Comparative Studies of the Aqueous and Ethanolic Extracts of *Ocimum gratissimum* and *Gongronema latifolium* Leaves on the *invivo* Antioxidant levels of Wistar Albino Rats

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ABSTRACT

This study comparatively investigated the effects of aqueous and ethanolic extracts of *Ocimum gratissimum* and *Gongronema latifolium* a mono or biherbal formula on *invivo* enzymatic and non-enzymatic antioxidants of male wistar albino rats after 28 days of treatment at various doses. In this study, a total of 42 rats were used and were placed in seven groups. The LD₅₀ by oral administration carried out showed that the lethal dose for aqueous and the ethanolic extracts of *O. gratissimum* is greater than 5000mg/kg and that of the aqueous and ethanolic extracts of *G. latifolium* is 3400mg/kg. Oral administration was 200mg/kg body weight for mono-herbal and 400mg/kg body weight for the bi-herbal extracts. All value are expressed as mean $\pm$ SD at $p < 0.05$ for various test groups against the control. The result above shows that there was no statistically significant difference between test groups and control group as determined by one-way ANOVA for Malondialdehyde, Vitamin E and Vitamin C. The activity of the enzymatic antioxidants like superoxide dismutase, glutathione peroxidase, catalase and that of the non-enzymatic antioxidant such as vitamin E and vitamin C of group 1, 2, 3, 4, 5 and 6 were raised in rats fed with aqueous and ethanolic extract of *G. latifolium* and *O. gratissimum* leaf compared to the control which is group 7. From the study, it can be concluded that the *G. latifolium* extract showed greater effect on the *invivo* enzymatic and non-enzymatic antioxidant than the *O. gratissimum* leaf.

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INTRODUCTION

The perennial plant, *Ocimum gratissimum* (Scent leaf) is widely distributed in Africa and Asia. It belongs to the genus *Ocimum*. The phytochemical evaluation of this plant has shown that it is rich in alkaloid, flavonoids, phenolics, glycosides, saponins and tannins and oligosaccharides. The ethanolic extract contained majority of the active compounds like alkaloid, flavonoids, phenolics, glycosides, saponins and tannins while aqueous extract contain oligosaccharides and reducing sugar. It has tolerable cyanogenic content (Ijeh *et al.*, 2004). The volatile aromatic oil from the leaves consist mainly of thymol (32 - 65%) and eugenol. It also contains xanthenes, terpenes and lactone (Ervanshings, 1986). Characteristics of its ethanolic extracts revealed the presence of non-cyclicsequesterpenes, phenols (Ervanshings, 1986). Oil from the leaves has been found to possess antiseptics, antibacterial and antifungal activities

(Ervanshings, 1986). It is used in the treatment of fungal infection, fever, cold and catarrh. Clinical trial in creams formulated against dermatological diseases have yielded favorable result (Edeoga and Eriata, 2001). Nutritional importance of this plant centers on its usefulness as a seasoning because of its aromatic flavour (Ervanshings, 1980). *O. gratissimum* have been reported to possess free radical scavenging (antioxidant properties (Ugochukwu *et al.*, 2006, Akinmoladun *et al.*, 2007). The presence of phenols has been reported in the ethanolic extract of the plant. Some of the pharmacological activities of the plant include antiseptics, antibacterial and anti-fungal and have been proved to be very effective microbial agent at low concentration (Ervanshings, 1986).

Gongronema Latifolium ("Utazi"), is a herbaceous shrub with yellow flowers and the stem that yields a characteristics milky exudates when cut. It belongs to the family

Asclepiadaceae and known locally as “utasi” by the Efiks, Ibibios and Quas, “utazi” by the Igbos and “aroakeke” by the Yoruba’s in Nigeria. It is primarily used as spices vegetables in traditional folk (Ugochukwu and Babady, 2002). The phytochemical evaluation of this plant has shown that it is rich in alkaloid, flavonoids, phenolics, glycosides, saponins and tannins and oligosaccharides (Schneider *et al.*, 1993; Morebise *et al.*, 2002).

