Medicinal Chemistry Comparative Phytochemical Screening on Three Growth Stages of *Pennisetum Pedicellatum Trin*

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Abstract- A comparative preliminary study on the extraction and phytochemical profile of methanolic extracts of the leave, stem and root of pennisetum pedicellatum Trin at three growth stages (i.e stage 1, stage 2, stage 3) was carried out. The extracts were analysed to determine the presence of phytochemicals in the plant. The study revealed the presence of alkaloids, flavonoids, saponins and steroids. Tanins were found only in the stems of the plants and steroids were found only in the leaves. The relevance of this difference in distribution is yet to be understood. More research is needed to explain this and other observations. Methanolic extraction of roots, stems and leaves of pennisetum pedicellatum indicated that leaves of stage 2 plants contain more phytochemicals (37.6g or 10.21%) as compared to all other plant parts and growth stages. The sample of stage 2 plants showed the least percentage yield (3.86%) as compared to all other parts and stages of growth.

Index Terms- Comparative Phytochemical, Pennisetum Pedicellatum Trin, Alkaloids, Flavonoids, Saponins, Steroids, Tanins.

I. INTRODUCTION

Man depends on plants as source of medicine. From a historical perspective, it is evident that the fascination for plants is as old as mankind itself. The plants are used as food, medicine, fuel and fibre through the beginning of civilization of human beings (Bukhah, Malik and Ahmadh, 2007). Medicinal plants are the richest bio-resources of traditional system of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube, Afolayan and Okah, 2008). About 80% of the population of developing countries of the world depend upon medicinal plants for primary health care (Bogham and Kocipal-Abyazan, 1994). Medicinal plant are easily available and less expensive (Catherine and Nagajan, 2011). Most of the medicinal plants are used as raw drugs and they possess beneficial properties (Mahesh and Sathish, 2008). Plant derived medicines are safer than the synthetic medicines, which have many adverse side effects (Nair and Chandra, 2007). The discovery of secondary metabolites has improved the understanding and use of plants in traditional medicines all over the world.

II. REVIEW OF SOME RELATED LITERATURES

Phytochemicals as medicine

Phytochemical are non-reactive plant chemicals that have protective or disease preventing properties. They are chemical compounds that occur naturally in plants and some are responsible for the colour and organoleptic properties of plants. These phytochemicals are non-essential nutrients, meaning that they are not required by human body for sustaining life. Human beings have been utilizing plants for basic preventive and curative health cares since time immemorial. It is well-known that plants produce these chemicals to protect themselves but recent research demonstrates that they can also be used to protect humans against diseases. Scientist estimated that there may be as many as 10,000 different phytochemicals having potentials to affect disease such as cancer, stroke, or metabolic syndromes. Although certain phytochemicals are available as diet supplements, some scientist speculate that potential health benefits of phytochemicals may best be derived from the consumption of the whole plant food (Glyconutrients Reference, 2014)

Botanical Classification of *Pennisetum pedicellatum*

Pennisetum pedicellatum Trin, belongs to the poaceae family and paniceae tribe. It is commonly known as kyasuwa grass in hausa and Fura in kanuri. It is also indigenous and occurs naturally in tropical and subtropical Africa (Bogdan, 1977). The stem height ranges from 40-150cm or more and in some cases may have up to 10 nodes. The stem is smooth, cylindrical, joined and encircled by the leaf sheath. The leaves are born on sheaths which arise at the nodes. (Wikipedia, 2014) kingdom: plantea, Sub kingdom: Angiosperm, Super Division: Monocot, Division Commellinids, Sub class: Poacea, Order: Poales, Family: Poacea, Genus: pennisetum, species: P. pedicellatum

III. ORIGIN AND HABITAT

The species is widespread in Sudan and Guinean savannahs of Africa though sparse in littoral areas, where it is a common agricultural weed, present on cultivated fields, also on managed grasslands as well as extensively grazed savannahs. It is a weed in tropical land and cropland following disturbance or forest

clearing, but is less common in forest lands and plantations, performing purely under dense shade. Introduced in India and Nepal; it is also found on cultivated and pasture lands (Shukla et al., 1988, siwokoti and Varma, 1997)

IV. NUTRITIONAL VALUE OF THE PLANT

Pennisetum pedicellatum Trin, has high crude protein and crude fibre contents. The high protein content was attributed to its leafy nature (i.e having abundant leaves) as crude protein is more concentrated in the leaves. The high fibre was attributed to its steamy nature particularly at the end of growing period. These workers also reported the presence of calcium and sodium in high quantities. The high calcium content in *pennisetum pedicellatum* was thought to be responsible for its high crude fibre content as calcium forms structural components of the cell wall in plants. In addition to these minerals. Ziblim et al. (2012) reported the presence of potassium, phosphorus and magnesium, and that seasonal change does not significantly changes the levels of calcium and magnesium. On the other hand the levels of phosphorus and potassium showed significant variations between the wet and dry seasons.

