Study of Total Phenol contents & Percentage antioxidant activities of Raw, Processed and Stored *Pergularia daemia* leaves for its processing and storage conditions

Abstract

Pergulariadaemia leaves are used for diet and ethnomedicinal remedies preparations in Nigeria. The concern of this study was to investigate raw, processed and stored *Pergulariadaemia* leaves for total phenols and antioxidant activities. Sample used were obtained from Benin City, then subsequently processed and stored at varying conditions.Storage duration was two months. Samples were noted to contain total phenols and further exhibited antioxidant activities.In raw *Pergulariadaemia* leaves, total phenols content was 2.18±0.51mg/gand percent antioxidant activities value was 94.37±3.66%. Findings also indicate that processing and storageinfluenced the values of the examined parameter. Statistical analysis $P<0.05$ indicatesignificant differences between the values of the studied parameters in raw samples and their corresponding values in the processed and stored samples. Hopefully,policy formulationspersonnel will find the results of this work useful.

Keywords: *Pergulariadaemia*, water activity(a_w),, antioxidant activites, total phenols, spectrophotometer.

Introduction

Pergulariadaemia leaves are commonly used in Nigeria both for its nutritional and pharmacological values. There are also reports (Makheswari and Sudarsanam, 2013; Sureshkumar and Mishra, 2006; Wahi *et al*., 2002; Packirisamy and Moorthy, 2014;Kirtikar, and Basu, 1999; Nadkarani, 1976; Karthishwaran and Mirunalini, 2010) indicating that elsewhere, the therapeutic relevance of *Pergulariadaemia*is well recognized. Notwithstanding some existing reports on the uses of *Pergulariadaemia* for ethnomedicinal purposes,literature search indicate that there is dearth of information with respect to the levels of nonnutritive bioactive substances contained in *Pergularimia*. Hence it isconsidered relevantthatresearchersinterest in studies relating to quantifications ofboth the yet investigated and the less investigated constituents of*Pergulariadaemia*, especially those factors that possess biological activities be enhanced. This,

it is hoped, will improve the use of *Pergulariadaemia* by a wider segment of the world populace. For instance, Sridevi *et al*. (2014) remarked that even in developed countries where pharmaceuticals are readily accessible, many still prefer herbal drugs ever over pharmaceutical drugs.It is imperative to mention that World Health Organization (WHO) (1993) noted that more than 80% of the world's population in poor and underdeveloped countries depends on traditional plant-based medicines for their primary healthcare needs.

The concern of this study is to determine the total phenols and total antioxidant activitiesof raw and subsequently processed, thereafter, stored *Pergulariadaemia*leaves. Scientific reports with respect to the concern of this work are scarce if in existence.Sun drying and grating as processing methods, would be used to produce the processed form of *Pergulariadaemia* leaves. Thereafter, samples of the processed *Pergulariadaemia*leaves would be stored under varying storage conditions and the levels of total phenols and total antioxidant activities of samples obtained from the different stages of this work would be investigated.

It is remarked by Sharafati-Chaleshtor*et al*. (2011) that phenolic compounds belong to a class of antioxidants that act as free radicals terminators. Viewed from this point, it would appear that in human body, via the pathway of free radicals termination,phenols could be relevant in the management of oxidative stress.Worthy of note is the remark of Pamplona-Rogers (2005)that the reactive free radicals species foster lipoprotein oxidation, arteriosclerosis, premature cellular aging, and even carcinogenic mutations. Therefore, the relevance of the free radicals terminating properties of phenols cannot be over emphasized. According toSolihab*et al*.(2012) phenols give protection against cardiovascular disease. It would therefore be nutritionally desirable, if phenols occur in safe form and amount in human diets. The imperative of the choice to investigate total phenols levels in the examined samples, especially as natural sources of phenols could be considered from this perspective.

