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PRELIMINARY STUDIES ON THE PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT PROPERTIES OF FOUR MEDICINAL PLANTS IN NIGERIA

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ABSTRACT

***Purpose**: The innumerability of un-researched medicinal plants has occasioned their exceptional and unlimited study. Without a continuous study, the importance and use of many medicinal plants will be spuriously occluded and hence marshal untold limitations to their medicinal value. This research therefore was conducted to unravel the phytochemical constituents and antioxidant activities of Petiveria alliacea L, Hoslundia opposita Vahl, Alternanthera brasiliana (L.) Kuntze, and Solanum erianthum D. Don.*

***Methods**: Standard laboratory procedures were dutifully followed and ensued data were analyzed using SPSS, version 23, 32 and 64 bit.*

***Results**: The four analyzed plants were rich sources of major phytochemicals like: tannins, terpenoids, steroids, saponins, phenolics, flavonoids and cardiac glycosides. All the plants also demonstrated high antioxidant activity in DPPH, Fe2+ Chelation, FRAP and Peroxides assays. The best antioxidant activity and IC50 values were obtained from S. erianthum which may be attributed to the highest quantity of phenols and flavonoids it contained in comparison to the rest evaluated plant samples.*

***Conclusion**: It was inferred that the observed high antioxidant activity of all the plant samples may be predicable to their plentious quantified phytochemicals. The plants were thenceforth recommended for further study on their acute toxicity (LD50) and with special emphasis on S. erianthum.*

INTRODUCTION

It has been asserted and confirmed over time that plants are very useful in the healthy survival of animals and humans because of many reasons imputable to the inherent constituents of their chemicals popularly called phytochemicals (Onuminya et al., 2017). Phytohemicals are chemicals that occur naturally in plants and are very useful in the defensive mechanism of the plants against pathogens. These phytochemicals are also useful to man as they contribute immensely to good immunological response and many biological activities in the body relating to sound health (Hasler and Blumberg, 1999). Plants also possess antioxidant property (Edewor et al., 2021) that helps the body to rid off free radicals and reactive species developing in living

tissues. Free radicals are a group of molecules capable of independent existence with unpaired electrons in their atomic orbital. These unpaired electrons result in certain common properties that are peculiar to most radicals. It has been reported that many of these radicals are unstable and highly reactive. They can donate electrons or receive electrons thereby manifesting the character of oxidants or reductants (Cheeseman and Slater, 1993). The highly reactive nature of free radicals empowers them to be able to cause damage to indispensable molecules within the living bodies such as DNA, proteins, lipids and carbohydrates, the outcome of which are cell homeostatic disruption and cell destruction. The following oxygen-embedded free radicals have been commonly identified and leading to oxidative stress in many disease conditions viz; superoxide anion radical, hydroxyl radical, peroxynitrite, hydrogen peroxide, nitric oxide radical, oxygen singlet, and hypochlorite radical (Young and Woodside, 2001). Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole are synthetic antioxidants supposedly reported to be harmful to health. It is therefore necessary to look for potent antioxidants from alternative sources of which medicinal plants were being discovered (Lobo et al., 2010). P. alliacea, H. opposita, A. brasiliana and S. erianthum are medicinal plants found in Nigeria but less had been reported about their phytochemical and antioxidant activities. This dearth of relevant information about them thus justified the essence of this study.

METHODOLOGY

Collection of Plant Samples, Identification and Authentication

The four medicinal plants were sourced in farms along Ita-Oniyan, off Ondo road Akure (Akure North Local Government, Ondo State, Nigeria in November, 2021. The samples were taken to the Medicinal Plant Herbarium, Department of Pharmacognosy, Faculty of Pharmacy Obafemi Awolowo University Ile-Ife Nigeria for formal identification and authentication. After the exercise, they were assigned Herbarium Deposited Voucher Numbers FPI 2269, FPI 2270, FPI 2271 and 2272 to *P. alliacea* L, *S. erianthum* D.Don., *A. brasiliana (L.) Kuntze,* and *H. opposita* Vahl respectively.

