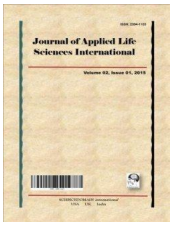

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Phytochemical Studies, Antioxidant Properties and Development of Dye Indicator from *Aspilia africana* Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Author HCCM designed the study, wrote the protocol and read the first draft. Author CEU managed the literature search and wrote the first draft of the manuscript; Authors ANO and PNO read the first draft; Authors CCD and COO did the statistical analysis, and read the first draft; Author CCIA carried out the laboratory analysis and managed the data. All authors read and approved the final manuscript.

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ABSTRACT

Background: Synthetic indicators are reported to be costly and could be toxic to both the user and the environment. This has necessitated the need for an alternative from natural sources. This study aimed to extract and apply natural dye as an indicator in acid-base titration and also to identify the phytochemical and antioxidant constituents of *Aspilia africana* flowers.
Methods: The indicator dye was extracted in ethanol and tested against standard phenolphthalein as an acid-base indicator. The antioxidant vitamins and phytochemical screening of the leave extract were also determined using conventional methods.

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Results: The acid-base titration of the extracted dye and the standard indicator showed titer values that compared favorably with the universal indicator. The results showed that the concentration of the antioxidant vitamins in the leave extract decreased in the following order vitamin c >vitamin E > vitamin A. The results also showed the presence of alkaloids, flavonoids and saponins.
Conclusion: The present study shows that the dye from *Aspilia africana* can serve as a good acid-base indicator. The study has verified the usefulness of *Aspilia africana* for phytochemical and medicinal purposes.

Keywords: Aspilia africana; dye; indicator; phytochemical; antioxidant.

1. INTRODUCTION

The phytochemical screening of medicinal plants has been the primary focus of many researchers in biological sciences in recent times [1]. The therapeutic values of plant is dependent on its chemical constituents that elicit definite physiological actions *in vivo* [2]. Some of these phytochemicals are alkaloids, saponins, flavonoids, tannins and phenols among others.

Aspilia africana is a bristle herbaceous plant that grows to about one meter tall [3]. Its wound healing [3] and morphological characteristics have been reported [4]. The pharmacological properties of the plant have been previously reported [3,5,6]. The phytochemical constituents of the plant leaves show the presence of alkaloids, tannins, saponins, flavonoids, and phenols. It is also reported to contain ascorbic acid, niacin, thiamine, and riboflavin [7].

Dye is a soluble compound which can be synthetic or natural. There are many reports on the extraction of dyes from different flowers of local plants [8-10] which can be used for different purposes. There are also reports on the use of some natural dye extracts as indicators in acid-base titration [11,12]. Synthetic indicators are costly and could be toxic to its users and the environment [13,14]. Some of the synthetic indicators have harmful effects on the users including diarrhea, pulmonary edema, and pancreatitis and could lead to abdominal cramps, dermatitis and environmental pollution [13, 15]. The toxicity notwithstanding, dye effluents, in addition, possess chemicals that are carcinogenic, mutagenic or teratogenic to many organs [16,17]. In addition, the direct dumping of untreated dye effluents resulting from synthetic dye manufacturing in water bodies can lead to water pollution. Natural dyes on the order hand, cause a minimal environmental hazard. They are renewable, safe and will not cause health challenges when unavoidably ingested. As result efforts are now towards sourcing for indicators from natural plants since they will be cheaper, easy to prepare and much more pollution friendly [8,14]. Some reports have shown that hibiscus flower extract can replace phenolphthalein as an indicator for weak base-strong acid titration [8] while dye extract from ginger can favorably replace methyl orange indicator [9]. *Aspilia africana* is a perennial herb with high medicinal value locally. To exploit the brightly colored yellow flowers of the plant and to expand the frontiers of research we sought to extract the dye present in the flowers. This study aimed to extract and apply natural dye as an indicator in acid-base titration and also to identify the phytochemical and antioxidant constituents of *Aspilia africana* flowers.

2. MATERIALS AND METHODS

2.1 Sample Collection and Treatment

Fresh leaves of *Aspirilia africana* were collected from a bush in Anambra State. The leaves were detached from the branches and cut into pieces, air dried at room temperature, powdered with a manual grinder and stored in airtight container.

2.1.1 Chemicals and reagents

All the chemicals and reagents used in the study were of analytical grade. They were purchased from the British Drug House (BDH) Poole England and Sigma Aldrich Chemical Co. Inc. Milwaukee Wis., U.S.A. They include analytical grade H_2SO_4, diethyl ether, acetic acid, tannic acid, gallic acid, HCl, ferric chloride, sodium sulphate, Na_2CO_3, absolute ethanol, and n-butanol.