Research works on antioxidants have been the concern of many scientists in recent time. It is hoped that more studies on antioxidants especially those of biological origin, will continue to attract the attentions of researches. The portion of work in the present study which focuses on theinvestigation of percentage antioxidant activities of *Pergulariadaemia*, is one of the aforementioned attractions.It should be emphasized that antioxidants play central role in preventing many human illness. In particular, there are literature reports (Yagi, 1987; Maxell,

1997; Thomas and Kalyanaraman, 1997; Scandalios, 1997; Jose and Janardhan, 2000; Tiwari, 2001 and Guest Editorial, 2002) which indicate that failure in the balance between Reactive Oxygen Species (ROS) production and antioxidant defenses, results in 'oxidative stress', with the consequential effects of deregulating the cellular functions and resultant manifestations of various pathological conditions.Significantly, aging,arthritis, carcinogenesis, asthma, autoimmune diseases, cataract AIDS, arteriosclerosis, broncho-pulmonary, liver disorder, dysplasia, cardiovascular dysfunction, skin disease, diabetes, genetic disorders, gastroduodenal pathogenesis, pulmonaryfibrosis, inflammatory diseases, ischemia reperfusion injury, lateral sclerosis muscular dystrophy, neurodegenerative diseases, stroke, Parkinsons dementia, Alzheimer's disease, radiation damage, retinopathy, amyotropicreheumatism, porphyria and snile dementia are implicated to be associated with conditions of oxidative stress.

A portion of this study tends to investigate the responses of the total phenol constituent and total antioxidant activities of*Pergulariadaemia* leaves to both processing and storage.Processing method of interest in this work is sun drying. With respect to storage conditions,the open laboratory and storage at water activities (a_w) of 0.23, 0.52 and 0.97 will be examined. All storages will be carried out at ambient conditions. It would appear that gap exists with respect to information on the effect of $a_w$on the compositional chemistry of *Pergulariadaemia* leaves. There is need to expand this frontier of knowledge, particularly with the increasing acknowledgement of the central role of water activity in food systems (Dibie, 2019). The relevance of water activity in food stability has also been remarked by some authors. For instance, Coultate (2002) opined that the concept of water activity is nowadays universally adopted by food scientists and technologists to quantify availability. Moreover, Belitz *et al* (2009) remarked that the storage quality of food does not depend on the water content, but on water activity (a_w). Furthermore, findings from the works ofsome researchers (Acker, 1969; Schoebel *et al*., 1969; Labuza, *et al*., 1970; Lajollo, *et al*., 1971; Eichner and Karel, 1972; Ukhun, 1986; Ukhun and Uwatse, 1988; Ukhun and Dibie, 1991; Dibie and Ukhun, 2020) indicate that food safety, stability and other properties can better be predicted from a_w than from water content. The need to subject total phenols constituent and total antioxidant properties of processed *Pergulariadaemia*to a_w studies is therefore imperative.

The total phenols constituentand total antioxidant activities of the *Pergulariadaemia*leaves samples will be determined usingspectrophotometric methods. Additionally, statistical analysis will be carried out using data that will be generated in this work. In particular, aspects of descriptive statistical evaluation of data and statistical evaluation of the relation between variables (ANOVA) will be done, using International Business Machine (IBM), Statistical Package for Social Sciences (SPSS)

Materials and Methods

2.1 Sample Collection

Fresh*Pergulariadaemia*leaves used in this study were purchased from some open markets in Benin City, Edo State.

2.2 Samples Inspection and Cleaning

The *Pergulariadaemia*leaves samples were pretreated in order obtain samples free from contaminants. Significantly, samples of *Pergulariadaemia* leaves used in this work were not diseased (i.e. they were not affected by bacteria, viral, or fungal infection).

2.3 Samples Preparation

The preparation of samples of *Pergulariadaemia* leaves used in this study entailed an initial process of sun drying. In particular, fresh*Pergulariadaemia*leaves were sun dried to constant weight andthe product obtained was recorded as sun dried samples. Thereafter, portion of the sun dried samples were grated with the aid of Black and Decker 650W, BX550 blender and subsequently, sieved using a 16 – mesh standard sieve (Pascall Eng. Co. Ltd. Sussex, England).