Preparation of Plant Samples

Healthy fresh leaves were plucked and rinsed in distilled water to clean up surface dirt on them. Air drying began immediately until a high level of crispness was attained before grinding to powder with the aid of an electric blender (3 Jar QC. NO: HP/14/001/0064). The powdered samples were stored in an air-tight plastic container and labeled accordingly till further action.

Preparation of the Extracts

An equal quantity (400 g) of each of the powdered samples was cold macerated in 2000 mL of ethanol (70%) in the ratio of 1:5 (w/v) exception for *S. erianthus* with a higher ratio of 1:8 (w/v) because the apportioned volume (ratio 1:5) that well soaked and covered the other plants was not sufficient for the same purpose in *S. erianthus*. Thus the volume has to be increased over the rest plant samples. The maceration lasted for 72 hours after which the samples were filtered with sterile muslin cloth and filter paper (Whatman filter paper No 1). The extracted samples were later concentrated to dryness under the electric fan in a clean laboratory environment. The final dried crude extracts were kept in small-sized plastics with lids at 4^{o}C until bio-assayed for phytochemical screening and antioxidant activity evaluation.

Phytochemical Screening

Standard procedures were followed as described by Sofowora, (1993), Wadood et al., (2013), Yakubu et al., (2019) and Mahmud et al. (2020) for qualitative screening while Harbone, (1973), Brain & Turner (1975), Brunner, (1984) and Chang et al. (2002) methods were adopted for quantitative screening.

Antioxidant Activity

The antioxidant activity of the four medicinal plant samples in this experiment was evaluated using DPPH (2,2-Diphenyl-1-Picrylhydrazyl), ferrous chelation (Fe^{2+}), Ferric-ion Reducing Antioxidant Power (FRAP) and Peroxide Free Radical (H_2O_2) assays having reconstituted each of the extracts to three different concentrations viz: 5 mg/mL, 7.5 mg/mL and 10 mg/mL.

DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) antioxidant activity

DPPH comprises stable free radicals, and hence commonly made use of in the determination of the free radical scavenging potential of compounds. Each plant's scavenging activity and that of the standard ascorbic acid was measured spectrophotometrically at 510nm (Optical density)as described by Yahaya et al. (2020) at the various reconstituted concentrations and expressed as percentage (%) inhibition (Yahaya *et al*., 2020) using the formula:

$$\text{Percentage inhibition (\%I)} = \frac{ABSORBANCE\ OF\ CONTROL - ABSORBANCE\ OF\ SAMPLES}{ABSORBANCE\ OF\ CONTROL} X\ 100$$

Chelation activity on Ferrous (Fe^{2+})

This entails the determination of the chelating effect on ferrous ions of the reconstituted extracts. The determination was carried out with the extracts, by the usage of methanol, 2 mM $FeCl_2$, in which the reaction was initiated by the addition of 5 mM ferrozine (Sudan et al., 2014). The mixture was thereafter kept at room temperature for 10 minutes and the absorbance was measured using a spectrophotometer at 510 nm. The percentage chelating power was calculated according to Gulcin and Alwasel (2022) using the formula:

$$\%\ \text{chelating power} = \frac{ABSORBANCE\ OF\ CONTROL - ABSORBANCE\ SAMPLES}{ABSORBANCE\ OF\ CONTROL} X\ 100$$

Ferric-ion Reducing Antioxidant activity (FRAP)

The ability of a substance or compound to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) in FRAP reagent (i.e. reduction of ferric tripyridyltriazine complexes to ferrous tripyridyltriazine) has also been very handy and useful in the determination of its antioxidant potential. This was carried out on the extracts as reported by Hossain et al. (2020) and values (mg/g) obtained in gallic acid equivalent (GAE) at 700 nm owing to differentials in blue colour complex with ferrous ion were taken as the extracts' antioxidant activity following the formula:

$$\text{Percentage inhibition (\%I)} = \frac{ABSORBANCE\ OF\ CONTROL - ABSORBANCE\ OF\ SAMPLES}{ABSORBANCE\ OF\ CONTROL} X\ 100$$

Peroxide free radicals (H_2O_2) antioxidant activity

This involved the scavenging potential of the experimented extracts on the free radicals of peroxide (H_2O_2). The ability of any compound or substance to be able to mop up H_2O_2 in any chemical reaction is a proof that such a substance will have protective activity against the hydroxyl radicals being generated by hydrogen peroxide. Hydrogen radicals have been reported to be engendering cytotoxicity in living tissues (Al-Owaisi et al., 2014). The solution and the reactions followed the procedure of Yahaya et al. (2020). Thereafter, the percentage scavenging potential was calculated at the absorbance of 532 nm using appropriate formula.

