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Processing Yield Percentage and Phytochemical Appraisement of the Leaf, Stem-Bark, Inflore-infructescence, and Root of *Petiveria alliacea* (Linneaus)

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***Abstract*-** The numerous pharmacological benefits of *Petiveria alliacea* Linneaus (Guinea Hen Weed) accentuated its further experimental studies, wherewith investigation of the sample processing yield percentage (SPY) and the phytochemical analyses of its leaf (LF), inflore-infructescence (INFLT), stem-bark (SB), and root were carried out using standard methods. Data were analyzed using IBM SPSS version 23 and results were presented as standard deviation (± SD) of duplicate determinations. The samples' processing yield (SPY) percentage result flows in the order stem-bark > root > inflore-infructescence > leaf indicating the highest (50.60 %) and the least (21.32 %) values obtained in the stem-bark and leaf preparations respectively. The phytochemical screening showed the evaluated plant parts to possess abundant phytochemicals (tannins, saponins, flavonoids, terpenoids, steroids, glycosides, and alkaloids) at varying amount with the INFLT and the R taking the lead. Keen observation divulged the seven phytochemicals screened to be present in the INFLT while other parts did not have them in full. This may be suggesting the INFLT as a better granary of phytochemicals than the other parts. It was concluded that all the parts of *P. alliacea* are bountiful sources of plants' secondary metabolites thereby justifying the fantastic use of the plant in folkloric medicine with special reference to the INFLT ampleness in glycosides and alkaloids. Additionally, the INFLT was recommended to be given unflinching attention in further experiments. The leaf also is to be more preferred (owing to its ability to re-sprout after several plucking) when the need for large quantity of extract is inevitably mandatory.

***Keywords*:** Phytochemicals, sample processing yield, plant parts, *Petiveria alliacea*

I. INTRODUCTION

Medicinal plants are known to be beneficial to Man and Animals throughout history. The study of folkloric utility of plants (ethnobotany) is an effectual method of medicines' exploration. Many chemical compounds are constantly being synthesized by plants which are very useful in performing innumerable quanta of biological functions and to defend against attacks from quite a number of external predators or nuisances among which herbivorous mammals, insects and microbes were identified. The above actually exemplifies the relevance of phytochemical analysis of various medicinal plants in use [1]. According to literature, thousands of compounds have been isolated from medicinal plants [2]. Without mincing words, so many available commercial drugs in the market today derived their source from medicinal plants recognized to have demonstrated herbal remedies against one ailment or the other. Opium, Digoxin, Aspirin, and Quinine are good examples of such drugs [3]. Medicinal Plants contain substances that have pharmacological (healing) potential. There are hundreds of remarkably common plants with therapeutic potentials and do serve as anti-bacterial, anti-viral, anti-inflammatory, anti-septic, anti-fungal, insect repellant, expectorant, detoxification, fever reduction and analgesic [4], [5]. *P. alliacea* is a tropical medicinal plant with a characteristic pungent and garlic-like odour that irrefutably fits into many of the above usefulness. The plant has many names depending on locality where it is situated. *P. alliacea* is popularly called 'guine' in Latin America, 'anamu' in South America, 'awogba arun' in Yoruba land and mapurite in Trinidad [6], [7]. In some other localities it is known as 'Guinea Hen Weed', 'apacin' and 'mucura'. Many authors reported *P. alliacea* as a broad spectrum antimicrobial, antifungal, antiviral, anticancer, immunomodulatory, and hypoglycaemic agent [8] – [11]. Despite the relevance of this plant in traditional herbal medicine, it is yet beset with scantiness of information on sample processing yield (SPY) percentage and phytochemical constituents of its leaf, inflore-infructescence, stem-bark, and the root. The necessity of carrying out this study thus become obviously indispensable as it will provide the basis to justify the traditional use of *P. alliacea* in the management of

ailments. This will probably be the first study to delve into the qualitative and quantitative phytochemical analyses of four main parts of this valued plant as such information in its entirety were not available/accessible/discovered from any academic archive or literature.

II. METHODOLOGY

Source and identification of the plant

The plant *P. alliacea* was discovered in a community in Akure, Ondo State Nigeria on Latitude: 7:15.80502, and Longitude: 5:9.06583. It was identified and authenticated by a Taxonomist in the Department of Pharmacology, Obafemi Awolowo University Ile-Ife Nigeria. The plant was processed and assigned Faculty's Herbarium Specimen Voucher Number "FPI 2269".

