AUTHENTICATION OF SOME FLORA SPECIES USING BASIC PHYTOCHEMICAL PROTOCOL AND THE NEED FOR CONSERVATION AROUND DENNIS OSADEBAY UNIVERSITY ANWAI, ASABA DELTA STATE-NIGERIA

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ABSTRACT: Plant species are the most useful biotic elements of the universe appearing in diverse life forms. These species are responsible for food, medicine, oxygen supply, ecosystem moderation and balancing in most cases. Plant species are constantly being destroyed for reasons best known to man. In addition to high rate of speciation in the tropics, habitat destruction, and overexploitation making the need for conservation all the more compelling. Whereas, the paucity of plant taxonomist with its inherent drawbacks constitutes key challenges to these species' conservation, as lack of proper species information can propel destruction. The species under study includes: *Myrianthus arboreus* P.Beauv, *Spondias mombin* Linn, *Tridax procumbens* Linn., *Newbouldia laevis* (L) , *Azadirachta indica* A. Juss and *Ixora coccinea* Linn.. The aim of the study was to establish authentication protocol for these species using basic phytochemical fingerprints, in the phase of inorganic reagents and to produce a taxonomic key to be used in authenticating species *in situ* and *ex situ*. These species information was obtained and documented, while the leaves were harvested, washed under running water, air dried and blended to powder for the purpose of their phytochemistry. Ten (10) grams of samples each were soaked in absolute ethanol for 24 hours, filtered, subjected to three routine phytochemical test methods. The changes observed in extracts were used in species authentication and construction of taxonomic key. The economic benefits derived from these species predispose them to overexploitation. Accordingly, sensitization campaign, seed banking, and habitat conservation is encouraged to enhance the conservation of these species in the Niger Delta region.

Key words: *Authentication, Basic phytochemical protocol, Conservation, Flora species, Taxonomic key*

INTRODUCTION

Plant species are among the most abundant and diverse organisms on earth, responsible for the oxygen we breathe, food supply, local climate regulation, carbon sink and air purification (Sharma, 2019). These all-important species have become poorly managed within the environment (Ahern *et al*., 2001), irrespective of their ecosystem service advantage (Gretchen *et al*., 1997). Plant species take off ample kilograms of carbon dioxide from the environment that is supposed to escape into the atmosphere, resulting in ozone layer depletion, deleterious to human health (Sharma, 2019). The destruction of these flora species may be attributed to ignorance on species ecosystem service advantage, which is implicated in the paucity of plant taxonomist within the country (Ahern *et al*., 2001). The reduced number of expert curators and taxonomists among the populace is referred to as "taxonomic crisis" (Dayrat, 2005). Nigeria like other third world countries is fraught with the challenge of species unsustainable management that is lethal to biodiversity. Going forward, proper species authentication will on the other hand reduce this menace that is grave to flora species existence while enacting effective laws for species conservation because every organism possesses the right to exist according to The General Assembly World Charter for Nature, (1982) and the U.S Endangered Species Act, (1973). Consequently, while plant taxonomy is the branch of science that deals with species authentication and identification, this discipline achieves this great task through observed differences recorded between species under investigation (Ebigwai *et al*., 2020). Furthermore, plant taxonomy utilizes different markers in plant authentication/identification, ranging from morphology, anatomy, cerology, palynology, molecular, cytology and phytochemistry (Akomaye *et al*., 2023; Ebigwai *et al*., 2020). While other markers have been extensively utilized for species authentication, phytochemistry has not been effectively utilized but yields abundant data for this purpose (Daiane and Cecilia, 2015). In addition, while other markers are easily influenced by environmental factors, pest and diseases among others, phytochemistry and molecular markers are not easily influenced (Akomaye *et al*., 2023; Eigwai *et al*., 2020; Daiane and Cecilia, 2015), making them extremely fit for taxonomic data gathering/generation and plant species authentication purposes. In addition, Daiane and Cecilia, (2015) recorded abundance of phytochemical among some Rubiaceae members in their active ingredient profiling among some selected species. This confirms the fact that plant species are endowed with potent active ingredients which makes a species unique in comparison with others. Accordingly, these active ingredients differ between species of the same genus, and more in species of different families. Every plant species subjected to phytochemical screening, yields important source of taxonomic data as plant taxonomy is always interested in differences observed between species to enable authentication. Meanwhile, these potent active ingredients have not found usage in species authentication.

