \times 100}{(W2-W1)}$$

$$(W2-W1)$$

Where:

W1 corresponds to the empty silica crucible

W2 corresponds to the sample including crucible for ignition

W3 corresponds to the sample including crucible after ignition

Acid insoluble ash content

To the vessel containing total ash content, 25 Milli liters of HCl were poured, the vessel was covered with its lid and heated for 5 minutes. Then, the vessel cover was cleansed using 5 ml of hot water and the liquid poured back into the vessel. The insoluble content was then obtained on a filter paper and boiling water used for cleansing. The ash less filter paper with the insoluble contents was placed back to the crucible, dried using a hotplate and ignited to constant weight. The residue's temperature was then cooled in a desiccating instrument for 30 minutes, and then the crucible was weighed immediately without any delay (W4)

$$\frac{(W4-W1) \times 100}{(W2-W1)}$$

$$(W2-W1)$$

Where:

W4 is the constant weight after addition of HCL

Water-soluble ash content.

To the vessel that contained the total ash content, 25 ml of water was poured. The vessel was then heated for a period of 5 minutes. The insoluble contents were collected in a filter. Boiling water was used for cleansing the contents and incinerated in a dry crucible for a period of 15 minutes at 450 °C. The residue's temperatures were cooled down in a desiccating instrument for 30 minutes and the weight taken once, weight 5 g. The weight of the total ash was subtracted from the weight of this residue to obtain the weight of the water insoluble ash, Weight 6 g. The water-soluble ash weight was determined by getting total ash weight minus the insoluble ash weight, Weight 7g. The water-soluble ash content of the dried *Pseudarthria hookeri* powder was computed as below:

$$\frac{(W7-W6) \times 100}{(W2-W1)}$$

Where:

W7 is the weight of ash, which is W3-W1

W6 is the weight of the residue, which is W5-W1

Loss on drying (gravimetric determination)

Five grams of the dried powder was introduced in a weighed preheated silica crucible and then introduced into a hot air oven for desiccation at 105°C until a constant mass was attained. The weight was taken after drying and was transferred to the desiccator to cool and then again, the crucible was reweighed. Percentage L.O.D was computed as below;

$$\frac{(W1-W2) \times 100}{W}$$

Where:

W is the weight of the sample

W1 is the weight of the sample before drying

W2 is the weight of the sample after drying

Extractive values for water, methanol, ethanol, chloroform and acetone.

All of the extractive values in the study were determined by maceration procedure. Thirty grams of the coarse powder were weighed and introduced into a 500 Milli liter conical flask, 300 ml of solvent added (1:10 ratio) and conical flask was covered well. The conical flask was frequently shaken during the first 6 hours and then kept aside without disturbing for 72 hours after which the content was filtered. Twenty-five ml were pipetted and heated till dry in a pre-weighed flat-bottomed dish on a water bath after which the residue was desiccated at a temperature of 105⁰ C to a constant weight in a hot air oven. Percentage extractive contents, expressed as percent w/w of powder material was then determined as below:

$$\% \text{ of Solvent soluble extractive} = \left[\frac{\text{weight of residue}}{\text{weight of the drug}} \right] \times 100$$

Fluorescence analysis

Five calibrated test tubes/flasks were labelled with the names of the five solvents to be used in the study. One ml of extract was weighed using a micropipette and placed in the test-tube, ensuring no bubbles were pipetted. The test tube was filled up to the mark with 10 ml of the first solvent using a second pipette and the content shaken to mix. A blank cuvette was prepared by adding the first solvent after rinsing with the same solvent twice. The transparent sides of the cuvette were cleaned using a clean chem wipe while holding the opaque sides. The cuvette was then transferred to the UV spectrophotometer, placing the transparent sides in the direction of light, and the content analyzed from the computer, after which the cuvette was removed. A second cuvette was rinsed with a small amount of the sample extract three times after which it was filled to the mark with the content from the previously prepared volumetric flask.