Free radicals including superoxide radical (O_2^-), hydroxyl radical ($OH^\cdot$), hydrogen peroxide (H_2O_2) and lipid peroxide radical are implicated in a situation where there are low activity of antioxidant and hyperlipidemia (Ugochukwu *et al.*, 2003). These reactive oxygen species (ROS) are normal consequence of biochemical process formed from the mitochondria and endoplasmic reticulum of aerobic organisms as oxygen is being reduced along the electron transport chain in the body and also as a result of increased exposure environmental xenobiotics (Allian and Miller, 1996; Cook and Samman, 1996; Kumpulainen and Salonen, 1999). Many medicinal plants including *G. Latifolium* and *O. gratissimum* have been reported to possess free radical scavenging (antioxidant) properties (Ogundipe *et al.*, 2003; Ugochukwu *et al.*, 2003; Trevisan *et al.*, 2006; Akinmoladun *et al.*, 2007). They contain several different pharmacological active compounds that may act individually additively or synergistically to improve health.

Lipid hydro peroxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. Their formation occur in enzymatic or non-enzymatic reactions involving activated chemical species known as “reactive oxygen species” (ROS) which are responsible for toxic effects in the body via various tissue damages. These ROS include among others hydroxyl radicals, lipid or peroxy radicals, singlet oxygen, and peroxynitrite formed from nitrogen oxide (NO), all these groups of atoms behave as a unit and are now named “free radical”. These chemical forms are defined as any species capable of independent existence that contains one or more unpaired electrons (those which occupy an atomic or molecular orbital by themselves). They are formed either by the loss of single electron from a non- radical or by the gain of a single electron by a non-radical. They can easily be formed when a covalent bond is broken if one electron from each of the pair shared remains with each atom, this mechanism is known as hemolytic fission. In water, this process generates the most reactive species, hydroxyl radical $OH^\cdot$ (Halliwell and Chirico, 1993). From the forgoing, this study investigated the comparative study of the Effects of aqueous and ethanolic Extracts of the *G. latifolium* and *O. gratissimum* on the *invivo* Antioxidants level of male Wistar Albino Rats.

MATERIALS AND METHOD

Collection and preparation of plant sample

The leaves of *O. gratissimum* and *G. latifolium* were collected fresh from the local Ochanga market around Onitsha, Anambra state and identified by Dr. A. Akinmbosun in the Department of Botany, University of Benin, Edo State, dried and stored in an air tight, opaque, well-sealed container

to avoid the destruction of the vitamins by sunlight dried leaves of the plants were coarsely powdered and weight taken. The *O. gratissimum* 700g and *G. latifolium* 700g of the powdered materials were soaked in one litre of ethanol and water respectively in a glass container with a plastic screwed cap and kept in a refrigerator for 48 hours at 4°C. The extracts were filtered and distilled on a water bath. The last traces of the solvent were evaporated under reduced pressure in rotary evaporator.

Feeding and administration of experimental animals

Forty two (42) male albino wistar rats weighing between 160-250g were obtained from the animal House of Anatomy, University of Benin. The animals were housed in a well-ventilated cages (wooden bottom and wire mesh top) where bedding was replaced every two (2) days and kept under controlled environment (room temperature of about 27 °C and 12 hours light/dark cycle). They were allowed to acclimatize for two (2) weeks and fed growers mash and water from tap *ad libitum*.

The rats were divided randomly into seven (7) groups of six (6) rats each.

Group 7 served as the control group while groups 1, 2, 3, 4, 5 and 6 served as the test groups.

Group 1: This group received 200mg/kg body weight of the aqueous extract of *O. gratissimum*.

Group 2: This group received 200mg/kg body weight of ethanol extract of *O. gratissimum*.

Group 3: This group received 200mg/kg body weight of aqueous extract of *G. latifolium*.

Group 4: This group received 200mg/kg body weight of ethanol extract of *G. latifolium*.

Group 5: This group received 400mg/kg body weight of aqueous extracts of both *O. gratissimum* and *G. latifolium*.