2.4 Samples Storage

Initially, air tight desiccators in which a_w of 0.23, 0.52 and 0.97 were established in accordance with the method prescribed by Rockland (1960) were prepared. Thereafter, three hundred grams of sun dried and grated *Pergulariadaemia*leaves were weighed in triplicates into different 500ml glass beakers (Pyrex glass). The beakers containing the weighed samples were subsequently placed in the separate air tight desiccators, after which samples were stored for 2 months. During storage and on monthly basis, samples were collected and used for the determination of the

parameters examined in this work. The storage desiccators were kept on laboratory bench at ambient conditions.

2.5 Measurement

2.5.1. Total Phenols Content Determination

The determination of total phenol wascarried out using the Folin – Ciocalteauspectrophotometric method as described by Kujala *et al*. (2000).

2.5.1.1 Procedure

Standard solutions of gallic acid were prepared (gallic acid solutions of concentration: 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10mg/l were the standards used in this work). Thereafter, to 1ml of methanol extract of sample and each of the standard solutions in separate test tubes, were added 5ml each of Folin–Ciocalteau reagent (1:10 dilution with distilled water) and subsequently, mixed thoroughly. This was followed by the addition of 4ml of 1M Na_2CO_3 to each of the test tubes. Thereafter, the separate test tubes and their contents were again thoroughly mixed. Subsequently, the various solutions were left to stand for 30min in the dark at ambient temperature. Blank preparation was also carried. At the completion of the 30min duration, absorbance reading of the content of each tube was taken at 765nm with the aid of aUv/visible spectrophotometer (Jenway spectrophotometer, 6715 Uv/vis). The total phenols content was calculated from the standard graph of gallic acid. Thereafter, the results were expressed as gallic acid equivalent (mg/g), which is a common reference compound.

2.5.2 Total Antioxidant Activity Determination.

In accordance with the method described by Liyana-Pathiranan and Shahidi (2005),the free radical scavenging activity of the extracts was determined, using the 1, 1-diphenyl–1–picrylhydrazyl (DPPH) assay. Gallic acid and ascorbic acid were the reference standards used.

2.5.2.1 Procedure

The reference standards viz – gallic acid and ascorbic acid were dissolved in methanol. The concentration of test extract and standards used for the determination was 250µg/ml. The aforementioned concentration of test extract and standards was obtained by serial dilution. 1.0ml

of freshly prepared DPPH solution (5.9mg/100ml methanol) was addedto each of the separate 2.5ml methanolic plant extracts and standards in various test tubes. Thereafter, the different reaction mixtures were incubated in the dark at ambient temperature for 30mins. Subsequently, the absorbance values of the various reaction mixtures were read at 517nm with aid of aUv/vis spectrophotometer (Jenway spectrophotometer, 6715 Uv/vis). Triplicate measurements were carried out. A lowered absorbance value (greater discolouration) showed higher radical scavenging activity. The calculation of percentage antioxidant activity was carried out using the expression below:-

% Antioxidant Activity = 100 – [(Abs sample/n Abs control) × 100]

Methanol was used as the blank.

Abs control = absorbance of DPPH radical + methanol.

Abs sample = absorbance of DPPH radical + sample extract.

The positive controls represented the values obtained with respect to the reference standards (ascorbic acid and gallic acids) measurements. The obtained percent antioxidant activity of the respective test extracts were compared with the positive control.

Results and Discussion

3.1: Qualitative Phytochemical Screening

Results for qualitative screening for total phenols constituents inthe examined crude extracts of*Pergulariadaemia* leaves are presented in Table1.As findings indicate, phenolics were present abundantly in the respective aqueous, methanol, ethanol, n-hexane, acetone and ethyl acetate crude extracts of *Pergulariadaemia*leaves.It would appear therefore, that either of the solvents used in this study could be used to extract the phenolic constituents of*Pergulariadaemia*leaves. It should be emphasized that phenolics have the tendency to exhibit both polar and nonpolar character; however, the extent of such solubility behaviour in experimentations depends on the ratio of the polar components to the nonpolar components. It seems therefore, that from the

findings in this work, particularly with respect to the solvent types used in the extraction of phenols from *Pergulariadaemia* leaves, there was a balance between the polar and nonpolar portions of the various phenolics which tended to positively influence their solubility in the solvent types used.