Furthermore, to enhance these species conservation, certain natural environmental challenges need to be understood and managed to achieve success. These natural environmental stressor elements include: erosion, volume change of materials within a habitat, dissolution of a material and the associated chemical changes, as well as biological processes (Moncmanová, 2007). Conversely, the anthropogenic activities that influence species conservation within an environment may include: poverty, ignorance, hunger and unemployment. Accordingly, when people are hungry and unemployed, they go any length to destroy flora species for financial values without considering the consequences thereof. This fact is consistent with the saying "A hungry man is an angry man". The unemployed youths within any environment mount great pressure on flora community just to make ends meet, with detrimental consequences on biodiversity. As such, the government should create jobs to enhance quality of life otherwise; conservation of natural

resources around Dennis Osadebay University, Asaba will always be compromised by the locals who have no other sources of livelihood.

It is in the light of these inherent challenges and drawbacks that this study was designed to establish a rapid and standardized authentication method suitable for field and off field studies using plant extract in the presence of inorganic reagents for observed differences to be utilized in species authentication and the construction of taxonomic keys.

Study methodology

Sample collection and preparation

Fresh leaves of six (6) flora species in six taxonomic families were harvested around Dennis Osadebay University Anwai, Asaba Delta State-Nigeria, and identified to species level using relevant literature (Keay, 2011). This task was carried out by a curator in the Department of Plant Science and Biotechnology, Dennis Osadebay University Anwai, Asaba Delta State. Flora species information was offered by the curator and presented in (Table 1). The samples were washed separately under running water in chemistry laboratory of the same institution. Samples were air-dried for sixty (60) days and ground to powder using electrical blender in faculty of Agriculture laboratory. The samples were then soaked in absolute ethanol for 24 hours, filtered through Whiteman's filter paper for utilization in basic phytochemical test.

Table 1: Flora species information with their conservation status and uses

Species name	Common Names	Family Names	Conservation Status	Local Names	Species Use
Myrianthus arboreus P. Beauv	Giant Yellow Mulberry	Urticaceae	LC	Ujuju	Fruit, vegetable and medicine
Spondias mombin Linn	Yellow Mombin/Hog Plum	Anacardiaceae	LC	Iyeye'/Akika	Oxidative stress reduction, fruit, live fence and fodder
Tridax procumbens Linn.	Coat Button	Asteraceae	NA	Igbalobe	Fodder and production of olive oil
Newbouldia laevis (L)	Boundary Tree	Bignoniaceae	LC	Aduruku'/Akoko	Live fence, fertility in women, sacred sites
Azadirachta indica A. Juss	Neem	Meliaceae	LC	Dongoyalo	Malaria, kidney treatment, timber and firewood
Ixora cocci	Flame of the Woods	Rubiaceae	NA	-	Ornamental and live fence

nea Linn.	

Key: NA=Not Assessed; LC=Least Concern.

Basic phytochemical tests

The plant extracts were subjected to chemical test adopting the standard methods for basic phytochemical tests (Table 2). The sampled flora extracts in transparent test tubes were observed visually for color change after introducing the inorganic reagent at specified concentration. Color change in extracts was recorded by matching observed colors with online color charts. The color of the ethanolic extract before treatment was noted as "initial color" while the colors after treatment were recorded as "final color" for utilization.

Table 2: List of the phytochemical test methods conducted

SN	Test conducted	Protocol
1	Flavonoid's test	Four (4) droplets of sulphuric acid (H_2SO_4) were mixed with 2 ml of the sample. Appearance of orange color confirms flavonoids in the sample (Wadood *et al*., 2013)
2	Coumarins test	The (3) ml of 10 % sodium hydroxide (NaOH) were mixed with 2 ml of sample. Yellow colour confirms coumarins in the sample (Wadood *et al*., 2013)
3	Anthocyanins	Precisely 1 ml of ammonia and 1 ml of hydrochloric acid (HCL) were mixed with 2 ml of extract. Early appearance of pink-red color turning into blue-violet indicates anthocyanins in the sample (Wadood *et al*., 2013)

Results

The results of basic phytochemical tests on ethanol extracts were presented in Table 3. The six (6) plant extracts presented different actions (color change) on flavonoids, coumarin and anthocyanin tests methods. Similarly, the extract of *M. arboreus* P. Beauv and *S. mombin* Linn showed unique colors in Coumarin tests that led to their authentication just as *T. procumbens* Linn., *A. indica* A. Juss, *N. laevis* (L), and *I. coccinea* Linn. exhibited peculiarities on anthocyanin tests that led to their authentication in the phase of inorganic reagent, useful for the design of taxonomic keys.