Group 6: This group received 400mg/kg body weight of ethanolic extracts of both *O. gratissimum* and *G. latifolium*.

The extracts was administered via oral gastric intubation at a dose of 200mg/kg body weight for single extract treatment and 400 mg/kg body weight combined extract treatment daily. The dosage of the extract use were according to the method of Sonia and Scrinvasan (1999) and Ebong *et al.*, (2006). The control group was given growers mash and water without treatment. The treatment lasted for 28 days (4 weeks).

Preparation of Serum

The male rats were sacrificed at the 28th day using light chloroform. The blood were taken using syringes of 5.0 ml into test tubes and allowed to stand for 30 min clotting time. It was then centrifuged at 8000xg for 10min. and the supernatant collected and used for the analysis.

Determination of phytochemicals of *O. gratissimum* and *G. latifolium*

Determination of alkaloid content was based on the method described by Chang *et al.*, (2002).

Determination of phenolic content was based on the method described by Siddhuraju and Beeker (2003).

Determination of flavonoid content was based on the method described by Zhishen *et al.*, (1999).

Determination of tannin content was based on the method described by Siddhuraj and Mancan (2007).

Determination of saponins content was based on the method described by Makkar *et al.*, (2007).

Determination of carotenoids content was based on the method described by Stalikas, (2007).

Acute toxicity study

Acute toxicity study (LD₅₀) was carried out according to the method of Lorke, (1983). The LD₅₀ was carried out according to the method employed by Lorke, (1983). A total of 52 mice, 26 for aqueous and ethanolic extract each of the two plants were used and the study was performed in two phases. In phase 1, a total of 9 mice was used, they were grouped into 3 groups of 3 mice per group. Group I received 10mg/kg extract per oral administration. Group 2 received 100 mg/kg, while group 3 received 1000 mg/kg. The animals were constantly monitored for the next 1 hrs, intermittently for the next 3hrs and finally over 9 period of 24hrs for mortality and behavioral changes. From result of the first phase, the second phase was carried out. Here a total of 4 mice was employed. They were grouped into 4 groups of one mice per group. Group 1 received 2000 mg/kg, group 2 received 3000 mg/kg, and group 3 received 4000 mg/kg while group 4 received 5000 mg/kg. The animals were monitored as in phase, for mortality and behavioral changes.

Biochemical Analysis for Enzymatic Antioxidant

Estimation of malondialdehyde was carried out by the method of Hunter *et al.*, (1963), modified by Gutteridge and Wilkins (1950).

Estimation of superoxide dismutase (SOD) content was by the method of Misra and Fridovich (1972).

Estimation of catalase activity was by the method of Cohen *et al.*, (1970).

Estimation of glutathione peroxide was by the method of Flohe and Gunzler method (1984).

Estimation of total protein was by the method of Weichselbaum, (1946).

Determination of Non-Enzymatic Antioxidant Activity

Estimation of vitamin C (ascorbic acid) content was by the method of Omaye *et al.* (1979).

Estimation of vitamin E (alpha tocopherol) content was by the method of Desai (1984).

Statistical Analysis

All data were expressed as mean±SD (and one way ANOVA used for the analysis of variance using IBM SPSS Version 21. Values were compared to control and p<0.05 was regarded as significant (Woodson, 1987).

RESULTS

The result obtained in the determination of LD₅₀ for rats fed with aqueous extract was presented in Table 1.

Table 1: The LD₅₀ result of aqueous extracts of *O. gratissimum* and *G. latifolium* leaf.

Phases	Doses (mg/kg)	Number of rats/Number of animals	behavioral
1	10	0/3	Normal
	100	0/3	Normal
	1000	0/3	Normal
2	2000	0/1	Normal
	3000	0/1	Normal
	4000	0/1	Normal
	5000	0/1	Normal

The result obtained in the determination of LD₅₀ for rats fed with ethanolic extract was presented in Table 2.

Table 2: The toxicity dose (LD50) result of ethanolic extracts of *O. gratissimum* and *G. latifolium*.