In their work on *Pergulariadaemia* leaves obtained from Ido-Ekiti, Ekiti State, Nigeria, Dosumu*et el*. (2019) reported the presence of phenols, which is consistent with the results obtained in this study for qualitative screening of phenols in *Pergulariadaemia* leaves. Furthermore, some researchers Srideri*et al*., (2014); Nithyatharani and Kavitha (2018); Maheshwari and Vijayarengan (2021) reported the occurrence of phenols in *Pergulariadaemia* leaves they variously investigated. The findings of the latter mentioned authors again, are consistent with our findings. It is of primary importance that phenols are present in *Pergulariadaemia* leaves as phenols possess therapeutic relevance. For instance, Dosumu*et el*. (2019) posited that phenolic compounds play a key role as antioxidants, or free radical scavengers. Also, Osawa (1994) opined that the antioxidant activity of the phenolic compounds is mainly because of their redox properties, which absorbs and neutralize free radicals, triplet oxygen or decomposing peroxides.

3.2: Quantitative Phytochemical Determination

Results for quantitative determinations of phenols in the examined raw, sun dried, grated and stored *Pergulariadaemia* leavesare presented in Table 2.

Table 1 Results for qualitative screening for total phenols constituents of*Pergularia daemia* leaves

		Extracts											
		Aqueous		**Methanol**		**Ethanol**		**n-Hexane**		**Acetone**		**Ethyl acetate**	
S/N	**Phytochemicals/ Samples**	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems	Leaves	Stems
1	Phenolics												
	*sample 1	++	+	++	+	++	+	++	+	++	+	++	+
	*sample 2	++	+	++	+	++	+	++	+	++	+	++	+
	*sample 3	++	+	++	+	++	+	++	+	++	+	++	+
	*sample 4	++	+	++	+	++	+	++	+	++	+	++	+
	*sample 5	++	+	++	+	++	+	++	+	++	+	++	+

+ = Slightly present, ++ = Largely present, - = Absent By visual inspection

Table 2 Results for quantitative determinations of total phenols constituents of investigated*Pergulariadaemia* leaves samples

S/N	Parameter	Raw (fresh) sample	Sun dried and pre-stored sample	Stored samples									
				Storage conditions/time (months)									
				a_w0.97		a_w0.52		a_w0.23		Open Laboratory			
										Covered container		Opened container	
				2-months	1-month	2-months	1-month	2-months	1-month	2-months	1-month	2-months	1-month
1	Phenols (mg/g)	2.18 ± 0.51	2.19 ± 0.82	4.77 ± 1.26	3.51 ± 0.16	6.33 ± 1.12	4.18 ± 0.47	8.89 ± 1.20	5.72 ± 0.66	5.10 ± 1.18	3.74 ± 0.51	2.44 ± 0.09	2.20 ± 0.61

It is discernible from results presented in Table 2that in raw *Pergulariadaemia*leaves, total phenols content was 2.18±0.51mg/g.Dosumu*et al.* (2019) noted that the total phenolic content in the extract of *Pergularia daemia* leaves they examined ranged from 15.898mg±0.111mg GA/g to 54.679±0.605mg GA/g. The difference between our findings and that of Dosumu*et al*. (2019) could be due to a number of factors. Notably, Sofowara (2008) posited that the age of plant and the season of harvest, determine the amount of bioactive ingredients in them. Additionally, it is imperative to mention that some other factors including method of cultivation, soil type, species, environmental and climatic conditions, as well as postharvest handling and storage conditions, influence the compositional chemistry of biological materials. These factors it would appear are responsible for the differences in the results reported by the various researchers.

It isfurther deducible from results (Table 2) that sun drying of *Pergulariadaemia* leavesled to increase in the level of total phenols. Notably, following sun drying of *Pergularia daemia* leaves, total phenols levels in the examined samples increased to 2.19± 0.82 mg/g. It would appear therefore, that during sun drying the series of reactions involved in the biosynthesis of phenols in *Pergularia daemia* leaves continued. The implication of this could be that varying concentrationsof total phenols exist in*Pergularia daemia* leaves sold to consumers, as this commodity is sold in Nigerian open markets unprotected from sunlight. Suggestively therefore, postharvest handling of *Pergularia daemia* leaves should be standardized to ensure safety.