V. TYPES OF PHYTOCHEMICALS

Phytochemicals are chemical compound formed during the plant normal metabolic processes. These chemicals are often referred to as secondary metabolites, it is composed of several classes including; alkaloids, flavonoids, steroids, phenols, Tannins, glycosides and Terpenoids (Harborne, 1973: Okwu and Okwu, 2004). In addition to these substances, plants contain other chemical compounds. These substances can act as agents to prevent undesirable side effect of the main active substances or to assist in the assimilation of the main substances (Anon, 2007).

1. **Tannins**: according to Global Britannica, (2014) Tannins (Tanic acid) are group of vegetable chemical products, amorphous, a crystalline in form obtained from various plants. Tannins have variable composition, some of these are described as condense tannins which are phenols of moderate complex structure, and others are esters of glucose or some other sugar with one or more trihydroxybenzoic acids. The empirical formula $C_{14}H_{14}O_{11}$, is often used to represent common tannins on the general structure.
2. **Saponins** are group of naturally occurring oily glycosides that foam freely when shaken vigorously with water. They occur in a wide variety of plants, including acacia, soapwort, soaproot, California pigweed, and many others. They have a bitter taste and when ingested orally are practically non-poisonous to warm blooded animals. When injected directly into the bloodstream, however, they are dengarous and quikly haemolyse red blood cells. Hydrolysis of a saponin, brought about by acids or by enzymes, gives a sugar (often, but not necessarily, glucose) and sapogenin, the latter being either a triterpene or a steroid. Some of this sugars and saponins are useful as raw materials for synthesis of steroids hormones (Harborne, 1973)
3. **Steroids** are a large group of naturally occurring and synthetic lipids, or fat-soluble chemicals, with a great diversity of physiological activity. Synthetic steroids are well known for their use by athletes as a performance – enhancing drug. There are many different steroids including certain alcohols (sterols), bile acids, hormones, plant compounds, and the poisons found in the skin of some toads, various sterols found in the skin of human beings are transformed into vitamin D when they are exposed to the ultraviolet rays of the sun. Cholesterol, a major contributor to arteriosclerosis, is a sterol.
4. **Flavonoids** according to Galeotti et al., 2008. Flavonoids (or bioflavonoids), are also collectively known as vitamin P and citrin representing another class of plant secondary metabolites. According to the IUPAC nomenclature, these metabolites can be classified into three sub classes:
   a. The flavonoid which are derived from 2-phynylchromen-4-one (2-phenyl-1,4-benzonpyrone) structure
   b. Isoflavonoids are derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure and
   c. Neoflavonoids derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure. The three flavonoids subclasses above are all ketone-containing compounds, and as such they are flavonoids and flavonols. The main class was the first to be termed ‘’bioflavonoids’’. The term flavonoids and bioflavonoid have also been more closely used to describe non-ketone polyhydroxy polyphenol compound which are more specifically termed flavonoids, flavan-3-ols, or catechins (although catechins are actually a subgroup of flavonoids) (Wikipedia, 2011).
5. **Alkaloids** according to answer.com, 2014. Alkaloids are basic organic compounds of plant origin, containing combined nitrogen. Alkaloids are amines, so their names usually end in ‘’ine’’ (e.g caffeine, nicotine, morphine, quinine). Most have complex chemical structures of multiple ring systems. They have diverse, important physiological effects on human and other animals like cows, goats, horses, sheep etc but their functions in the plants that produce them are poorly understood (Raymond et al, 2010). Some plants (e.g., opium poppy, ergot fungus) produce many different alkaloids, but most produce only one or a few. Certain plant families, including the poppy family (papaveraceae) and the nightshade family (solanaceae) are particularly rich in them. Alkaloids are extracted by dissolving the plant in dilute acid (answer.com, 2014).