Collection of *Petiveria alliacea* plant

Young seedlings were uprooted. The roots were harvested and rinsed under a running tap water. Fresh, disease free leaves, inflore-infructescence (inflorescence together with the infructescence) were plucked from the plant at the flowering/fruiting stage. The stems of the plant were cut using secateurs, and the bark scrapped with the aid of a clean and sharp knife.

Processing

All the samples (root, leaf, inflorescence and the stem bark) were chopped into small sizes (in order to fast-track air-drying), weighed (recorded as fresh weight) and air dried under the shade. The processing of the samples involving pulverization using Bajaj® electric blender commenced after the attainment of constant weight of all the samples. After weighing of the samples and recorded as air dried weight, the samples were kept individually in different plastic container having firm lids at 4^oC until analyzed. Sample processing yield (SPY) percent was calculated using the formula $SPY = \frac{\text{Air-dried weight}}{\text{Fresh weight}} X\ 100$

Qualitative Tests

The qualitative tests of the phytochemicals in *P. alliacea* were carried out following standard procedures [12] – [17].

Tannins

The test for tannins involved the measurement of 0.5 g each of the samples into different laboratory test tubes and 10 mL distilled water added to each of the test tubes. The test solutions were boiled and filtered. Thereafter, drops of 0.1 % ferric chloride were added to the solutions leading to the formation of brownish blue or black colour as an indication of tannins presence.

Saponins

An approximate of 6 mL distilled water was mixed with 0.2 g of the extract. Formation of persistent bubbles (or foams) was noticed after boiling and hence showed that saponins were in the sample.

Flavonoids

Aqueous filtrates each of the extracts were prepared and 5 mL dilute ammonia added. Thereafter 1 mL concentrated sulphuric acid was added in turn to each of the solutions. Yellow colouration was observed indicating the presence of flavonoids.

Terpenoids

This was carried out by weighing 0.5 g of sample and the addition of 20 mL of chloroform to form a solution which was filtered and, 3 mL of concentrated H_2S0_4 was introduced into the filtrate. The consequent solution gave a reddish brown colour at the interface that was taken as the confirmatory test for the presence of terpenoids.

Steroids

A colour change from violet to blue or green was taken as confirmatory test for steroids presence in the sample. This was brought about by adding 2 mL acetic anhydride to 0.5 g of the prepared extract in the test tube and final addition of sulphuric acid at a volume of 2 mL.

Glycosides

Exactly 0.5 mL of glacial acetic acid, 3 drops of 1 % aqueous ferric chloride solution, and 1 mL of concentrated Sulphuric acid were introduced into 1 mL of the extract. When brown ring at the interface was observed, it was taken as indication of the presence of glycosides.

Alkaloids

Marquis reagent was used in the confirmation of the presence of alkaloids in the samples. This was accomplished by the mixture of 1 mL of the extract, 1 mL of Marquis reagent, 2 mL of concentrated sulphuric acid and few drops of 40 % formaldehyde. Alkaloids were confirmed by the appearance of dark orange or purple colour in the solution.

Quantitative Tests

The quantitative determinations were carried out following the tabulated standard methods depicted in Table 1.

Table 1: Tabulated quantitative phytochemical screening methods

Phytochemicals	Reagents	Absorbance (nm)	References
Glycosides	Chloroform, pyridine, 29 % sodium nitroprusside, 20% NaOH	510	[18]
Tannins	Folin Phenol, 35 % sodium carbonate, tannic acid (Folin and Ciocalteu method).	725	[17]
Saponins	20 % ethanol, diethyl ether, n-butanol and 5 % aqueous sodium chloride.	-	[19]
Flavonoids	80 % aqueous methanol.	-	[20]
Terpenoids	Chloroform and methanol (ratio 2:1), 10 % Sodium Dodecyl Sulphate (SDS) solution and 0.01M ferric chloride.	510	[18]

Steroids	Pyridine and metallic copper powder or copper (1) oxide.	350	[18]
Alkaloids	10 % acetic acid in ethanol, concentrated ammonium hydroxide, and dilute ammonium hydroxide	-	[21]

Statistical analysis

The phytochemicals' data were analyzed employing descriptive statistics using IBM SPSS computer software package [22] and results presented as ± SD (standard deviation) of duplicate determinations.