Phases	Doses (mg/kg)	Number of rats/Number of animals	behavioral
1	10	0/3	Normal
	100	0/3	Normal
	1000	0/3	Normal
2	2000	0/1	Normal
	3000	0/1	Normal
	4000	1/1	Calm
	5000	1/1	Calm

Phytochemical analysis results of ethanolic and aqueous extracts of *G. latifolium* was presented in Table 3, 4 and 5.

Table 3: Preliminary phytochemical screening of ethanolic and aqueous extracts of *G. latifolium*

Phytochemical	Ethanolic extract	aqueous extract
Reducing Sugar	+	++
Polysaccharides	+	++
Glycosides	+	+
Saponins	+	++
Phenolics	++	+
Flavonoids	+	+
Tanins	+	-
Steroids	++	-
Terpenoids	-	+
Alkaloids	++	+

Key: - = Absent; + = present, ++ = Highly present.

Table 4: Preliminary phytochemical screening of ethanolic and aqueous extracts of *O. gratissimum*

Phytochemical	Aqueous Extract	Ethanolic Extract
Reducing sugar	+	+
Polysaccharides	+	+
Glycosides	++	+
Saponins	++	+
Phenolics	-	+
Flavonoids	+	++
Tanins	+	+
Steroids	-	-
Terpenoids	-	-
Alkaloids	+	+

Key: - = Absent; + = present, ++ = Highly present.

Table 5: Comparative quantitative result of the phytochemicals found in ethanolic and aqueous extracts of *O. gratissimum* and *G. latifolium*.

Phytochemicals (%)	A ₁ (aqueous)	A ₂ (ethanol)	B ₁ (aqueous)	B ₂ (ethanol)
Alkaloids	2.43	5.03	0.36	2.47
Flavonoids	1.84	3.92	2.83	10.18
Saponins	7.47	2.08	9.07	6.82
Tannins	-	0.49	1.03	4.84
Phenolics	3.87	10.74	-	0.48
Glycosides	0.84	3.79	5.21	0.73
Steroids	-	8.43	-	-
Terpenoids	0.34	-	-	-
Reducing sugar	4.11	0.87	7.84	6.08
Polysaccharides	6.31	1.88	10.34	6.77

A₁ is the aqueous extract of *G. latifolium*, A₂ is the ethanolic extract of *G. latifolium*, B₁ is the aqueous extract of *O. gratissimum*, B₂ is the ethanolic extract of *O. gratissimum*.

Figure 1: The SOD activity in rats for different groups used in the study.

Figure 2: The glutathione peroxidase activity in rats for different groups used in the study.

Figure 3: The activity of catalase in rats for different groups used in the study.

Figure 4: The activity of malondialdehyde in rats for different groups used in the study.

The result presented in Table 6 shows a comparative result obtained from one-way ANOVA for both enzymatic and non-enzymatic antioxidants.

DISCUSSION

The LD₅₀ by oral administration carried out with the method of lorke, (1983) showed that the lethal dose for aqueous and the ethanolic extracts of *O. gratissimum* was greater than 5000mg/kg and that of the aqueous and ethanolic extracts of *G. latifolium* was 3400mg/kg (Table 1 and 2). Results obtained from the phytochemical analysis (Table 3, 4 and 5) revealed that phenols, flavonoids, alkaloids were higher in the ethanolic extracts of the leaf samples than that of aqueous extracts. This could be because they are more soluble in ethanol than water.