The transparent sides of the cuvette were cleaned using a chem wipe while handling the opaque sides of the cuvette. The cuvette was transferred to the UV-visible spectrophotometer and the content analyzed from the computer. The procedure was performed in triplicates to obtain the mean absorbance values and standard deviation. All of the apparatus used were washed properly and the same procedure was followed for all the remaining four solvents. Results of the spectrum graphs were printed from the computer.

Quality Assurance and quality control

The local name of the plant which had been provided by a traditional healer was confirmed by a taxonomist to ensure that the correct plant was collected and used for the study. The equipment was first calibrated beforehand to ensure the accuracy of measurements and results and were labeled where necessary to avoid confusion and mix ups. The results obtained were expressed as a mean of three determinants \pm the SEM. Clean protective gear like gloves and laboratory coats were used to avoid contamination of the specimen and standard operating procedures and Good Laboratory Practice were followed throughout the whole study.

RESULTS

Determination of extractive values.

Table 1 Extractive values

EXTRACT	PERCENTAGE YIELD MEAN \pm SEM (%)	STANDARD DEVIATION
Distilled water	6.02 \pm 0.0811	0.1411
Methanol	3.96 \pm 0.0706	0.1222
Ethanol	7.63 \pm 0.0376	0.0651
Acetone	4.58 \pm 0.0493	0.0854
Chloroform	1.79 \pm 0.0493	0.0854

n=3

Determination of Ash Values.

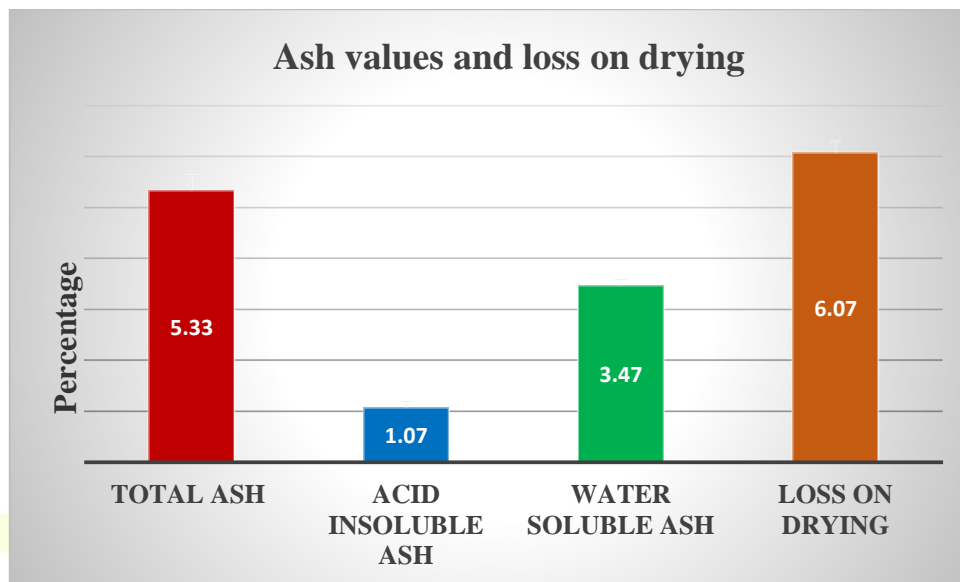
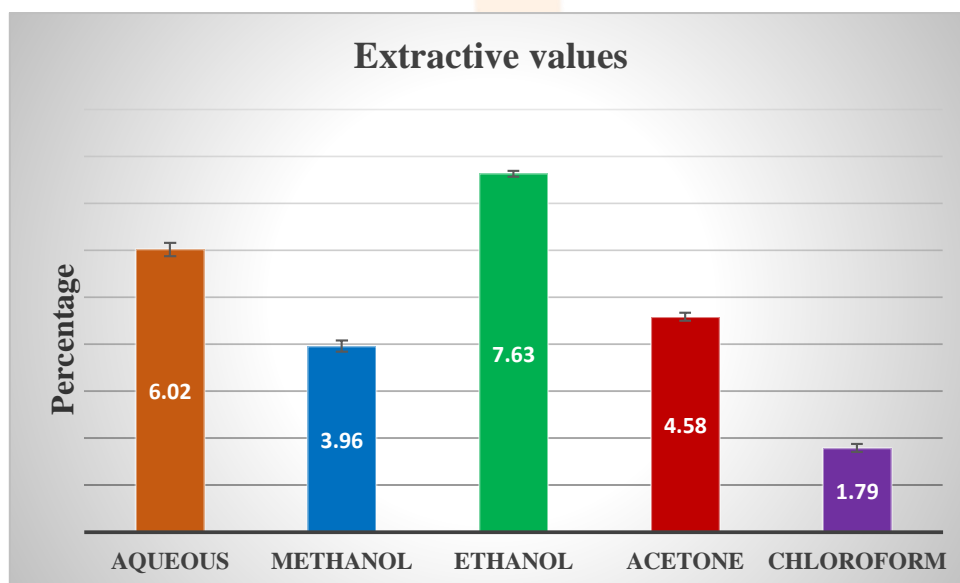
Table 2 Ash values

