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COMPARATIVE PHYTOCHEMICAL AND *IN VITRO* ANTIOXIDANT ANALYSIS OF SOME INDIGENOUS PLANTS

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ABSTRACT
This study was carried out to determine and compare the phytochemical and antioxidant activities of *Allium fistulosum*, *Bryophyllum pinnatum*, *Cymbopogon citratus* and *Heinsia crinita*. The leaves were washed, dried and powdered. The powdered leaves were macerated in methanol for forty-eight hours (48hrs) after which the filtrate obtained was concentrated in an oven at 40^{o}C. The methanol extract of the leaves, were then screened for phytochemicals. Antioxidant activities of the leaf extracts were ascertained using the ferric reducing antioxidant power (FRAP) method and hydrogen peroxide scavenge assay. Results show that *A. fistulosumB. pinnatum, C. citratus* and *H. crinita*contains phytochemicals including flavonoids, reducing sugars, terpenoids, steroids, saponins, alkaloids, tannins, amino acids, proteins and cardiac glycosides. Fixed oils were absent while saponins were present in all fourextracts. FRAP and hydrogen peroxide scavenging assay revealed the potent antioxidant activity of all plant extracts. However, *B. pinnatum* showed the highest ferric reducing antioxidant power, followed by *H.crinita*, *A. fistulosum* and *C. citratus*, at varied concentrations of ferric cyanide. *H. crinita* at varied concentration of Hydrogen peroxides, revealed the highest percentage scavenging ability followed by *A. fistulosum, B. pinnatum*and then *C. citratus*. This study has revealed that the leaf extracts of *A. fistulosum*, *B. pinnatum*, *C. citratus* and *H.crinita* possess potent antioxidant activity, phytochemical and nutritional benefits, with *B. pinnatum* having the highest phytochemical and nutritional content.It also supports their use in folk medicine for the management of diseases. Further research should be carried out to ascertain specific active principles in these plants.

Keywords: Phytochemicals, Antioxidants, Hydrogen Peroxide scavenging, ferric reducing antioxidant power

INTRODUCTION
Green leafy vegetables are an important part of human and animal diet, as they are vital sources of mineral nutrients needed for proper metabolic functions of the body. Many plant-derived substances, collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity. Antioxidants are capable of stabilizing, or deactivating, free radicals before they

attack cells. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being(Mark, 1998). Antioxidants present in vegetables are therefore able to prevent and stop the deleterious effect of the free radicals by various known mechanisms. Among such popularly consumed vegetables are *Allium fistulosum, Bryophyllum pinnatum, Cymbopogon citratus* and *Heinsia crinita.*

Allium fistulosum, (spring onion) is consumed in many parts of the world including Nigeria. Its leaves and bulb are sliced as seasoning for cooking meat, as dressing for salads, and as part of food recipe. It is also known as salad onions, green onions and spring onions scallium (Lucia *et al*., 2014). It is a rich source of vitamins and minerals, which aids in curing various ailments. It is seen to have helped in reducing the harmful impacts of various diseases. Michael and Smith (2005) reported the antioxidant abilities of onions in averting ulcerations. Onions also has anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties (Michael and Smith, 2005). It can reduce high blood pressure and insulin resistance, aid in weight loss, fight chronic bronchitis and infection, fever, etc. (Edeogun *et al*., 2007).