Table 6: Comparative results of the enzymatic and non-enzymatic antioxidants

GP	SOD Unit/g.	GLUT. P. unit/g.	CAT. unit/g.	MDA unit/g.	VIT.E Mg/dl	VIT. C Mg/ml
1	0.05 ±0.00	0.02 ±0.00	0.71 ±0.16	0.00 ±0.00	567 ±46	0.03 ±0.02
2	0.06 ±0.01	0.02 ±0.00	1.56 ±0.28	0.00 ±0.00	578 ±68	0.02 ±0.00
3	0.06 ±0.00	0.02 ±0.00	0.78 ±0.24	0.00 ±0.00	524 ±88	0.02 ±0.00
4	0.06 ±0.01	0.03 ±0.00	1.14 ±0.59	0.00 ±0.00	589 ±59	0.02 ±0.00
5	0.03 ±0.00	0.04 ±0.00	0.61 ±0.29	0.00 ±0.00	462 ±49	0.02 ±0.00
6	0.06 ±.001	0.02 ±0.00	1.13 ±0.14	0.00 ±.00	523 ±237	0.02 ±0.00
7	0.03 ±.001	0.01 ±0.00	0.48 ±-0.19	0.00 ±.00	508 ±52	0.02 ±0.00

Figure 5: The concentration of vitamin E in rats for different groups used in the study. **Figure 6: The vitamin c concentration in rats for different groups used in the study.**

Again, saponins, reducing sugars and polysaccharides were high in the aqueous extracts than in the ethanolic extracts. The aqueous extract of *G. latifolium* leaf contained more quantity of the phytochemicals like flavonoids, saponins, tannins, glycosides, reducing sugar, polysaccharides than that of *O. gratissimum*. This is in agreement with that reported by Morebise *et al.*, (2002), who reported that aqueous extract of *G. latifolium* contained more phytochemicals than *O. gratissimum*.

Results obtained from the antioxidant analysis effects on superoxide dismutase (SOD) activity (Table 6) revealed that rats fed with aqueous and ethanolic extracts of the individual leaf samples exhibited higher activity at $p < 0.05$ than the control (Fig. 1). The ethanolic and aqueous extract of the bi-herbal mixture of both plant extracts fed to rats in groups 5 and 6 have higher activity than the mono-herbal aqueous and ethanolic extract of each of the extracts fed to rats in groups 1, 2, 3 and 4. From the SOD analysis, it shows that the ethanolic extracts are more effective than the aqueous extracts of either plants. This is in agreement with that reported by Ugochukwu *et al.* (2003), who suggested that ethanolic extracts are more effective than the aqueous extracts on SOD. The analysis also shows that extracts of *G. latifolium* is more effective than *O. gratissimum*.

In the glutathione peroxidase (GLUT.P) assay (Fig. 2), there were significant glutathione peroxidase activities compared to the control. The mean glutathione peroxidase concentration of group 2 was not significantly higher than group 1 when compared to group 7. In the group 3 fed with the aqueous extract of *G. latifolium* and group 4 fed the ethanolic extract of *G. latifolium*, the mean glutathione

peroxidase value of group 4 was significantly higher than group 3 when compared to group 7. In the group 5 fed with the aqueous extract of *G. latifolium* with *O. gratissimum* and group 6 fed the ethanolic extract of *G. latifolium* with *O. gratissimum* leaf, the mean glutathione peroxidase concentration of group 5 was significantly higher than group 6 when compared to group 7. Here, the result shows that ethanolic mono-herbal formula of either plant was more effective than its aqueous mono-herbal formula of either plant. Again, the ethanolic bi-herbal formula of *G. latifolium* and *O. gratissimum* fed to rats in group 6 was low in activity compared to groups 2, 3, 4 and 5 fed aqueous mono-herbal formula, ethanolic mono-herbal formula, aqueous mono-herbal formula and bi-herbal formula respectively. This shows that there could be complication resulting from herbal combination. This is in line with the report of Nwanchukwu and Iweala (2014). They reported similar result in their publication.

In catalase (CAT) analysis (Fig. 3), rats fed with the various concentrations of the both plant extracts (aqueous and ethanolic) and bi-herbal extracts exhibited a significant higher activities than the control which is group 7. In the group 1 fed the aqueous extract of *O. gratissimum* and group 2 fed the ethanolic extract of *O. gratissimum*, the mean catalase (CAT) values of group 2 was significantly ($p < 0.05$) higher than group 1 when compared to the control. In the group 3 fed the aqueous extract of *G. latifolium* and group 4 fed the ethanolic extract of *G. latifolium*, the mean catalase (CAT) values of group 4 was not significantly higher than group 3 when compared to group 7. The effect of the various extracts on the activity of malondialdehyde in rats for different groups (Fig. 4) indicates that there were less malondialdehyde in the various groups to compare with control. This is an indication of antioxidant activities in preventing lipid peroxidation.