Results (Table 2) further indicate that storage of sun dried and grated *Pergulariadaemia* leaves in the open laboratory under ambient conditions whether stored in opened or closed containers, positively favoured continued biosynthesis of phenols constituents of the examined sample at the end of the two months storage period. Remarkably, total phenols level of 5.10±1.18mg/g was obtained for samples stored in closed containers at the end of two months storage time.With respect to sun dried and grated *Pergularia daemia* leaves stored in opened container and kept in the open laboratory under ambient conditions, results indicate that at the end of the two months storage period, total phenols level was 2.44±0.09mg/g. Clearly, with respect to the reported increase in total phenols constituents of stored sun dried and grated *Pergularia daemia* leaves in the open laboratory and under ambient conditions, the noted increment was more in samples kept in closed containers than in samples kept in opened container. This observation even if partly, emphasizes the relevance of standardizing methods for postharvest and post processing handling

ofsun dried and grated *Pergulariadaemia* leaves in storage, with particular concern on their total phenols constituents.

In biological materials postharvest, series of reactions both synthetic and degradation in nature could take place. What is deducible from the observed increase in total phenols levels of stored sun dried and grated *Pergulariadaemia* leaves, is that synthetic reactions were favoured over degradative reactions in the stored samples.The variations in the levels of total phenols constituents of *Pergulariadaemia* leaves at the end of the storage time, for samples stored separately in the closed and opened containers and as noted, the samples stored in the closed containers had higher increment, is ascribed to the fact that the conditions in the closed containers accentuated the biosynthesis of total phenols in the stored samples, better than the prevailing conditions in the opened storage container.Presumably if the conditions in the opened containers favoureddegradations reactions more than what was obtained in the closed container, then the samples stored in the closed container should have higher total phenols level.Apparently, if the degradations reactions were either photo-catalyzed, oxidative in nature or perhaps both, then more of degradation reactions would have taken place in samples stored in opened container; as the closure of the containers would have minimized the interaction of the samples with the prevailing environment.The possible influence of moisture exchange between the stored samples and the environment that prevailed over them could also be relevant in attaining the results obtained.

Results (Table 2) also indicate that at the end of the two months storage duration, with respect to storage of sun dried and grated *Pergulariadaemia* leaves at a_w0.23, 0.52 and 0.97, marked increases in the values of total phenols in the stored samplesoccurred. It was particularly observed thatthe highest increase in total phenols level was recorded in samples stored at a_w 0.23. On the other hand, at the end of the two months storage time, the lowest increase in the value of total phenols wasobtained in samples stored at a_w 0.97. What is deducible from this observation is that firstly, the various storage $a_w$positively influenced the synthesis of the phenolics in sun dried, grated and stored *Pergulariadaemia* leaves. Additionally, the trend of the results obtained from the $a_w$studies indicate that at low a_w,there is greater production and accumulation of the phenolics in the stored samples.Apparently, this should be the case if at low a_w synthesis reactionsthat led to increase in total phenols constituents of the studied samples, prevailed most

and over degradation reactions.It is significant to mention that at the higher storage $a_{w,}$ there is increased available water, which it would appear, exhibited lower positive effect in further biosynthesis of the phenolics constituents of sun dried, grated and stored *Pergulariadaemia* leaves. Clearly, if the reactions that led to the degradations of phenolics, or their further utilizations in reactions are water catalyzed, then the greater available water at higher $a_{w,}$ should favour reduced accumulations of phenolics in the stored samples. It is also important to consider the dilution effect of water on reaction rates. Significantly, greater amount of water in the reaction system could lead to reduced reactants mobility and therefore their subsequent conversion to products. This even if partly, could have contributed to the observed pattern of results in the a_w studies.At $P<0.05$, the reported storage increases at the different storage conditions were noted to be statistically significant.

According toUkhun (1984), in milled cowpea flour, physical attributes such as large surface area, high degree of porosity, enzyme decompartimentalization following milling, and the milling operation which is a form of stress, could have promoted chemical responses. Viewed from this perspective, it could also mean that grating of the studied sun dried *Pergulariadaemia* leaves prior to storing, fostered a wide array of chemical reactions which impacted positively on the total phenols constituents of the sun dried and grated *Pergulariadaemia* leaves. This is particularly so, grating operations are accompanied by histological disintegration and enhanced enzyme decompartmentalization.