2.2 Extraction of Indicator Dye

This was as described by Eze and Ogbuefi [14]. Ten grams of the ground leaves were soaked in 30ml of ethanol and allowed to stand for 3 hours. The reaction mixture was vortexed for 5 minutes and filtered using Whatman number 1. The

filtrate was put in a beaker and allowed to evaporate to get a more concentrated solution.

2.3 Experiment with Natural Indicator

The acid used was 0.1M HCl inside a 25ml burette which was initially rinsed with the acid. The base used for the titration was 0.1M NaOH and three drops of the dye as an indicator. The acid was titrated against the base until the neutralization point was reached. Standard indicator phenolphthalein was used as the control using 5ml of the base 0.1M NaOH and 0.1M Hcl in a 25ml burette and three drops of the conventional indicator. The experiment was conducted at room temperature in triplicate.

2.4 Quantitative Phytochemical Screening of the Leaves

2.4.1 Alkaloid determination

The alkaline precipitation gravimetric method was used [18]. Five grams of the processed sample was dispersed in 100mls of 10% acetic acid in ethanol solution. The mixture was shaken well for 30 minutes and allowed to stand for 4 hours at room temperature after which it was filtered using Whatman number 45 grade filter paper with the known weight(W). The filtrate was concentrated by evaporation to a quarter of its original volume and was treated with dropwise addition of concentrated ammonia solution to precipitate the alkaloid. Dilution was done until the ammonia was in excess. The alkaloid precipitate was removed by filtration using Whatman filter paper. After washing with 1% NH_4OH solution, the precipitate in the filter was dried at 60^{o}C and allowed to cool in a desiccator and weighed. The alkaloid content was calculated thus;

Percentage alkaloid = $(W_2 - W_1 \times 100)/W$

W = Weight of sample
W1 = weight of empty filter paper.
W2 = weight of filter paper + alkaloid precipitate

2.4.2 Tannin determination

The Folin-Denis spectrophotometric method was used as described by Pearson [19] and reported by Onwuka [18]. A measured weight of the sample (1.0 g) was dispersed in 10 ml distilled water and agitated. This was left to stand for 30 minutes at room temperature, being shaken every 5 minutes. At the end of the 30 minutes, it was centrifuged and the extract gotten. Then 2.5 ml of the supernatant(extract) was dispersed into a 50 ml volumetric flask. Similarly, 2.5 ml of the standard tannic acid solution was dispersed into a separate 50 ml flask. A 1.0 ml Folin-Denis reagent was measured into each flask, followed by 2.5 ml of saturated Na_2Co_3 solution. The mixture was diluted to mark in the flask (50 ml),, and incubated for 90 minutes at room temperature. The absorbance was measured at 250 nm. Readings were taken with the reagent blank at zero. The tannin content was given as follows:

Percentage tannin = (An × C × 100 × Vf)/As × W × Va

An = absorbance of test sample, As = absorbance of standard solution, C = concentration of standard solution, W = weight of sample used, Vf = total volume of extract, Va = volume of extract analyzed.

2.4.3 Saponin determination

This was done by double solvent extraction gravimetric method [20]. Fifty grams of the processed sample was mixed with 50ml of 20% aqueous ethanol solution and incubated for 12 hours at 55^{o}C with constant agitation. The mixture was filtered through Whatman filter paper number 1. The residue was re-extracted with 50ml of ethanol solution for 30 minutes, and the extract weighed. The combined extract was reduced to about 40 ml by evaporation and transferred to a separating funnel and equal volume (40 ml) of diethyl ether was added to it and vigorously shaken. The aqueous layer was separated carefully after settling down the solution. The purification process was repeated again. Saponin in the extract was taken up in excess extraction with 60 ml and 30 ml portions of n-butanol. The precipitate was washed with 5% NaCl solution and evaporated to dryness in a previously weighed dish. The saponin was dried in the oven at 60^{o}C (to remove residual solvent), cooled in a desiccator and re-weighed. The saponin content was calculated as shown below;

Percentage of saponin = $(W_2 - W_1)/W$

W = weight of the sample
W1 = weight of empty evaporation dish
W2 = weight of dish plus saponin extract

2.4.4 Flavonoid determination

The total flavonoid content of the extract was evaluated as reported by Boham and Kocipal

[21]. Five grams of processed sample was boiled in 10ml of HCl solution under reflux for 40 minutes and allowed to cool and filtered. The filtrate was heated with an equal volume of ethyl acetate and the mixture was transferred to a separate funnel. The flavonoid extract contained in the ethyl acetate portion was recovered by filtration using weighed filter paper. The weight was obtained after drying in the oven and cooling in a desiccator. The flavonoid content was calculated as shown below

Percentage flavonoid = $(W_2 - W_1)/W$

W = weight of the sample
W1 = weight of filter paper
W2 = weight of filter paper and flavonoid precipitate.