VI. MATERIALS AND METHODS

Qualitative analysis of the plant extracts were carried out using standard phytochemical methods as described by Harborne (1973)

Apparatus used:

Watman No. 1 filter paper, electronic weighing balance (model PA214), thermostatic drying oven (model DNG9202) and soxhlet extractor borosicate glass were used.

Chemicals used:

Methonal used was from Loba Chemic PVT Ltd, Mumbai. India, iodine crystal, potassium iodide, mercuric and bismuth nitrate are from Hopkins and Williams Co. Ltd, England. All other chemicals used are of high analytical grade produced by BDH, Poole, England.

Collection and Preparation of Plant Samples

The fresh leaves of *Pennisetum pedicellatum* Trin were collected at Shehu sule College of Nursing compound in Damaturu, yobe state. Nigeria. The plant was identified by a traditional healers attached to biology department of Yobe State University. The plants were grouped according to the stage of growth viz (e.g stage 1, stage 2 and stage 3). Stage 1 represents the very young plant at 6 inches tall; in stage 2 the plant has reached flowering stage but has not started; while stage 3 represents the fully grown plant with well-developed flowers. The sampled plants from each of the stages are further sorted into; root, stem and leaves so that the part of the plant with higher concentration of phytochemical distribution in these parts can be established. The samples were dried in the laboratory at ambient temperature and were grinded to coarse powder. At the end of the drying and grinding process each of the samples was subjected to extraction by soxhlet extraction using methanol as a solvent.

Extraction

Each plant sample (i.e. root, stem, leaf) was extracted with 250ml of methanol using soxhlet apparatus. The volume of the solvent was reduced to about 50ml using the procedure outline below:

Recovery of Extracted Phytochemicals

Each extract were filtered through whatman no. 1 filter paper into a washed, oven dried and pre-weighted 1000cm^3 round-bottomed flask. This was then mounted to the rotary evaporator which is attached to a receiving flask and condenser, and carefully inserted into water bath (attached to a rotary evaporator) which was then activated and solvent distillation carried on until the volume reduced to about 50cm3 at the end. The extract was transferred into another washed, oven-dried and weighted basin. The remaining solvent was allowed to evaporate in the fume chamber and the extract was then cooled at room temperature and re-weighed.

Phytochemical Screening Test

The methanolic extracts were separated and tested for the presence of Alkaloids, Flavonoids, saponins, Tannins and as per the procedures outlined below.

Test for Alkaloids

One gram each of the dried methonolic extract was weighed and placed into two separate test tubes. To the first test tube, 2-3 drops of Dragenoff's reagent were added while to the second tube 2-3 drops of meyer's reagent. The development of orange-red precipitate (turbidity) in the first test tube (with Dragedogff's reagent) or white precipitate (turbidity) in the second test tube (with meyer's reagent) were indicative of the presence of alkaloids. The same procedure was repeated using aqueous extract (cuilel, 1994)

Test for Saponins

Five grams of the aqueous extract was weighted and placed in a test tube. This was followed by the addition of 5ml de-ionised distilled water. The content was vigorously shaken. The appearance of a persistent froth that lasted for 15 minutes was indicative of the presence of saponins (Brain and Turner, 1975).

Test for Flavonoids

3ml of each extract was added to 10ml of distilled water and the solution was shaken well, 1ml of 10% NaOH solution was added to the mixture. The appearance of yellow coloration was taken to indicate the present of Flavonoids.

Test for Steroids

The presence of steroid wasted by the Salkowski's test: 5 drop of concentrated H_2SO_4 was added to 1ml of each extract in a separate test tube. The appearance of a red coloration indicates the present of steroids.

Test for Tannins

2ml of each extract in a separate test tube were boiled gently for 2 minute and allowed to cool. To each tube 3 drop of ferric chloride solution were added. The appearance of orange coloration was taken to indicate the presence of Tannins.

VII. RESULT AND DISCUSSION

The samples were subjected to solvent extraction with methanol and subsequently screened for phytochemicals. The results of the methanol extraction of total phytochemicals were presented in table 1. It indicates that there were more phytochemicals found in leaves (37.6g or 10.21%) of stage 2 *P. Pedicellatum* compared to all other parts and stages. This is followed by the stage 2 stems (22.1g or 6.91%). Comparable percentage quantities were also showed. Stage 2 roots showed the lowest percentage (3.86%) of extracted phytochemicals.