Based on the results obtained in Table 3, a dichotomous taxonomic key was designed for authentication of the species under study:

1a Sample extract + flavonoid test, light green color formed

- - - - - - - - - -Go to 2

1b. Sample extract + flavonoid test, brown color formed

- - - - - - - - - -Go to 3

2a. Lime + green ring in coumarin test

- - - - - - - - - - *M. arboreus* P. Beauv

2b. Light yellow + slight ring in coumarin test - - - *S. mombin* Linn

3a. Sample extract + coumarine test, yellow color formed -Go to 4

3b. Sample extract + coumarine test, green color formed -Go to 5

4a Sample + Anthocyanin test, pink red + slight blue color formed

- - - - - - - - - - *T. procumbens* Linn.

4b Sample extract + Anthocyanin test, honey color formed

- - - - - - - - - - *A. indica* A. Juss

5a. Sample extract + Anthocyanin test, blue color formed at meniscus

- - - - - - - - - - *N. laevis* (L)

5b. Sample extract + Anthocyanin test, rust red and pink color formed at bottom

- - - - - - - - - - *I. coccinea* Linn.

Table 3: Color change in the study plant's extracts on basic phytochemical test

Species	*M. arboreus* P. Beauv	*S. mombin* Linn	*T. procumbens* Linn.	*N. laevis* (L)	*A. indica* A. Juss	*I. coccinea* Linn.
Initial Color	Dark Green	Light Green	Olive	Light Green	Green	Light Green
Flavonoid Test	Light Green (-)	Light Green (-)	Brown (-)	Green (-)	Brown (-)	Orange (+)
Coumarin Test	Lime + Green ring formation (-)	Light Yellow + Slight ring formation (+)	Mustard Yellow (+)	Fresh Green (-)	Camel Yellow (+)	Dark Green + Chocolate red ring at meniscus (-)
Anthocyanin Test	Dark Brown (-)	Forest Green + Pink red at meniscus (+)	Pink red + Slight blue color (+)	Blue at meniscus (+)	Honey color (-)	Rust red and Pink at the bottom (+)

Key: (+ =Phytochemical present, - = Phytochemical absent).

Discussion

The changes in physical property of plant extract when exposed to inorganic reagent is a function of its chemical contents, which could serve as valuable chemotaxonomic marker for species authentication (Daiane and Cecilia, 2015; Amita, and Shalini, 2014). Going forward, the exhibition of unique behavior among phytochemical fingerprints in voucher specimens or plant extract could present a quick, time-saving and/or rapid method for species authentication, as taxonomic data acquisition depends mostly on observed differences among sample species to enhance authentication (Jaiswal, *et al.*, 2014).

In this research, the phytochemical fingerprint of six (6) plant species in six (6) taxonomic families authenticated to species level using relevant literature (Keay, 2011) as: *M. arboreus* P. Beauv (Giant Yellow Mulberry), *S. mombin* Linn (Yellow Mombin/Hog Plum), *T. procumbens* Linn. (Coat Button), *N. laevis* (L) (Boundary Tree), *A. indica* A. Juss (Neem) and *I. coccinea* Linn. (Flame of the Woods) were investigated to establish differences that are useful for species discrimination. When sample extracts were independently subjected to basic phytochemical test methods, specimen was discriminated using the taxonomic keys drawn based on flavonoid, coumarin, and anthocyanin tests.

Similarly, the six (6) test plants' extracts showed unique behavioral actions on coumarin test, implying that the test plants possess different quantities of coumarin and anthocyanin phytochemicals profiles (Daiane and Cecilia, 2015). This on the other hand, explains why flavonoids active ingredients could not be utilized for species discrimination. As such, the aforesaid active ingredient was only recorded in *I. coccinea* Linn (Flame of the Woods).

Conversely, the presence of coumarin and anthocyanin active ingredients in sample species explains why flavonoids were not effectively utilized for species authentication. The presence of diverse phytochemical groups among sample species have been reported (Daiane and Cecilia, 2015; Jaiswal, *et al.*, 2014; Wu *et al.*, 2014; De *et al.*, 2014; Yang *et al.*, 2013; Eswaraiah and Elumalai, 2011; Dinda *et al.*, 2008; Silva *et al.*, 2008), but these potent active ingredients have not found usage in species authentication. But their presence in sample species for this study was utilized for species authentication as a quick and rapid method. Whereas, morphological authentication among other taxonomic markers is fraught with error due to certain environmental challenges, leading to the rearrangement of species structural morphology. This on the other hand presents challenges to young curators and scholars in the field leading to species identity misplacement if not carefully managed. However, molecular, anatomical, cerological, palynological and cytological authentication methods are quite expensive. This claim was confirmed some authors (Akomaye *et al.*, 2023; Ebigwai *et al.*, 2020) when determining accurate nomenclature of five Ixoroideae subfamily members using DNA Barcoding. The implication of this basic phytochemical approach implies that a cheaper but accurate and fast means of species authentication is discovered through basic authentication methods. This method of study presents a scenario where test plant extracts will be subjected to inorganic reagents for the changes elicited to be utilized in species authentication. With this quick authentication method, flora species conservation would be enhanced since plants with known identity are likely to be conserved and managed.