2.4.5 Total phenol determination

The total phenol content was estimated as reported by Obadoni and Ochuko [22]. A fat-free extract was prepared by mixing 5g of crude extract with 100 ml n-hexane and defatted using a soxhlet apparatus for 2 hours. The resultant extract was used for the assay of total phenol content. The defatted extract was boiled with 50 ml of ether for 15 minutes. The resulting solution was filtered and 5 ml of the filtrate was pipetted into a 50 ml flask. Ten ml of double distilled water was added to it then followed by addition of 2 ml of NH_4OH solution and 5 ml of concentrated amyl alcohol solution with constant stirring. For color development, the solution was incubated for 30 minutes at room temperature. The absorbance of the solution was read at 550 nm against a suitable blank. The phenolic content was evaluated from a gallic acid standard curve.

2.5 Estimation of Vitamin A

This was as described by Okerulu et al. [23]. Twenty (20 g) of the sample was weighed into a 250 ml conical flask into which a mixture of acetone and low boiling petroleum ether (1:1v/v) was added to the sample. The mixture was shaken for 3 hours with the aid of a shaker. The mixture was allowed to cool then filtered and 2ml trichloroacetic acid added. The absorbance was obtained using UV/Visible spectrophotometer at 390nm with acetone and diethyl ether blank. The concentration of vitamin A was calculated using the formula:

Concentration of vitamin A = (Absorbance x volume of extracted sample x dilution factor)/(Volume of curvette x volume of sample used)

2.6 Estimation of Vitamin C

Twenty (20 g) of the sample was weighed into a 250 ml conical flask. One hundred (100 ml) of 20% aqueous ethanol was poured into the sample and allowed to stand for 3 minutes. The mixture was shaken continuously for four hours and allowed to stand overnight. The sample mixture was filtered with Whatman filter paper number 1. The filtrate (50 ml) was measured into a conical flask followed by the addition of 10 ml concentrated tetraoxosulphate (VI) acid and 20 drops of 1% starch indicator. The sample mixture was then titrated with 0.05N iodine solution until a blue-black color was seen. The concentration of vitamin C was calculated thus [23]:

Concentration of vitamin C = (Titre value x 0.00886x N x 1000)/Volume of sample used

2.7 Estimation of Vitamin E.

The vitamin E content of the extract of *Aspilia africana* was determined as described by Agiang et al. [24]. One gram (1 g) of the extract was weighted into a 250 ml conical flask containing 10 mls of absolute alcohol and 20 mls of 1M alcoholic sulphuric acid. The condenser and flask were wrapped in aluminum foil and refluxed for 45 minutes and then cooled for 15 minutes. A volume of 50 mls distilled water was added to the mixture and transferred to a 250 ml separating funnel covered with aluminum foil. The unsaponifiable matter in the mixture was extracted with 30 mls of dimethyl ether. The combined extracts were washed free of acid and dry-evaporated at a low temperature and the residues obtained, immediately dissolved in 10mls of absolute alcohol. Aliquots of solutions of each sample and standards (0.3-3.0 mg vitamin E) were transferred into a 20ml volumetric flask and 5 ml absolute alcohol added, followed by a careful addition of 1 ml concentrated HNO_3. The flask was allowed to cool rapidly under running water and volume was adjusted with absolute alcohol. The absorbance was read at 470nm against a blank solution containing 5mls absolute alcohol and 1ml HNO_3 treated in a similar manner. The vitamin E content was calculated thus:

Vitamin E (mg/100g) = Absorbance of sample × Dilution factor × gradient factor/weight of sample

2.8 Statistics

Results obtained were subjected to analysis using SPSS version 20.0. The results were presented as mean ± SD and difference in mean were compared using ANOVA at a probability threshold P=0.05.

3. RESULTS

The results of the phytochemical component of the leaves of *Aspilia africana* are shown in Table 1. The results show copious presence of alkaloids, flavonoids, and saponins with fewer amounts of tannin and phenol. The results show that the plant is capable of exerting biological effects. The results also show that the leaves contained vitamins A, C and E suggesting that the plant extract has antioxidant which can be exploited in the management of oxidative stress (Table 2). The acid-base titration of the extracted dye and the standard indicator phenolphthalein showed titre values that could compare favorably with the universal indicator (Table 3).