IC_{50} determination

IC_{50} implies the concentration of the antioxidant substance that will reduce initial concentration by 50 % or that produced 50 % antioxidant activity. The lower the value of the $IC_{50,}$ the better it is for the antioxidant reaction and the higher the antioxidant activity of the test agent. (Mukta *et al*., 2020). This was calculated according to Mahmud *et al*. (2020) using the formula:

$$IC_{50} = \frac{Concentration\ of\ the\ extract}{\%\ inhibition} X\ 50$$

Statistical analysis

The experiments were carried out in duplicates and the results are presented as ± standard deviation. (SD). Collated data were analyzed using SPSS, version 23, 32 and 64 bit (2015) through compare means among the extracts, followed by one-way ANOVA and post hoc test. Duncan Multiple ranges and multiple E-test (2009) of the same package were used to test for significant difference and p-values less than 0.05 ($p < 0.05$) were considered to be statistically significant.

RESULTS AND DISCUSSION

The results of the qualitative and quantitative phytochemical screening are presented in Tables 1 and Table 2 while that of the antioxidant activity embarked upon by making use of four different assays are shown in Tables 3 to Table 6. The results of the IC_{50} obtained are graphically presented in figure I to figure IV.

Table 1. Qualitative Phytochemical Result

Phytochemicals	**Leaf samples**			
	P. alliacea	***H. opposita***	***A. brasiliana***	***S. erianthum***
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Saponins	+	+	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	–	–	–	–
Phlobatannins	–	–	–	–
Anthraquinones	–	–	–	–
Cardiac Glycosides				
Keller-killiani's test	+	+	+	+
Salkowski's test	+	+	+	+
Lieberman's test	+	+	+	+

+ = Absent, – =Present

Table 2. Quantitative phytochemical of the samples

Phytochemicals (mg/g)	**Leaf samples**			
	P. alliacea	***H. opposite***	***A. brasiliana***	***S. erianthum***
Tannins	3.77 ± 0.01^{b}	3.60 ± 0.01^{c}	5.15 ± 0.04^{a}	2.78 ± 0.01^{d}
Terpenoids	15.53 ± 0.04^{b}	14.73 ± 0.04^{c}	22.34 ± 0.04^{a}	10.69 ± 0.04^{d}
Steroids	17.28 ± 0.02^{a}	13.10 ± 0.02^{d}	16.20 ± 0.02^{b}	13.96 ± 0.02^{c}
Saponins	38.91 ± 0.26^{c}	85.45 ± 0.26^{a}	33.45 ± 0.26^{d}	49.27 ± 0.26^{b}
Glycosides	31.45 ± 0.05^{a}	31.45 ± 0.05^{a}	20.03 ± 0.05^{c}	23.76 ± 0.05^{b}

Phenols	2.42±0.43^{c}	6.04±0.43^{b}	6.04±0.43^{b}	17.21 ±0.43^{a}
Flavonoids	0.31±0.05^{b}	0.38±0.05^{b}	0.42±0.05^{b}	1.15±0.05^{a}
Alkaloids	–	–	–	–
Phlobatannins	–	–	–	–
Anthraquinones	–	–	–	–

Means±SD of duplicate determinations, – = not found. Means with different superscripts in the same row are significantly different ($p < 0.05$) from one another.