Analysis involving vitamin E revealed that rats fed with the various concentrations of extracts of both plant extracts shows that they were not significantly higher than the control which is group 7 (Fig. 5). In the group 1 fed with aqueous extract of *O. gratissimum* and group 2 fed the ethanolic extract of *O. gratissimum*, the mean concentration of vitamin E of group 2 was not significantly higher than group 1 when compared to group 7. In the group 3 fed with aqueous extract of *G. latifolium* and group 4 fed with ethanolic extract of *G. latifolium*, the mean concentration of vitamin E concentration of group 4 was not significantly higher than group 3 when compared to group 7. In the group 5 fed the aqueous extract of *G. latifolium* with *O. gratissimum* and group 6 fed the ethanolic extract of *G. latifolium* with *O. gratissimum*, the mean concentration of vitamin E of group 6 was not significantly higher than group 5 when compared to group 7. From this analysis, there is suggestion that the ethanolic extract of the plant are more effective than the aqueous extract of the plants. Again, the aqueous extracts bi-herbal

combination of *O. gratissimum* and *G. latifolium* seem to be less effective than the ethanolic extracts bi-herbal combination of *O. gratissimum* and *G. latifolium*. From this, it could be suggested that the aqueous bi-herbal combination of *O. gratissimum* and *G. latifolium* is less effective in enhancing the activity of vitamin E than the mono-herbal aqueous and ethanolic of each plant. This could result from complication of the combined plant extract. This is in line with the report of Nwanchukwu and Iweala, (2014). They reported similar result in their work.

In vitamin C analysis, rats fed with the various extracts revealed that Vitamin C concentrations were not significantly higher than the control which is group 7 (Fig. 6). In the group 1, rats fed the aqueous extract of *O. gratissimum* and group 2 fed with ethanolic extract of *O. gratissimum*, the mean concentration of vitamin C of group 1 was not significantly higher than group 2 when compared to group 7. In the group 3 fed with aqueous extract of *G. latifolium* and group 4 fed with ethanolic extract of *G. latifolium*, the mean concentration of vitamin C concentration of group 3 was not significantly higher than group 4 when compared to group 7. In the group 5 fed the aqueous extract of *G. latifolium* with *O. gratissimum* and group 6 fed with ethanolic extract of *G. latifolium* with *O. gratissimum*, the mean concentration of vitamin c of group 5 was not significantly higher than group 6 when compared to group 7. From this analysis, it could be suggested that the vitamin C ethanolic extracts of the plants are less effective than the vitamin C aqueous extract of the plants. This could be that the aqueous extract of the plant contain phytochemicals that support the activity of vitamin C. This is in line with the report of Halvoresen *et al.*, (2006), who reported similar observation. Again, the aqueous extract of bi-herbal combination of *O. gratissimum* and *G. latifolium* seem to be more effective in supporting the vitamin c activity than the ethanolic extracts bi-herbal combination of *O. gratissimum* and *G. latifolium* and so, it could be suggested that *O. gratissimum* have more potential of enhancing the vitamin c activity. It is clear that *invivo* antioxidant enzymes like superoxide dismutase, catalase and the glutathione peroxidase are more effective when present in the ethanolic extract than in aqueous extract but the reverse is the case when it comes to non-enzymatic antioxidants like vitamin C and E.

CONCLUSION

The result reveals that the ethanolic extracts of *O. gratissimum*, and *G. latifolium* were more active increasing the enzymatic and non-enzymatic antioxidants than the aqueous extract of the two leaves. The bi-herbal extract of both leaves shows higher activity on the enzymatic and non-enzymatic antioxidants than the mono-herbal formula of either leaves when fed to the rats. An extension of this work needs to be carried out to determine the toxicity level of the bi-herbal extract on animal and human subjects.

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