In this work, the reported occurrence of phenols in *Pergulariadaemia*leaves, as well as theobserved increases in the phenolsconcentration of the storedsamples could be nutritionally and therapeutically relevant assuming threshold values for individual consumers is not exceeded.Hollman, (2001)posited that plant phenols can protect against lipoprotein oxidation. Additionally, Wattenberg (1992) remarked that plant phenols are group of antioxidants that inhibit various stages of cancer process. Obviously, these are desirable nutritional and therapeutic qualities.

Results for Percentage Antioxidant Activities Determinations

Results for determinations of percentage antioxidant activities of raw, sun dried, grated and stored*Pergulariadaemia* leaves extracts are presented in Table3.

Table 3 Results for **Percentage Antioxidant Activities Determinations** of investigated*Pergulariadaemia* leaves samples

S/N	Storage Condition / Sample Description	Total antioxidant capacity (%) / Time (months)		
		Pre-storage	1-month	2-months
1	a_w0.25, stored sample extract (0.25mg/ml)	93.16± 1.85	86.16± 1.62	79.27± 0.28
2	a_w0.52, stored sample extract (0.25mg/ml)	93.16± 1.85	82.41± 1.44	72.52± 0.91
3	a_w0.97, stored sample extract (0.25mg/ml)	93.16± 1.85	77.73± 0.80	64.52± 1.12
4	Open Laboratory (covered container) , stored sample extract (0.25mg/ml)	93.16± 1.85	81.48± 2.03	70.89± 1.54
5	Open laboratory (uncovered container) , stored sample extract (0.25mg/ml)	93.16± 1.85	74.92± 1.00	59.94± 2.11
6	Sun dried pre-stored sample extract (0.25mg/ml)	93.16± 1.85	ND	ND
7	Raw sample extract (0.25mg/ml)	94.37± 3.66	ND	ND
8	Ascorbic acid (0.25mg/ml)	93.61± 4.15	93.41 ± 2.95	92.17 ± 1.55
9	Gallic Acid (0.25mg/ml)	91.74± 1.88	91.68 ± 0.74	90.25± 1.02

ND = Not Determined

Findings indicate (Table3) that the percentage antioxidant activity of the examined fresh*Pergulariadaemia*leaves is 94.37±3.66%. Following sun drying and grating of

Pergulariadaemia leaves,and subsequent storage of the processed*Pergulariadaemia* leaves at different storage conditions, findings from this work indicate reduction in antioxidant activities of the examined samples. The noted reductions in antioxidant activities retention of the stored samples were observed to be progressive with storage time. Furthermore, varying percent antioxidant activities valuesas results (Table3) indicate were obtained for the different samples stored at different storage conditions. This is an indication that storage conditions influenced the rate of reductions in sun dried, grated and stored *Pergulariadaemia* leaves.In this study, the reported percentage antioxidant activity of 94.37±3.66% for fresh *Pergulariadaemia*leaves is considered high, especially as the percentage antioxidant activity of *Pergulariadaemia*leaves (94.37±3.66) at a concentration of 0.25mg/ml, were noted to be higher than those of the standards (ascorbic and gallic acids) at the same concentration of 0.25mg/ml, in the DPPH free radical scavenging activity test. Blois (1958)posited that DPPH is a relativelystable nitrogen centered free radical, that easily accepts an electron or hydrogen, aswellas react with suitable reducing agents; following which the electrons become paired with a subsequent loss in colour of the solution, depending on the number of electrons taken up.