Table 3. DPPH (%) free radicals scavenging activity of the samples

Concentrations	**Leaf samples**			
	P. alliacea	***H. opposita***	***A. brasiliana***	***S. erianthum***
5 mg/mL	53.89±0.11^{d}	62.92±0.11^{c}	67.79±0.11^{b}	70.23±0.11^{a}
7.5 mg/mL	54.28±0.11^{c}	68.42±0.11^{b}	71.72±0.11^{a}	72.03±0.11^{a}
10 mg/mL	54.75±0.11^{d}	74.86±0.11^{c}	75.65±0.11^{b}	80.60±0.11^{a}

Means ± Standard deviation (SD) of duplicate determinations. Means with different superscripts in the same row are significantly different ($p < 0.05$) from one another.

Table 4. Fe chelation (Fe^{2+} %) power of the samples

Concentrations	**Leaf samples**			
	P. alliacea	***H. opposita***	***A. brasiliana***	***S. erianthum***
5 mg/mL	31.00±0.24^{d}	35.50±0.24^{c}	43.67±0.24^{b}	46.50±0.24^{a}
7.5 mg/mL	33.50±0.24^{d}	40.67±0.24^{c}	44.00±0.24^{b}	58.50±0.24^{a}
10 mg/mL	42.00±0.24^{c}	49.50±0.24^{b}	51.00±0.24^{b}	60.33±0.24^{a}

Means ± Standard deviation (SD) of duplicate determinations. Means with different superscripts in the same row are significantly different ($p < 0.05$) from one another.

Table 5. FRAP (mgGAE/g) of the samples

Concentrations	**Leaf samples**			
	P. alliacea	***H. opposita***	***A. brasiliana***	***S. erianthum***
5 mg/mL	29.14±0.21^{c}	29.59±0.21^{c}	30.05±0.22^{b}	32.76±0.21^{a}
7.5 mg/mL	29.29±0.21^{c}	30.95±0.21^{b}	30.95±0.21^{b}	34.88±0.21^{a}
10 mg/mL	29.74±0.21^{d}	33.67±0.43^{b}	31.10±0.21^{c}	36.69±0.21^{a}

Means ± Standard deviation (SD) of duplicate determinations. Means with different superscripts in the same row are significantly different ($p < 0.05$) from one another.

Table 6. Peroxide (H_2O_2 %) free radicals scavenging activity of the samples

Concentrations	**Leaf samples**			
	P. alliacea	***H. opposita***	***A. brasiliana***	***S. erianthum***
5 mg/mL	42.86±1.12^{c}	71.43±1.12^{b}	73.81±1.12^{b}	93.65±1.12^{a}
7.5 mg/mL	53.97±1.12^{c}	83.33±1.12^{b}	85.71±1.12^{b}	96.83±1.12^{a}
10 mg/mL	80.16±1.12^{d}	85.71±1.12^{c}	88.89±1.12^{b}	97.62±1.12^{a}

Means ± Standard deviation (SD) of duplicate determinations. Means with different superscripts in the same row are significantly different ($p < 0.05$) from one another.