Ash value.	Mean \pm SEM	STANDARD DEVIATION
Total ash value	5.3333 \pm 0.1764	0.3055
Water soluble ash value	3.4667 \pm 0.0667	0.1155
Acid insoluble ash value	1.0667 \pm 0.0667	0.1155

n=3

Determination of Moisture content**Table 3 Loss on drying**

L.O.D (%) ± SEM	STANDARD DEVIATION
6.0667	0.1313

**Figure 1** Ash values and loss on drying**Figure 2** Extractive Values.

Fluorescence characteristics

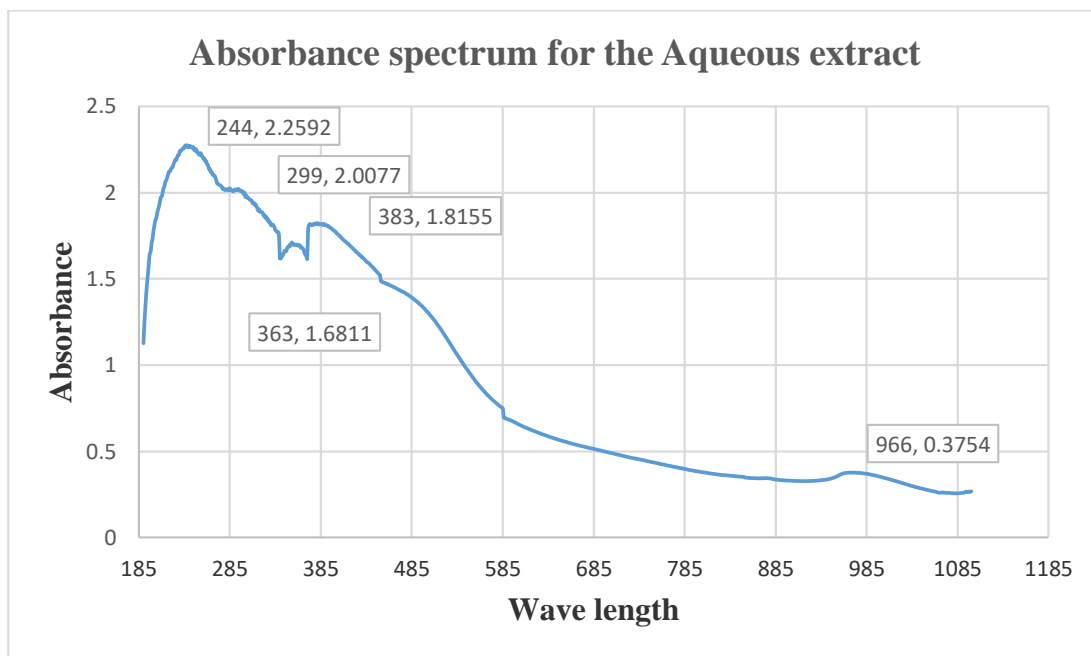


Figure 3 Absorbance spectrum for Aqueous extract.