III. RESULTS AND DISCUSSION

The fresh and air dried weight of the samples led to the calculation of the processing yield percentage results presented in Table 2 while that of the photochemical screening of the various parts of *P. alliacea* L. are presented in Table 3 and 4.

Table 2: Sample processing yield (SPY) of different parts of *P. alliacea*

Parameters	Leaf	Inflore-infructescence	Stem-bark	Root
Fresh weight (g)	2831.2	118.50	334.00	34.00
Air-dried weight (g)	603.8	29.00	169.00	16.20
Weight differential (g)	2227.40	89.5	165	17.8
Sample processing yield (%)	21.32	24.47	50.60	47.65

SPY= Sample processing yield

Sample processing yield (SPY) percentage follows the order stem-bark > root > inflorescence > leaf indicating highest and least values in the stem-bark and leaf preparations respectively (Table 2). The woodiness of the stem-bark, root and inflore-infructescence may be a considerable factor among others here. Likewise, it is a rational expectation that the leaf should be higher in moisture content than the rest parts; hence the lowest sample processing yield (SPY) recorded for the leaf sample. Tannins, saponins, flavonoids and terpenoids were found present in all the parts of *P. alliacea* screened. Steroids were detected only in the stem-bark but absent in the other respective parts. Glycosides were absent in the leaf but available in the inflore-infructescence, stem-bark and root while alkaloids presence was only visible in the inflorescence but undetected in the rest of the parts considered (Table 3). The presence of flavonoids and terpenoids corroborates earlier reports [23], [24]. Similarly, the absence of alkaloids and presence of flavonoids, tannins, terpenoids, saponins, and steroids in the root extract agrees with [25] with the exception of undetected saponins from the aqueous (water) fraction in their study. The phyto-constituents in *P. alliacea* parts as observed in the current research is in consonance with [26] in similar study, though only the leaf and the root were examined by in their study. Quantitative phytochemical results (Table 4) revealed the phytochemicals to be in the order of tannins quantity in the inflore-infructescence > root > leaf > stem-bark, saponin: root > stem-bark > inflore-infructescence > leaf, Flavonoids: root > stem-bark > inflore-infructescence > leaf, terpenoid: root > stem-bark > leaf > inflore-infructescence, steroid: leaf > inflore-infructescence > root > leaf, Glycosides: inflore-infructescence > root > stem-bark > leaf and alkaloids only present in the inflore-infructescence. Quantitatively, the results for saponins (7.09 mg/g) and terpenoids (14.84 mg/g) in the leaf were higher than those observed by [27], who reported 6.71 mg/g and 3.27 mg/g respectively. However, alkaloids were undetected in the leaf which contradicts their observation. The value for flavonoids (0.45 mg/g) in this study was equally lower than theirs (9.56 mg/g). Differences in the results cannot but be geographically and environmentally induced. It was observed that all the seven phytochemicals screened in this study were found present only in the inflorescence; suggesting that the inflore-infructescence might be better granary of phytochemicals than other parts of the investigated plant. Ironically, this part is grossly un-researched by the scientists. Table 5 presented where (parts of the plant) each of the phytochemicals was highest or lowest at a glance. The highest and lowest values for tannins were recorded in the inflore-infructescence and stem bark respectively. Saponins and flavonoids were highest in the root and lowest in the leaf. Terpenoids had the highest value recorded in root and lowest in the inflorescence. The leaf showed the highest value for steroids and least in the root. The inflore-infructescence ranked first in glycosides and alkaloids, while the lowest in glycosides quantity was the stem-bark. Alkaloids were not detected in the leaf, stem bark, and the root samples. Tannins with their natural displeasing taste are very useful in the tanning of leathers [28]. They have anodyne, wound healing, anti sepsis, disinfecting, anti ulcer, antifungal potentials. The tannic acids in tannins are indispensable bio-compounds for plasma loss prevention in patients with wounds originating from burn [28] – [30]. Industrially, tannins are used as starter culture for drugs, herbicides and pesticides production [31]. Saponins are glycosides of triterpenes and steroids [28]. Tannins are bitter in taste and foam like soap. Their foaming ability underscores their essentiality in bio-surfactants and vaccines adjuvants preparation. Their potentials includes; anti-inflammatory, anti-cancer, antifungal and anti-viral [32], [33]. Saponins and alkaloids are particularly important in the prevention of cardiovascular diseases because they are able to modulate the absorption rate of cholesterol in the gastro intestinal tract [34]. Flavonoids are known to be excellent natural oxidant and free radicals scavenger. Their activities are very advantageous, as they regularly prevent cell damages orchestrated by oxidation [28]. Flavonoids and sub-classess have also been reported to possess anti-tumour activity in cells infected with HIV in an *in vitro* cell culture experiment. It was reported that alkaloids and their derivatives contain indispensable active and useful