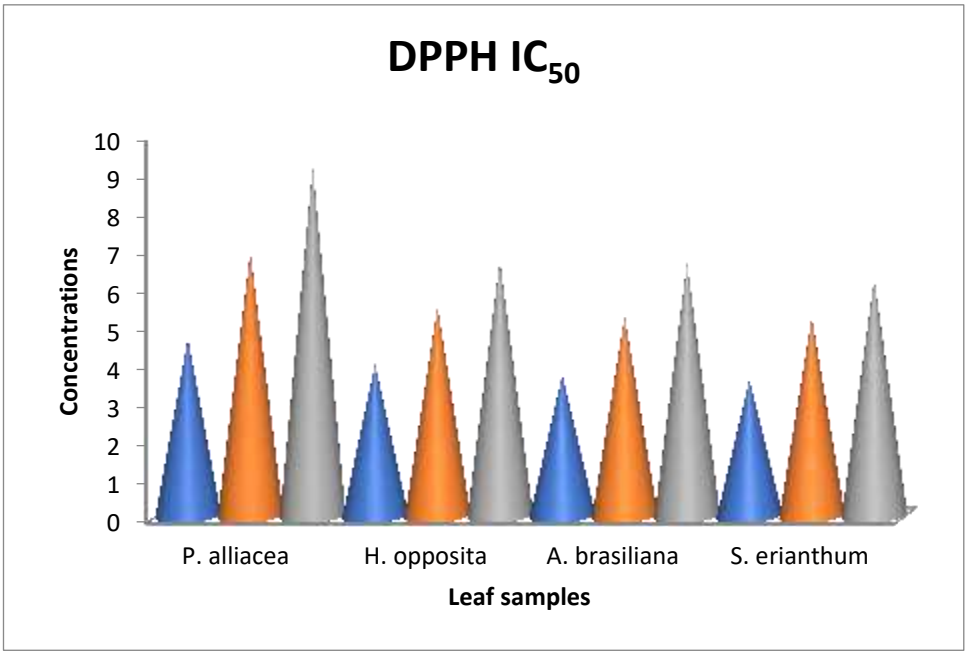

Figure 1. DPPH IC_{50}

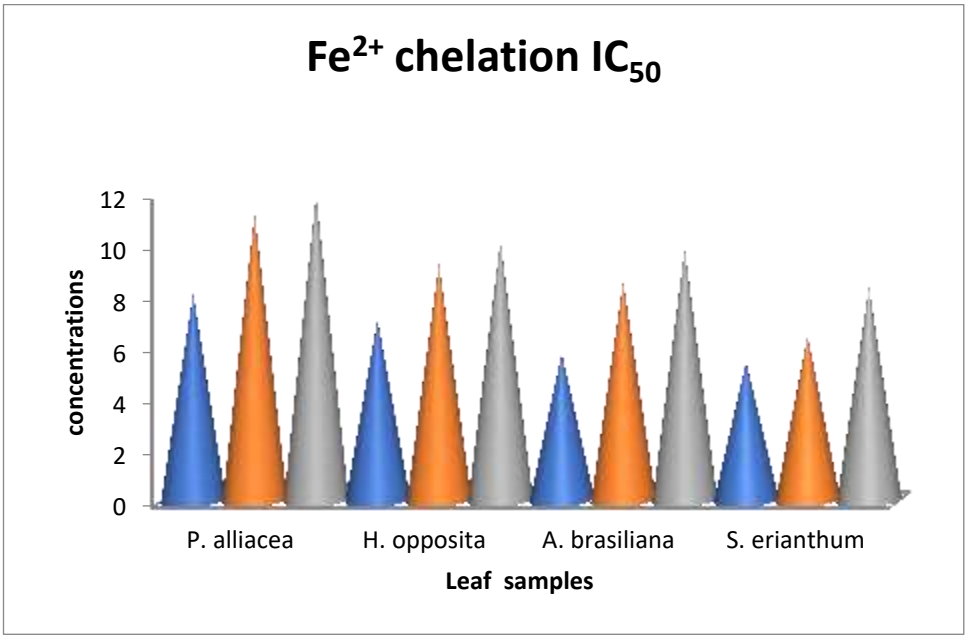

Figure 2. Fe^{2+} chelation IC_{50}

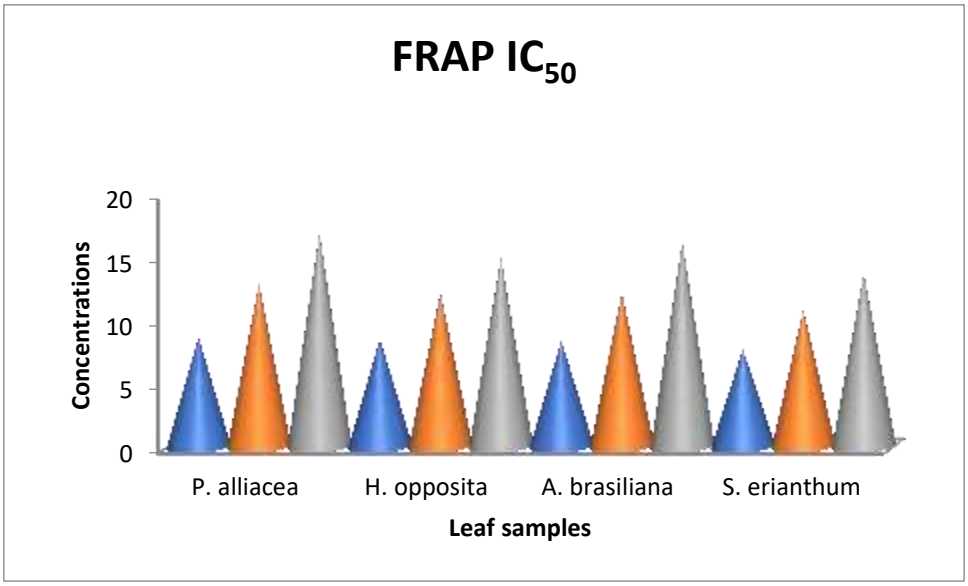

Figure 3. FRAP IC_{50}

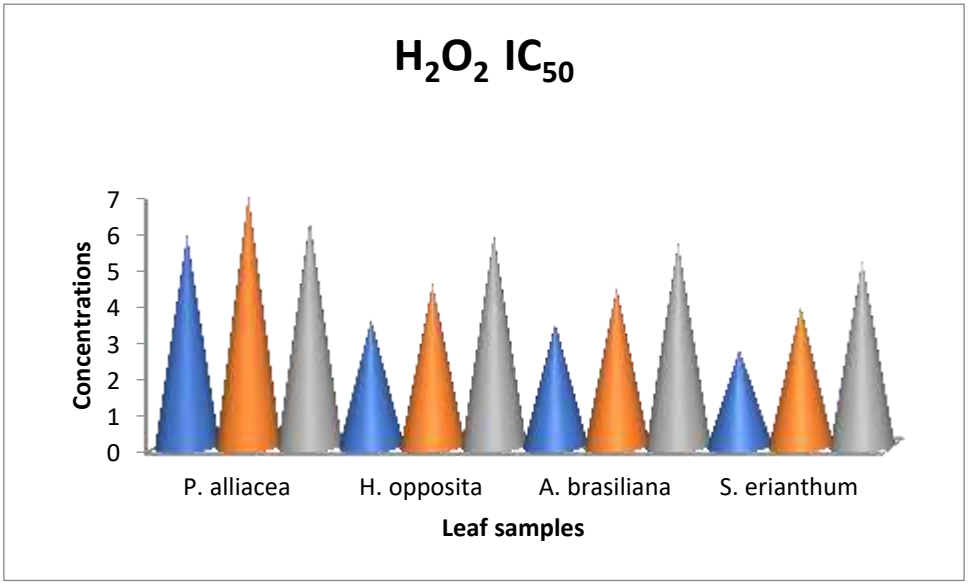

Figure 4. H_2O_2 IC_{50}

In this study, ethanolic extracts of *P. alliacea, H. opposita, A. brasiliana and S. erianthum* showed the presence of major phytoconstituents (Table 1) which are; tannins, terpenoids, steroids, saponins, phenols, and flavonoids while alkaloids, anthraquinones, and phlobatannins were not found in all the samples. This indicates that all the evaluated plant samples are rich source of plant phyto-chemicals. The absence of anthraquinones and alkaloids as well as the presence of terpenoids in this study disagrees with the report of Sathiyabalan et al. (2017) but agrees in terms of the presence of tannins, flavonoids, phenolics, saponins, steroids and glycosides even though the samples in both studies were ethanolic extracts. Also the presence of saponins, tannins, flavonoids, terpenoids and steroids in this study agrees with findings about our earlier experiment on aqueous extract of the leaf of *P. alliacea* but at variance with glycosides (Arogbodo et al., 2021) which may probably be due to solvents' peculiarity. The qualitative result (Table 2) shows a varying quantities of the phytochemicals across the samples as significant differences ($p < 0.05$) were observed. Tannins and terpenoids were in the order *A. brasiliana* > *P.* alliacea > *H. opposita* > *S.*

erianthum. Steroids: *P. alliacea* > *A. brasiliana* > *S. erianthum* > *H. opposita*. Saponins: *H. opposita*> *S. erianthum* > *P. alliacea* > *A. brasiliana*. Glycosides: *P. alliacea* > *H. opposita*> *S. erianthum* > *A. brasiliana*. Phenolics: *S. erianthum* > *A. brasiliana* > *H. opposita* > *P. alliacea*. Flavonoids: *S. erianthum* > *P. alliacea* = *H. opposita* = *A. brasiliana*. The quantity of saponins and terpenoids obtained in this study was higher than those reported by Ayodele et al. (2015) while the quantity of flavonoids and phenolics was lower than those reported by the same author.