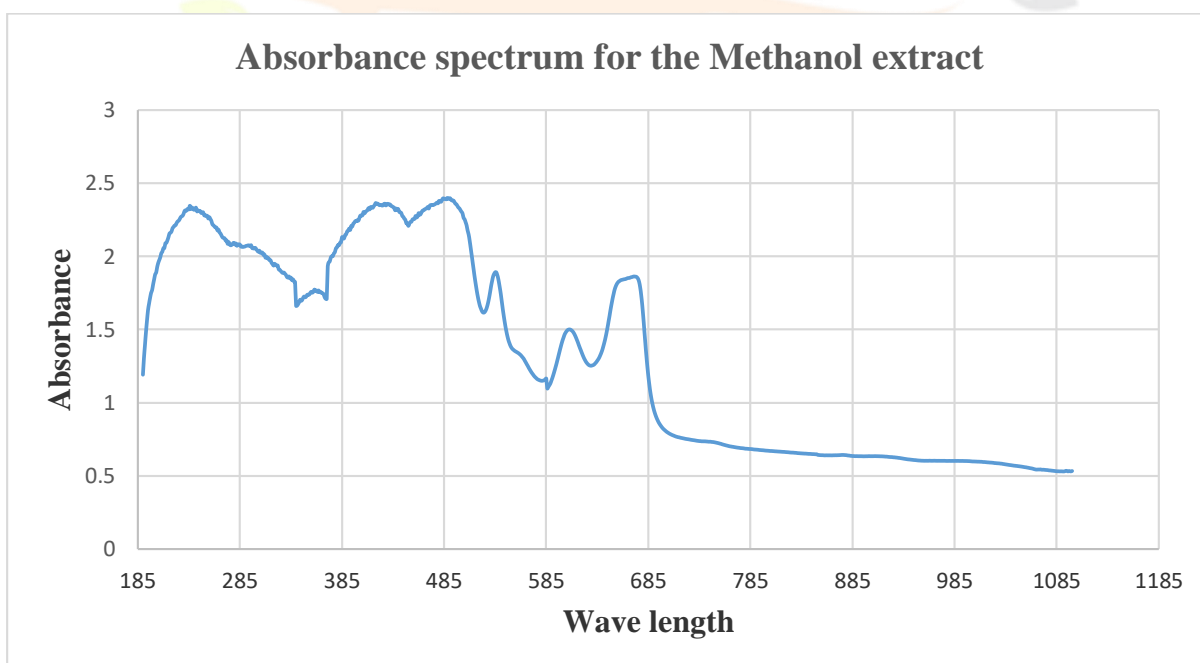


Figure 4 Absorbance spectrum for Methanol extract

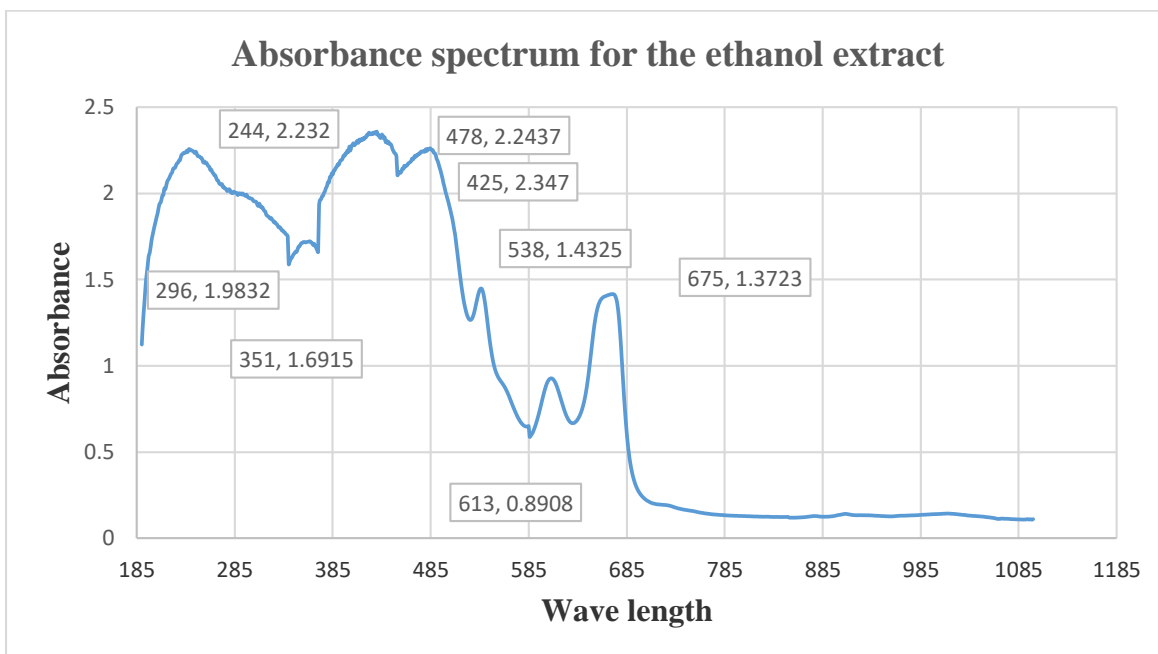


Figure 5 Absorbance spectrum for Ethanol extract

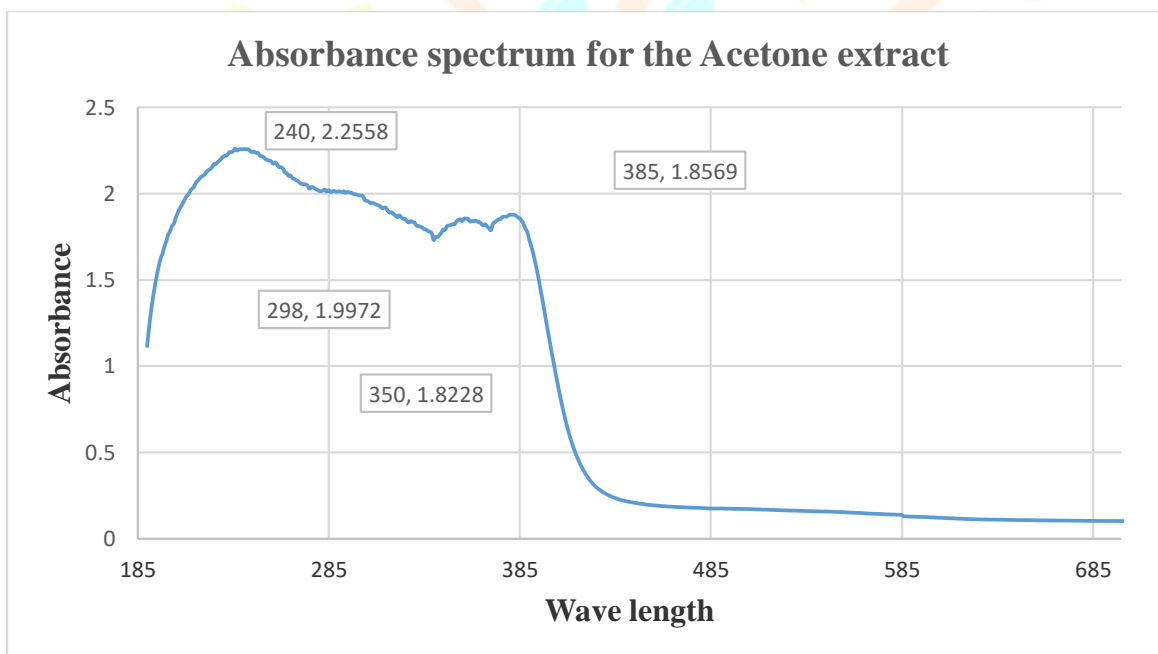


Figure 6 Absorbance spectrum for Acetone extract

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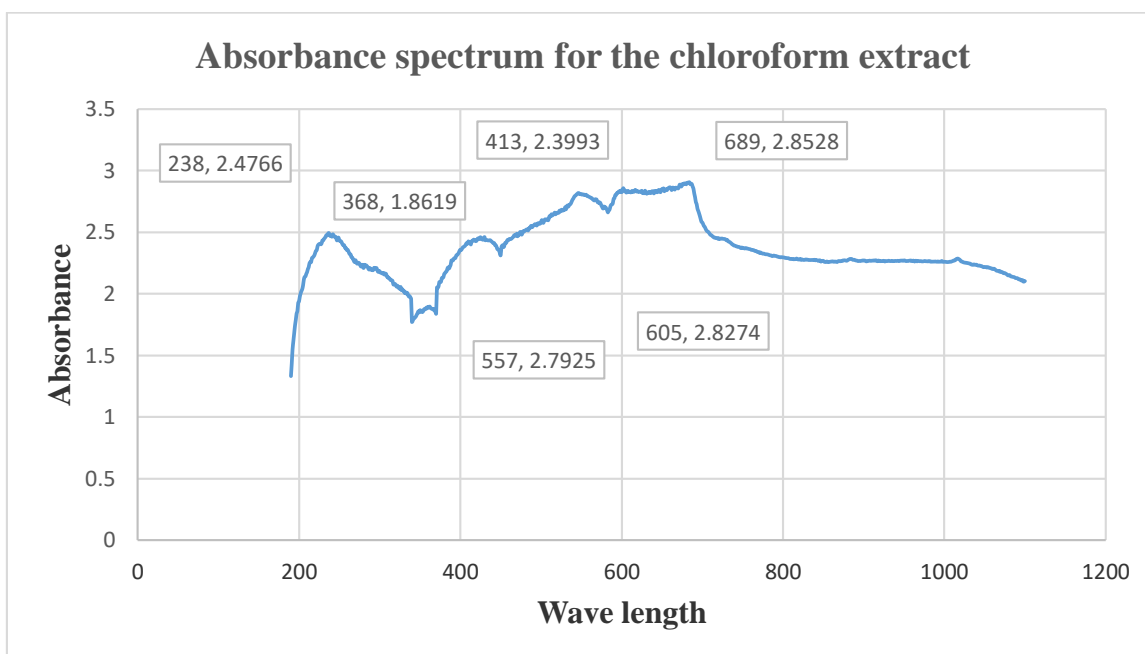


Figure 7 Absorbance spectrum for Chloroform extract

Discussion

Currently, *Pseudarthria hookeri* is being employed as an herbal medicine for numerous ailments, albeit, its use in such medicines has not been properly authenticated or evaluated. Hence, in the present study, the leaf of the plant was analyzed for its physico-chemical properties including ash and extractive value contents and fluorescence properties. Consequently, the results and findings obtained from this study can, therefore, be used as a reference due to the identification, collection/harvesting and investigative procedures which had been performed.

Extractive values

Extractive values provide an insight regarding the character of the chemical compounds present in the plant. They may also assist when estimating particular compounds that are soluble in a particular menstruum utilized in the extraction procedure, as well as the detection of exhausted extracts (Kabra et al., 2019). In this study, ethanol solvent extracted the highest amount of active constituents from *Pseudarthria hookeri* (7.63 ± 0.0376), followed by water (6.02 ± 0.0811), acetone (4.58 ± 0.0493), Methanol (3.96 ± 0.0706) and lastly, chloroform (1.79 ± 0.0493) as shown in Table 1. This indicates that most of the active constituents in *Pseudarthria hookeri* are polar compounds in nature while a few are non-polar compounds. Ethanol, giving the highest extractive value also shows that it is the best solvent for extraction procedures for components from *Pseudarthria hookeri*.