Cymbopogon citratus, commonly known as lemon grass, is a perennial grass plant, distributed worldwide, most especially, in tropical and subtropical countries (Francisco *et al*, 2011). The aqueous extract is commonly used as an aromatic drink while the whole plant is well incorporated into traditional food for its lemon flavor. It also enjoys wide application in folk medicine (Figueirinha *et al*., 2008a).Traditionally, teas made from lemon grass leaves is popular among countries of South Americans, Asia, and West Africa, having been widely utilized as antiseptic, antipyretic, antidyspeptic, carminative and anti-inflammatory (Figueirinha *et al*., 2008b).It is an important species of poaceae family. The leaf blade is linear, tapered at both ends and can grow to a length of 50cm and width of 1.5cm (Geetha and Geetha, 2014). *B. pinnatum* is commonly known as air plant, love plant, miracle leaf, life plants etc. It has been accepted as a herbal remedy in almost all parts of the world (Nwali *et al.*, 2012). It is a crassulescent herbs that is about 1 metre in height with opposite, glabrous leaves (with 3-5 deeply crenulated, fleshy leaflets), distributed worldwide but growing primarily in the rain forest (Jain *et al*., 2010). It grows widely and is used as folk medicine in tropical Africa, India, China, Australia and tropical America, Madagascar, Asia and Hawail (Lans, 2006). It is astringent sour in taste, sweet in the post digestive effect and has hot potency. It is well known for its haemostatic and wound healing properties. The leaves and stem of *B. pinnatum* is used for treatment of diarrhea, vomitus, ear ache, burns, abscesses, gastric ulcers and insect bites, (Okwu and Njoku, 2009). The juice from fresh leaves is used to treat small pox, Otitis, cough, asthma, palpitations, headache, convulsion and general debility, antimicrobiial (Okwu, 2011; Ubemese and Falana, 2013).

Heinsia crinita belongs to the family Rubiaceae. It is locally called Atama leaf (Okokon, 2009). It is a shrub with woody stem and branches, and is indigenous to West Africa, especially the

Southern part of Nigeria (AkwaIbom and Calabar), tropical regions of Africa, from Guinea to Western Cameroon and Equatoria Guinea and across the Congo basin to East and South Central Africa (Charles *et al*., 2014). Almost all parts of the plant can be utilized as food or for medicinal purpose (Charles *et al*. 2011; Mashesh, 2008). Studies have shown that *H.crinita* has antidiabetic and antiplasmodia properties (Okokun, 2009),and antioxidant property (Nwanna *et al*., 2015) Hence its use in folk medicine.

Aims and Objectives of the Present Study

The aims and objectives of the present study was to determine and compare the phytochemical constituent and *in vitro* antioxidant capacity of the *B. Pinnatum, C. citratus, A. fistulosum* and *H. Crinite* leaves.

MATERIALS AND METHODS

Collection of Plant Material

B. pinnatum and *C. citratus* were harvested from Ogba Zoo in Benin City, Edo State, *A. fistulosum* was purchased from Oba market, Benin City, Edo State, while *H. crinita* was purchased from fruit garden market Port-Harcourt, River State. The leaves were washed, and dried in an oven at 10 °C. Dried leaves were crushed with a blender to fine powder and preserved in an air tight container.

Preparation of Leave Extracts

300g of each sample was macerated in 200ml ethanol for 48hours, while stirring at intervals. The mixture was fitted using a filter cloth to obtain the filtrate. The filtrate obtained was concentrated using a rotary evaporator and thereafter an oven at 40°C to obtain the dried extract, which was stored at 4°C in a refrigerator until when needed for analysis.

Phytochemical Screening

Each plant extract was analyzed for the presence of tannins, saponins, flavonoids, terpenoids, steroids, alkaloids, reducing sugar, glycosides, amino acids, proteins and fixed oils, according to the methods of Soforwora (1993), Trease and Evans (1989) and Harborne (1998).

***In vitro* Antioxidant Analysis**:

i. Ferric Reducing Antioxidant Power (FRAP) method was carried out according to Aiyegoro and Okoh, 2010. 1ml of the extract at various concentrations (0.2-0.6mg/ml) was mixed with 2.5ml of 0.2M phosphate buffer pH 6.6 and 2.5ml of 1% potassium ferricyanide. The reaction mixture was incubated in a water bath at 50ºC for 20minutes. Afterwards, the reaction mixture was rapidly cooled and 2.5ml of 10% Trichloroacetic acid was added to stop the reaction. The mixture was then centrifuged at 3000rpm for 10minutes. 2.5ml of the aliquot was pipette out and 2.5mll of deionized water and 0.5ml of 0.1% ferric chloride solution was added. The colour changes to green. The mixture was allowed to stand for 10minutes and absorbance was measured at 700nm, using a UV- visible Jasco V-630 spectrophotometer. The blank was obtained by mixing blank and solvent. 0.1% Ascorbic acid was used as standard. This procedure was done in triplicate.
ii. Hydrogen peroxide scavenging assay was carried out according to Ruch *et al*., 1989. A solution of 40mM hydrogen peroxide was prepared in

phosphate buffer (50mM pH7.4). 1ml of different concentration of the extract (0.2-0.6mg/ml) was added to hydrogen peroxide and absorbance read at 230nm after 10minutes, against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The percentage of hydrogen peroxide scavenging was calculated as follows:

%scavenging (H_2O_2) = [(*Ai-At*)/ *Ai*] x 100

Where *Ai = absorbance of control and*

At = absorbance of the test

RESULTS

The results for qualitative phytochemical screening of *A. fistulosum, B. pinnatum, C. citratus* and *H. crinite* are shown in Table 1. These results reveal the presence of phytochemicals in all the extracts including alkaloids, cardiac glycosides, flavonoids, reducing sugars, tannins, terpenoids, steroids, amino acids and proteins. Fixed oils were absent in all three extracts.

Table 1: Qualitative phytochemical screening of *A. fistulosum, Bryophyllum pinnatum, C. citratus* and *H. crinita*

TESTS	*A. fistulosum*	*B. pinnatum*	*C. citratus*	*H.crinita*
Alkaloids	-	+	-	-
Cardiac glycosides	-	-	-	+
Flavonoids	+	+	-	+
Reducing sugars	+	-	+	+
Tannins	-	+	-	+
Terpenoids	+	+	+	-
Steroids	+	+	+	+
Amino acids	-	+	-	-
Proteins	-	+	-	-
Fixed oils	-	-	-	-

Key: + = present; - = absent

Figure 1 shows the hydrogen peroxide scavenging capacity of *A. fistulosum, B. pinnatum, C. citrates* and *H. crinita.* The results indicate that *H. crinita* has the highest hydrogen peroxide scavenging capacity at all concentration, compared to the other extracts. However, the scavenging capacity of each extract increased with increasing concentration.

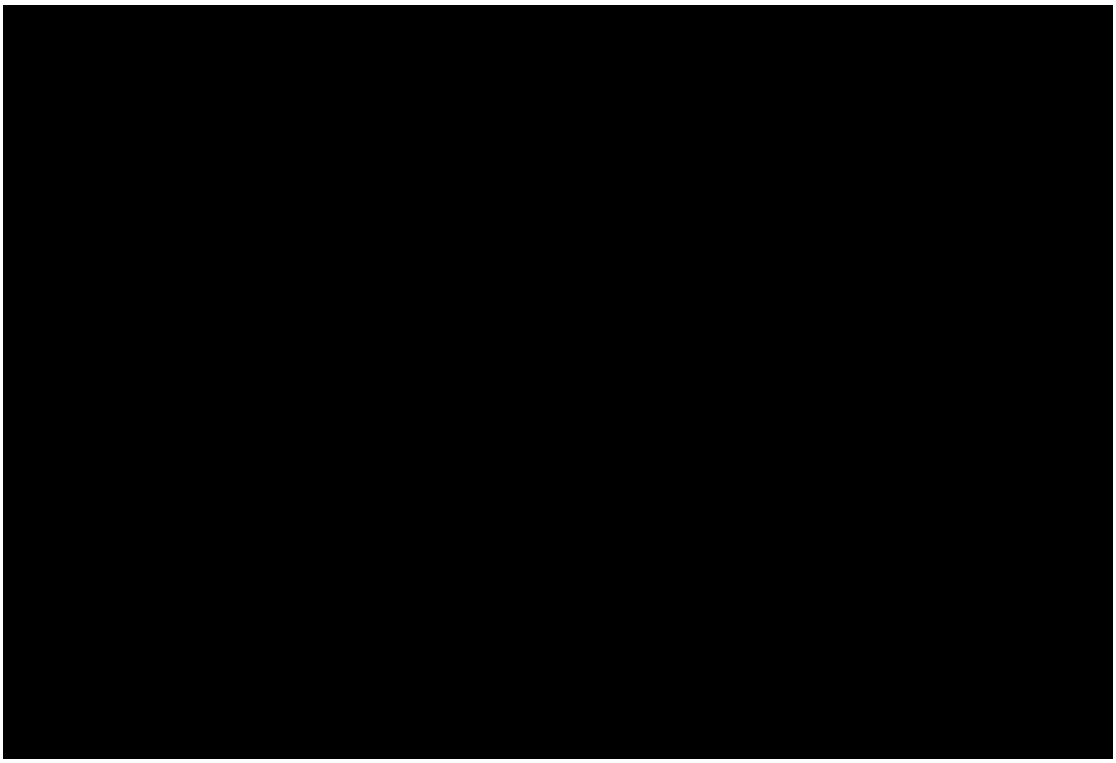

Fig. 1: Hydrogen peroxide percent scavenging capacity for *A. fistulosum, B. pinnatum, C. citratus* and *H. crinita*