Climatic factors, environmental differences, the solvent used, the season of the year, period of harvest, etc may be adducible for values disparity. However, relevant information on the phytochemicals of ethanolic extracts of *S. erianthum*, *A. brasiliana, H. opposita, P. alliacea* is scarce in the literature. The little reports accessed used other solvents in their extraction methods. In the phytochemical evaluation of *H. opposita* Ojo et al. (2010) made use of aqueous, methanol and ethyl acetate as solvents. Kannan, (2014) and Pratab, (2017) used hexane, chloroform and methanol for the extraction of *A. brasiliana* while Francis-Xavier et al. (2013) used methanol, petroleum ether, ethyl acetate, chloroform, and aqueous for the extraction of *S. erianthum*. Nevertheless, irrespective of the solvent used, the phytochemical screening results from this study and reports from other authors corroborate the fact that the experimented plants contain essential and relevant phytoconstituents of high pharmacological value.

Phytochemicals are known to have protective functions in plants against the assaults of pathogenic agents and ultraviolet (UV) rays (Mathai, 2000). They also act as defensive chemicals against human diseases like cancer (Narasinga, 2003) and those of bacterial, protozoal, and viral origin. The effectiveness of the usage of medicinal plants in the treatment and prevention of diseases is similar to synthetic drugs. Medicinal plants sometimes produce excellent results most importantly the case of chronic diseases in the long run (Saxena et al., 2013). The measured capableness of an extract in scavenging DPPH radicals, reduction of Fe^{3+} /ferricyanide complex (Mahmud, 2020) to ferrous (Fe^{2+}), metal chelating, free radical scavenging of H_2O_2 (Yahaya et al., 2020) among others denotes its antioxidant strength (Lawal *et al*., 2015). Antioxidants possess the physiological role of maintaining and preventing cellular constituents' damage arising from free radicals (Young and Woodside 2001).

The antioxidant activities of the experimental plants as evaluated using DPPH, Fe^{2+} Chelation, FRAP and Peroxides assays (Tables 3 to 6), shows that *S. erianthum* has the highest antioxidant activity 80.60±0.11, 60.33±0.24, 36.69±0.21 and 97.62±1.12 while *P. alliacea* recorded the least values 54.75±0.11, 42.00±0.24, 29.74±0.21 and 80.16±1.12 at 10 mg/mL extracts' concentration for DPPH, Fe^{2+} chelation, FRAP and peroxides respectively. The values for *H. opposita* and *A. brasiliana* were between the highest and the least values range. The IC_{50} results as shown in Figures I to IV followed a reversed pattern to those seen above, which is scientifically normal. The highest and the lowest values (IC_{50}) were recorded in *P. alliacea* and *S. erianthum* respectively. The lower the IC_{50} value, the higher the antioxidant activity and *vice versa* (Mahmud *et al*., 2020). It was observed from this study that *S. erianthum* has the highest quantity of phenols and flavonoids which corresponds to its best antioxidant activity. This observation agrees with the reports from earlier authors (Rice-Evans *et al*., 1997; Li *et al*., 2006; Faeji and Oladunmoye 2017; Mudasir *et al*., 2019) that the phenolic content of a plant is directly proportional to its free radicals scavenging capability. Nevertheless, the four plant samples demonstrated a very high antioxidant potentiality.

CONCLUSION

The four medicinal plants investigated in this study are rich sources of major phytochemicals (tannins, terpenoids, steroids, saponins, phenolics, flavonoids and cardiac glycosides) which could be the major reason for their high antioxidant activity, with S. erianthum taking the lead. Further study is therefore recommended on the toxicity of the test plants at the sub-chronic and chronic level to unravel and make a sufficing utilization of their medicinal endowment.

Conflict of Interest

The authors do not have any conflict of interest to declare.

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