Ash Values and Loss on drying

The total ash content, acid insoluble ash content and water-soluble ash content of *Pseudarthria hookeri* were, in percentage (5.33 ± 0.1764), (1.07 ± 0.0667) and (3.4667 ± 0.0667) respectively as indicated in Table 2. Loss on drying value was found to be 6.0667 ± 0.1313 as shown in Table 3. Low total ash value indicates the low amount of inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium while high values indicate high amounts of the mentioned components. Samples with higher than regulatory contents of loss on drying may not have been dried sufficiently in the course of processing. Consequently, these samples may have been affected by humidity during storage and distribution. Greater than normal moisture levels expose crude materials to microbial organisms, including bacteria, fungi and others (Folashade et al., 2012).

Fluorescence

The fluorescence analysis is a tool for the determination of constituents in the plant that provides an insight regarding the chemical nature of the compounds. The fluorescence shown by the samples was attributed to the chemical components available in the crude drug powder (Mahadevi & Madhavan, n.d.). The absorbance spectra of the aqueous, methanol, ethanol, acetone and chloroform extracts were represented by figures 1-5 above. The absorbance spectra in the five solvents were performed using a UV-Visible

Spectrophotometer using a wide scan range of 190.0 – 1100.0nm, a scan step of 1.0nm and scan filter of 10. The different peaks of the absorbance spectra indicate different constituents in *Pseudarthritis hookeri* leaves at corresponding wave lengths. The aqueous extract absorbed UV-visible light at 244, 299, 363, 383, and 966 nm indicating presence of active constituents at the corresponding wave lengths. The methanol extract absorbed UV-visible light at 240, 296, 353, 417, 487, 553, 604, and 656 nm indicating the presence of several active constituents at the corresponding wave lengths. The ethanol extract absorbed UV-visible light at 244, 296, 351, 425, 478, 538, 613, and 675nm indicating the presence of constituents at those wave length. The acetone extract absorbed UV-visible light at 240, 298, 350, and 385 nm indicating a few constituents at the corresponding wave length. The chloroform extract absorbed UV-visible light at 238, 368, 413, 557, 605, and 689 nm indicating the presence of constituents at the corresponding wave lengths. Ethanol and methanol extracts produced the highest number of peaks followed by chloroform, water, and lastly, acetone. This indicates that *Pseudarthritis hookeri* leaf contains more polar constituents than non-polar or partially polar compounds.

Conclusion

The use of medicinal plants offers a feasible therapeutic method for treating populations from mainly developing countries, since their health sectors are not adequately equipped to meet the health care needs of their population due to lack of access or expense. In addition, these medicinal plants could act as alternatives to synthetic medicines in this era where diseases and disease organisms are becoming resistant to current medications. However, for this to happen, these plants need complete standardization to avoid adulteration, substitution and to ensure overall high quality, safety and efficacious medicines if they are to be of benefit to patients. WHO has highlighted the need to ensure that the quality of crude drugs and material used should use contemporary methods and by incorporating the needed parameters and quality levels or grades to check the quality (WHO, 2007). Regulatory authorities are obliged to make sure that patients and consumers obtain medications that are safe and effective. As prescribed by the WHO, evaluations and determination of physicochemical properties are crucial to standardize various crude drugs. Therefore, the ash and extractive value contents in conjunction with the fluorescence characteristics of *Pseudarthritis hookeri* which are being reported for the first time could be useful in its standardization.

Recommendations

For complete standardization of *Pseudarthritis hookeri*, more evaluation parameters that remain hitherto unknown should be researched. Regulatory authorities such as the NDA and other related bodies should strongly encourage and participate in research involving evaluation and standardization of all medicinal plants in Uganda especially those collected from the wild. Findings from evaluation of these medicinal plants can then be compiled via a national herbal pharmacopoeia which can be used as a standard reference.

Limitations of the study

The plant leaves used for this research derived from wild regions. Furthermore, the terrain where the plant leaves were collected was difficult to travel as it was mainly hilly.

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