Table 2 illustrates the ferric reducing power assay results for *A. fistulosum, B. pinnatum, C. citrates* and *H.crinita.* The results show that *B.pinnatum*has the highest while *C. citratus* show the lowest ferric reducing power compared to the other extracts.

Table 2: Ferric reducing antioxidant power (FRAP) assay results for *A. fistulosum, B. pinnatum, C. citratus*and *H.crinita*

Concentration (mg/ml)	***A. fistulosum***	***B. pinnatum***	***C. citratus***	***H.crinita***
0.2	9.05±0.25	10.75±0.11	6.25±0.14	10.64±0.07
0.4	10.05±0.11	11.61±0.03	6.39±0.31	10.49±0.03
0.6	11.29±0.26	13.20±0.04	7.13±0.32	11.77±0.10

Values are given as mean ± standard deviation

DISCUSSION

A. fistulosum, B. pinnatum, C. citratus, and H. crinita leaves extracts indicated the presence and absence of certain phytochemicals including Tannins, saponins, flavonoids, terpenoids, steroids, alkaloids, reducing sugar, glycosides, amino acids, protein, and fixed oils.

Alkaloids have been associated with some medicinal uses. Many alkaloids are still used in medicine usually in the form of some drugs such as caffeine, codeine and morphine (Neha, 2015). Alkaloids

have a wide range of pharmacological activities including antimalarial, antiasthma, anticancer properties as reported by Kittakoop *et al.,*(2014).It was also reported to have vasodilatory, analgesic and antibacterial properties (Russo *et al.,* 2013). *B.pinnatum* revealed the presence of alkaloids which was in accordance with work done by Nwali, *et al.,* 2012. It has been known to be used in treatment of burns, gastric ulcers, vomiting, diarrhoea, astringent and analgesic. Alkaloids were however absent in *A. fistulosum, C. citratus*, and *H. crinita.* This finding is similar to work done by Adegbegi *et al*., 2012.

It has been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Pourmorad*et al.,* 2006,Ugwu*et al.,* 2013) and they might induce mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al*., 2008). *A. fistulosum, B. pinnatum,* and *H.crinita* revealed the presence of flavonoids but was absent in *C. citratus.* Although this result is at variance with previous work by Adegbegi *et al*., 2012, other researchers have also shown the presence of f1avonoids in *A. fistulosum, B. pinnatum, H. crinita* (Nwali, *et al.,*2012, Ugwoke and Ezugwe, 2012,).

Cardiac glycosides are an important class of naturally occurring drugs whose actions help in the treatment of congestive heart failure (Yukari *et al.,* 1995). It is used for the treatment of cardiac infections along with other ailments such as cough, and chest pain as reported in Technical Data Report for Graviola, 2005 and Taylor, 2002.In this study, cardiac glycosides were present in *H. Crinite* but absent in *A. fistulosum, B. pinnatum, and C. citratus.*

Excess Consumption of sugar-sweetened beverage is associated with risk of type 2 diabetes, obesity, hypertension, and coronary heart disease (CHD) (Tekeshe *et al*., 2013*).* The presence of reducing sugars in *A. fistulosum, C. citrates* and *H. crinita* indicates the ability of these plants to be effective in sugar reduction if consumed. Reducing sugar was absent *B. pinnatum.*

Tannins may be employed medicinally as an antidiarrhoeal, haemostatic, and antihemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, oesophagitis, enteritis, and irritating bowel disorders. Diarrhoea is also treated with an effective astringent medicine that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine (Cheng *et al.,* 2002). Tannins were absent in *A. Fistulosum* and *C. citratus* but present in *B. Pinnatum* and *H. crinita.* The presence of tannins in these plants indicates their ability to act as anti-inflammatory agent.