In addition, among the sample flora species, flavonoid was present in *I. coccinea* Linn. (Flame of the Woods) while other species recorded the absence of flavonoid in sample extract (Table 3). Contrariwise, *S. mombin* Linn (Yellow Mombin/Hog Plum), *T. procumbens* Linn. (Coat Button), and *A. indica* A. Juss

(Neem) recorded the presence of coumarin in sample extract while other species lack the presence of this active ingredient. The presence of potent phytochemical in sample extract owe promise to the use of flora species in pharmaceutical industries for drug production, which will as well lead to sources of income for Dennis Osadebay University Anwai, Asaba Delta State. Due to the phytochemical values of these plant species sampled around the project area, efforts should be made for this plant species conservation in order to ensure sustainability around the University community. Accordingly, projects around the University community should be conducted in line with International Best Practice (IBP), a situation where Environmental Impact Assessment (EIA) projects are conducted around the University community before the commencement of any building project, just to enhance the conservation of gene pool.

Furthermore, the presence of these active ingredients in plant extracts reaffirms the potent use of this flora sample for the treatment of different ailments and in the management of oxidative stress (Adu *et al*., 2017; Alzohairy, 2016). In addition, anthocyanin compound was recorded in four different sample extracts namely: *S. mombin* Linn (Yellow Mombin/Hog Plum), *T. procumbens* Linn. (Coat Button), *N. laevis* (L) (Boundary Tree), and *I. coccinea* Linn. (Flame of the Woods) while others lack this active ingredient. The presence of these active ingredients in the presence of inorganic reagent led to unique behavior which was useful in species authentication. Meanwhile, these active ingredients if extracted may be utilized in pharmaceutical industries according to the findings of Adu *et al*., (2017). Accordingly, for the purpose of chemotaxonomic marker, useful for species authentication, these active ingredients have been exposed for utilization in the pharmaceutical industries.

Be that as it may, species with similar behavior in the presence of inorganic reagents are closely related (Gorthi *et al*., 2017; Nishimura *et al*., 2016; Singh, 2016; Ndam *et al*., 2014; Mongrand *et al*., 2004; Rockenbach *et al*., 1992). The findings of the basic phytochemical test showed that sample species contains exceptional active ingredient that compel them to maintain unique behavior in the presence of inorganic reagents, a pointer to non-relatedness among study samples (Ndam *et al*., 2014; Tigoufack *et al*., 2010). The basic phytochemical test has also led to the development of taxonomic keys for field and off field species authentication with great taxonomic benefits. Students of plant science will explore this quick and rapid means of authentication to solve plethora of flora identity misplacement among voucher specimen in the herbarium and field studies. In addition, sensitization campaign, seed banking and habitat conservation is encouraged in order to protect and enhance sustainability around the fragile ecosystem of the Niger Delta.

Conclusion

The findings of this study revealed that basic phytochemical test was utilized for rapid species authentication. This method of study is an excellent alternative in plant species authentication as all the sampled flora species subjected to this method were authenticated based on the differences elicited in the presence of inorganic reagent (color change), evident in the construction of taxonomic key using species chemical finger prints. This method is cheap and rapid compared to other methods of species' authentication.

Recommendation

Further basic phytochemical studies should be carried out in the study samples in order to validate the findings of this research

Laws should be made against indiscriminate tree destruction around the project area while recruiting the unemployed who destroy trees to function as conservation personnel, in order to enhance conservation around Dennis Osadebay University Asaba Delta State.

Authors' Contributions

D.A.O was the project leader who conceptualized, designed, supervised and wrote the manuscript. O.I.A made conceptual contributions; E.K and E.O provided laboratory reagents and assisted in the research work. O.I.K made literature search and edited the manuscript.

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Competing interest

Authors declared that there is no competing interest that may have resulted in the writing of this manuscript.

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