The negative influence of sun drying, grating and storage of *Pergulariadaemia*leaveson the antioxidant properties of*Pergulariadaemia*leaves is evident from the results (Table 3) obtainedfor percentage antioxidant activity valuesof various derived products of*Pergulariadaemia* leaves in this work. In particular, findings (Table 3) indicate that the percentage antioxidant activity of the sun dried samples was 93.16±1.85%. The apparent negative influence of sun drying on the antioxidant properties of *Pergulariadaemia* leaves is in part, ascribed to the possible photo-sensitive nature of some antioxidant species in *Pergulariadaemia* leaves. Presumably also, the noted reduction in percentage oxidant activities of the sun dried *Pergulariadaemia* leaves could be due to the occurrence oflight assisted or initiated reactions, that are associated with antioxidant species depletion. Apparently, if such reactions occurred during the sun drying of the plant materials, then there should be reduction in the percentage antioxidant activities of the sun dried *Pergulariadaemia* leaves.It is imperative to mention that some antioxidants found in plants are biological in nature and could be thermally labile. Significantly, depending on the magnitude of the adverse consequences of the heat from the sun on the thermally labile antioxidant species, it could be considered as one of the factors that led to reduction in antioxidant properties of *Pergulariadaemia* leaves following sun drying.

Part of the processing operations carried out in this study entailed grating. It is imperative to mention that grating creates greater surface area for reactions to take place. Therefore, if the overall effects of the reactions enhanced by grating of the samples are degradative in nature, then grating of the samples prior to storage should foster loss in some of the grated plants constituents. Significantly, the collapse of cell walls that accompanies grating would promote contact of certain exogenous enzymes with some protected substances. The result of this would be enzymatic transformation of the protected materials into other forms. There are indications in some cases where certain characteristics of the new compounds formed differ from that that of the parent compound. In particular, the contact of ascorbic acid with ascorbic acid oxidase will promote the conversionof ascorbic acid into compounds with little or no reducing properties, even when ascorbic acid is one of the most powerful antioxidant provided by nature.

As earlier mentioned,progressive storage losses with respect to antioxidant activitiesof the examined samples was observed inall the samples stored at the various storage conditions. It is particularly discernible from resultsthatthe degree of reducedretentionofantioxidantactivityvariedwith storage condition. Notably, withrespect to samples stored in the open laboratory under ambient conditions, results indicate that those stored in opened containers suffered greater loss in antioxidant activities, compared to samples stored in closed containers. This could be an indication that the continuous contact of the storedsamples with atmospheric factors including oxygen and moisture, fostered greater loss in antioxidant activities of the sun dried, grated and stored samples. Presumably therefore,the noted loss in antioxidant activities of the stored samples took place via some oxidative and hydrolytic degradation processes.

In this work, investigation of influence of water activity on the antioxidant activities of the stored samples, indicate progressive losses of antioxidant activities with storage time. Additionally, it was further deducible from findings that the loss in antioxidant activities occurred more in samples stored at higher a_w. What is envisaged here is that at higher a_w, the greater amount of available water enhanced the rate of hydrolytic reactions, as well as increased solubilization of water soluble antioxidant entities, which it would appear led to greater loss of antioxidant activities in samples stored at high a_w. Clearly, the breakdown of crystalline regions of the samples which accompanies increased levels of available water shouldpromote oxygen diffusion

into them and therefore, would have led to increased antioxidant species degrading reactions. In their work on stored cassava and garri,Ukhun and Dibie(1991) opined that increased levels of available water could have promoted increased oxygen dissolution in the food materials they investigated, which according to them, led to increased oxidative loss of ascorbic acid. Furthermore, Labuza (1972) posited that elevated a_w may act to lower the activation energy for ascorbic acid destruction. Viewed from this, then, if the activation energy for antioxidant species degradation is lowered in samples stored at higher a_w due to greater amountof available water,greater reduction in antioxidant species retention should occur in those samples. This is consistent with the findings in this work.

Conclusion

In this study, percentage antioxidant activities and total phenols constituents of raw, processed and stored *Pergulariadaemia* leaves were investigated. Findings indicate that the examined samples of *Pergularia daemia* leaves exhibited antioxidant activities. Results also revealed the occurrence of phenolics in the investigated samples. It was further observed from results, thatantioxidants activities of *Pergularia daemia* were adversely affected by the investigated processing methods and storage conditions; however, the total phenols values were favoured by the investigated processing methods andstorage conditions.The noted losses in antioxidants activities and the observed increases in total phenols constituents of the stored samples were found to be progressive with storage duration. It is hoped that this findings would be taken into considerations by handlers of *Pergularia daemia*.

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