Terpenoids have proven to have antimicrobial, anticarcinogenic, antioxidant, analgesic, anti-inflammatory and muscle relaxing properties (Ayoola, 2008). The presence of terpenoids in *A. fistulosum, B. pinnatum,* and *C. citrates* supports its effectiveness in managing some ailments. Terpenoids was absent in *H. crinata.*

Recent meta-analyses have concluded that doses of plant sterol or stanols of 1-2 g daily can effectively lower LDL-cholesterol levels 8%-12% (Katan*et al.,* 2003). Steroids were

present in *A. fistulosum, B. pinnatum* and *H. crinita* and absent in *C. citratus.*

Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Roa *et al.,* 1995). Saponins have the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011). Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.,* 2000). Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Just *et al.,* 1998; Okwu and Emineke, 2006, Liu and Henkel, 2002). Saponins were present in *A. fistulosum, B. pinnatum, C. citratus* and *H. crinita.*

Fixed oils were absent in *A. fistulosum, B. pinnatum, C. citratus* and *H. crinita.* This suggests that these plants are healthy and may have a role in decreasing cholesterol levels.

Diets high in plant protein, such as the vegetarian diet, are linked with many health benefits. Studies suggest vegetarians tend to have a lower body weight, lower cholesterol and lower blood pressure levels. They also have a lower risk of stroke, cancer and death from heart disease than non-vegetarians (Craig, 2010). Protein and amino acid were present in *B. pinnatum* and absent in *A. fistulosum, C. citrates* and *H. crinita.*

B. pinnatum has the highest phytochemical content compared to *A. fistulosum, C. citratus, and H. crinita*. It is the only extract with amino acids and proteins. Thus, making it a good alternative source of plant protein. It is therefore an excellent nutritional vegetable compared to the other leaves. *H.crinita* has the second highest phytochemical constituent compared to *A. fistulosum, B. pinnatum,* and *C. citratus.* It may not be a good source of amino acids, fixed oils and proteins. Phytochemicals present include cardiac glycosides, flavonoids, reducing sugars, tannins, steroids and saponins. *A. fistulosum* has the least content of phytochemicals compared to the others. It is not also a good source of amino acids and proteins and oils. Phytochemicals present in *A. fistulosum* include flavonoids, reducing sugars, terpenoids, steroids and saponins.

The ferric reducing antioxidant power (FRAP) assay is often used to evaluate the ability of an antioxidant to donate an electron (Yildirim, *et al.,* 2000). *B. pinnatum* was observed to have a higher value for FRAP assay compared with other extracts, followed by *H. crinita, A. fistulosum, and C. citratus.* However, these values obtained showed no significant difference ($P > 0.05$) with *A. fistulosum, B. pinnatum, C. citratus,* and *H. crinita.* These results shows that may have a higher chance to reduce Fe^{3}- to Fe^{2}+ and may act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions (Alam *et al*., 2012a). This may be attributed to the presence of alkaloids in *B. pinnatum* which was absent in the other leaf extracts.

Humans are exposed to hydrogen peroxide (H_2O_2) indirectly via the environment. Hydrogen peroxide (H_2O_2) enters the body through inhalation, vapor

or mist and through eye or skin contact. H_zO_2 is rapidly decomposed to OH^- which can initiate lipid peroxidation and cause DNA damage (Alam, 2012b). *H. crinita* at all three concentrations of hydrogen peroxide showed the highest percent hydrogen scavenging ability compared to *A. fistulosum, B. pinnatum,* and *C. citratus.* This may be attributed to the presence of cardiac glycosides which was conspicuously absent in the other leave extracts and tannins which was absent in *A. fistulosum,* and *C. citratus. A. fistulosum* showed the second highest percent hydrogen scavenging activity followed by *B.pinnatum* then *C. citratus.* It was also observed that the hydrogen scavenging ability of each extract was increased with increasing concentration of hydrogen peroxide.

CONCLUSION

The investigation carried out on the leaves of *A. fistulosum, B. pinnatum, C. citratus, and H. crinita* proven to be beneficial. The phytochemicals present has revealed that these leaves possess potent antioxidant properties. Also the nutritional content of the leaves support their use as food. The presence of bioactive compounds and pharmacological activities proved the potency of plant in the development of novel drugs in the future.

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