Table 1: Comparative Percentage Quantities of the Plant Parts Used in the Three Stages

S/N	Plant Part Used	Wt.of Plant Part Used (g)	Wt. of Extract (g)	Percentage Yield (%)
				STAGE 1
1	Leaf	390.1	24.6	6.31
2	Stem	427.0	26.2	6.14
3.	Root	290.7	16.5	5.68
				STAGE 2
1.	Leaf	368.2	37.6	10.21
2.	Stem	319.6	22.1	6.91
3.	Root	334.2	12.9	3.86
				STAGE 3
1.	Leaf	409.0	22.8	5.58
2.	Stem	420.0	24.8	5.91
3.	Root	279.0	13.2	4.73

Based on the above finding it can be concluded that *pennisetum pedicellatum* leaves could be more effective as

medicinal plant with more phytochemical. The present study corroborates the traditional use of fresh leaves of this plant in stopping bleeding from fresh cuts and preventing infection.

Phytochemical Screening

Methanolic extracts of *P. Pedicellatum* leaves, stem and roots were subjected to phytochemical screening using established method described elsewhere (Harborne, 1973). The results revealed presence of flavonoids, alkaloids, tannins, saponins and steroids in all the plant parts in the 3 stages studied as shown in table 2-4. The result also revealed that tanins are not present in all the leaf samples of the three stages studied. These phytochemicals are found only in stem extracts of the stages of growth. NB furthermore, steroids are only found in the leaves of *P. Pedicellatum* as they were not detected in the stems and roots. More alkaloids were detected in the roots of *P. Pedicellatum*. It should be noted that no phytochemical screening was performed on *P. Pedicellatum* prior to this study. Most of the research works carried out earlier was centred on the nutritional value of this plant as a good fodder for animals (Ziblim et al, 2012, Waziri et al, 2013). However, some workers (Evans, 2005, Okaraonye and Ikewuchi, 2009 and Purewal, 2014) who worked on two other pericettum species, namely *P. purpureum* and *P. glaucum* have revealed the presence of flavonoids, saponnins in this species. Tannins and alkaloids are found in *P. purpureum* but were not detected in *P. glaucum* (purewal, 2014). The importance of these differences in the distribution of phytochemical is yet to be clearly understood, but further research works may reveal the secret.

TABLE 2: *Phytochemical screening of the leaves, stem and root of the stage 1 (pennesetum pedicellatum*

S/N	SAMPLES	LEAVES	STEM	ROOT
1.	Alkaloids	+	+	++
2.	Flavonoids	+	+	+
3.	Saponins	+	+	+
4.	Tannins	-	+	-
5	Steroids	+	-	-

Key: + = present; - = absent

TABLE 3 Phytochemical Screening of the leaves, stem and roots of stage 2 *pennesetum pedicellatum*

S/N	SAMPLES	LEAVES	STEM	ROOT
1.	Alkaloids	+	+	++
2.	Flavonoids	+	+	+
3.	Saponins	+	+	+
4.	Tannins	-	+	-
5	Steroids	+	-	-

TABLE 4: Phytochemical screening of the leaves, stem and roots of stage 3 *pennesetum Pedicellatum*

S/N	SAMPLES	LEAVES	STEM	ROOT
1.	Alkaloids	+	+	++
2.	Flavonoids	+	+	+
3.	Saponins	+	+	+
4.	Tannins	-	+	-
5	Steroids	+	-	-

The metabolites are of various pharmacological importances which have been reported in some genius of the plant like *pennisetum purpureum* by Okoraonye and Ikewuchi, 2009; these secondary metabolites are reported to have varied in uses as antimicrobial and other physiological activities (Sofowara, 1980). There is the need to carry out studies on antimicrobial activities as well as determining factors responsible for bleeding.

VIII. CONCLUSION

The present study has revealed that the leaves stems derived from *pennisetum pedicellatum* plants just before flowering stage (i.e stage 2) generally contained higher amounts of phytochemicals. Phytochemical screening has shown the presence of alkaloids, flavonoids, saponins and steroids in leaves, root and stems of the plant at the three stages of growth studied. Tannins are absent in *P. pedicellatum* and steroids are only found in the leaves. These findings indicate that stage 2 plant leaves and stems can be used for medical purposes.

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