Table 1. The phytochemical components of *Aspilia africana*

Parameter	Concentration (DW%)
Alkaloid (mg/g)	5.79 ±0.17
Flavonoid (mg/g)	2.35 ±0.15
Phenol (mg/g)	0.10 ±0.00
Saponin (mg/g)	2.44± 0.37
Tannin (mg/g)	0.16 ±0.14

Results are mean± standard deviation of two replicates

Table 2. The antioxidant vitamins in *Aspilia africana* leaf extract

Parameter	Concentration (DW %)
Vitamin A (mg/100 g)	10.92±0.00
Vitamin C(mg/100 g)	24.91± 12.01
Vitamin E(mg/100 g)	20.37 ±0.09

Results are mean± standard deviation of two replicates

4. DISCUSSION

The phytochemical screening carried out with *Aspilia africana* leaves revealed the presence of alkaloids, saponin, flavonoids, tannin, and phenol which is in agreement with the study of Obadoni and Ochuko [22] that identified saponins and tannins to be the major phytochemicals in the leaves. Alkaloids have the highest concentration of 5.79% followed by saponins with 2.44% and flavonoids with 2.35%. Tannins have 0.16% and phenols have 0.16%.

The phytochemical analysis on *Aspilia africana* leaves shows that the leaves are very rich in alkaloid and saponins which are known to have antimicrobial activities [7]. It contains flavonoids which are super antioxidants that provide protection against oxidative cell damage [25] and against allergies, viruses, ulcers, and inflammation [26]. Terpenoids have been isolated from the leaves while sesquiterpenes and monoterpenes were also isolated from the essential oil of the leaves [27]. Phenols and their oxidative products are corrosive to living bacteria cells and are considered to be potentially toxic to the growth and development of pathogens. Tannins have astringent properties which hasten the healing of the wound and inflamed mucous membrane. This is due to their ability to bind to the protein of exposed tissue and precipitating the protein. This then forms a mild antiseptic protective coat under which regeneration of new tissues take place leading to wound healing [25]. The presence of these phytochemicals explains why the leaves of *Aspilia africana* is used by tradomedical practitioners to stop bleeding, cure wound, allergies, rheumatism, inflammation and ulcer. Our results showed that alkaloid content of our extract (5.79±0.17 mg/100 g) was higher than results obtained by Oko and Agiang [1] (4.27 mg/100 g) and much higher than results of Okwu and Josiah [28] (1.24±0.11mg/100g). We also observed higher concentration of flavonoid and tannins than previous reports [1,28] while having a lower concentration of phenol in our study relative to previous studies. The phytochemical result was in line with results of Abii and Onuoha [7] while the vitamin content was higher in this study. These differences in the concentrations of the phytochemicals could be because of the use of different extraction solvents. The identified constituents are reported to exhibit strong antioxidant scavenging activity for radicals involved in the lipid peroxidation [29]. Polyphenol compounds have an essential role in preventing lipid peroxidation and are also involved in antioxidant activity [30]. Phytochemicals such as flavonoids, phenols, alkaloids, and tannins are well known free radicals scavengers and possessing multiple biological activities including antioxidant activity [31].

The vitamin composition reveals the presence of a high concentration of vitamin C which is reported to be implicated in the synthesis of anti-inflammatory steroids and also in wound healing. The presence of vitamins A C and E in reasonable concentrations in the flower extract

Table 3. The acid-base titration of *Aspilia africana* leaf dye and standard indicator

Titration (titrate vs titrant)	Strength in moles	Indicator	Mean±SD
HCl vs NaOH	0.10±0.04	*Aspilia africana*	5.00±0.30
Hcl vs NaOH	0.10±0.06	Phenolphthalein	4.76±0.34

Results are mean± standard deviation of two replicates

showed that the extract can boost endogenous antioxidant capacity that has been reported to lower the chance and or incidence of oxidative damages linked with oxidative stress. Vitamins C E and flavonoids (phenols) are the main exogenous antioxidants that are available in plants [32]. Vitamins A, C, and E are antioxidant vitamins due to their roles in scavenging free radicals. They are used as a standard in antioxidant assay because of their roles as antioxidants. The presence of these vitamins in *Aspilia africana* makes it an important tool in herbal medicine and helps to account for its use in wound healing, treatment of skin disease and eye infection [25]. The dye extracted from *Aspilia africana* leaves was screened for its use as an acid-base indicator in acid-base titrations and the result compared with the standard. This was done using strong acid and strong base (HCl and NaOH) with 0.1M strength of acid and alkalis. At the equivalent point, the indicators were able to effect a sharp color change.

5. CONCLUSION

The present study has verified the usefulness of leaves of *Aspilia africana* for phytochemical and medicinal purposes due to the presence of antioxidant compounds in the flower. It has also verified that the dye gotten from the plant can serve as a good acid-base indicator and may be used because of its economy, simplicity, safe to human and availability.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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