compounds that are of great medical significance in drug formation e.g. antibacterial and antifungal [35]. Steroids are naturally occurring substances (hormones). They are very useful in the treatment of brain tumour, brain inflammation or cerebral oedema [36]. Cardiac glycosides are steroids and have been reported useful in potential drugs development [37] for the treatment of heart related ailments (e.g. heart failure, ranging from acute to chronic). They have the ability to display some positive governing actions on cardiac muscle [38] thereby increasing the efficiency of the blood pumping capability of the myocardium [39]. According to [40], [41] some cardiac glycosides can act as anti-tumour and anti-viral respectively, but higher doses can cause cardiac arrest of the systole. The plethora of phytoconstituents in medicinal plants and of course in various parts of *P. alliacea* suggests its relevance, criticalness and cruciality in medicine and phyto-pharmacological industries. This actually agrees with the submission of some authors [42], [43] exemplifying the medicinal utility of virtually all the parts of *P. alliacea*. The indispensability of medicinally priceless plant like *P. alliacea* underscores the sustainability of its cultivation and management [44] in order to avert the extinction of our naturally endowed resources.

Table 3: Qualitative phytochemical constituent of *P. alliacea* parts

Phytochemicals	Different parts of *P. alliacea*			
	Leaf	Inflore-infructescence	Stem-bark	Root
Tannins	I	I	I	I
Saponins	I	I	I	I
Flavonoids	I	I	I	I
Terpenoids	I	I	I	I
Steroids	I	I	NF	I
Glycosides	NF	I	I	I
Alkaloids	NF	I	NF	NF

Legend: I= Identified, NF= Not found

Table 4: Quantitative phytochemical constituent of *P. alliacea* parts

Phytochemicals	Different parts of *P. alliacea*			
	Leaf	Inflore-infructescence	Stem bark	Root
Tannins	3.30 ± 0.26	5.71 ± 0.01	2.28 ± 0.01	4.03 ± 1.16
Saponins	7.09 ± 0.26	23.27 ± 0.26	23.73 ± 0.39	25.36 ± 1.16
Flavonoids	0.45 ± 0.01	0.98 ± 0.01	1.09 ± 0.01	1.52 ± 0.02
Terpenoids	14.84 ± 0.04	3.14 ± 0.04	15.70 ± 0.06	19.20 ± 0.04
Steroids	12.23 ± 0.02	5.36 ± 0.02	ND	2.37 ± 0.11
Glycosides	ND	44.63 ± 0.05	9.98 ± 0.07	12.15 ± 0.05
Alkaloids	ND	39.75 ± 0.04	ND	ND

Values are presented as ± Standard deviation (SD) of duplicate determinations

Where ND= Not detected

IV. CONCLUSION AND FUTURE SCOPE

This study unveiled the various parts of *P. alliacea* to be garners of phytochemicals with the inflore-infructescence and the root taking the lead. The opened-up quantities of phytochemicals in each of the parts of *P. alliacea* is *sine qua non* to the purpose (s) to which member (s) of its parts could be pharmacologically explored. The inflore-infructescence of *P. alliacea* present cornucopia arrays of medicinally important phyto-chemicals and despite the lowest SPY of the leaf and inflore-infructescence in this study, the leaf is still recommended for study where large quantity of extract is needed. The unbeatable advantage of the leaf and the inflore-infructescence over all the other parts is their ability to sprout again and again after much repeated plucking. The harvest of the roots and at times the stem-bark terminate the life span of the plant concerned and should therefore be sparingly involved unless under utmost necessity or expediency. It can therefore be concluded that all the parts of *P. alliacea* are rich sources of phytochemicals (with special reference to the inflore-infructescence ampleness in glycosides and alkaloids) which might be responsible for its efficacy in prophylactic and therapeutic management of ailments in traditional herbal medicine.

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