International Journal of Biochemistry, Bioinformatics and Biotechnology Studies
Vol.6, No.1, pp.30-54, 2021
Print ISSN: 2397-7728,
Online ISSN: 2397-7736

COMPARATIVE STUDY OF ANTIOXIDANT AND ANTIPYRETIC ACTIVITIES OF LEAF EXTRACTS OF *OCIMUM GRATISSIMUM* AND GONGRONEMA *LATIFOLIUM*[*]

Chukwunwike Eze , Gloria N. Okwu, Chinwe S. Alisi.
Department of Biochemistry, Federal University of Technology, Owerri, Imo State, Nigeria.

ABSTRACT*: This study investigated the antioxidant and antipyretic activities of ethanolic leaf extracts of Ocimum gratissimum and Gongronema latifolium. Although non-steroidal anti-inflammatory drugs, opiates and various synthetic drugs have been used classically in these conditions, but some adverse reactions occur with these drugs such as gastrointestinal disturbances, renal damage, respiratory diseases and possible dependence. In search of inexpensive but effective and readily available drugs, with little or no side effects, several investigations have been conducted on indigenous plant materials. Antioxidant and antipyretic study was done using a total of 56 Wistar albino rats, divided into 7 groups of 4 rats per group, each groups administered different concentration of extract and standard. Oxidative stress biomarkers such as glutathione, superoxide dismutase, malondialdehyde and catalase were used to determine antioxidant activity in acetaminophen induced oxidative stress in rats while rectal temperature measurement was used to determine the antipyretic effect of these leave extracts in baker yeast induced pyrexia. The results from the antioxidant analysis in comparison to leave extracts of Ocimim gratissimum and Gongronema latifolium, showed that bi-herbal combined ratio of (Ocimum gratisssimum + Gongronemalatifolium) showed a higher antioxidant and antipyretic activity followed by Ocimum gratissimum, and Gongronema latifolium respectively. In conclusion, Ocimum gratissimum and Gongronema latifolium leaves have antioxidant and antipyretic potentials, which could be harnessed for clinical applications***.**

KEYWORDS: **antioxidant, antipyretic, leave extracts, ocimum gratissimum, gongronema, latifolium**

INTRODUCTION

Aerobic organs such as the liver generate reactive oxygen species that induce oxidative tissue damage. These radicals, which react with cell membranes and thus induce lipid peroxidation or cause inflammation, have been implicated as important pathological mediators in many clinical disorders such as heart disease, diabetes, gout and cancer. A major defence mechanism is the antioxidant enzymes, which convert active oxygen molecules into non-toxic compounds. Oxidative stress is a condition of excess formation and insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Cade, 2008; Giacco and Brownlee, 2010).

Pyrexia or fever is a disease caused as a secondary impact of infection, malignancy or other diseased states (Sharm et al., 2010; Chattopadhaya et al., 2005). It is the body's natural function to create an environment where infectious agents or damage to tissue cannot survive (Chattopadhyay et al., 2005). Normal body temperature is regulated by centres in the hypothalamus that ensures balance between heat loss and heat

gain. Fever occurs where there is a disturbance of this hypothalamic "thermostat" which therefore leads to the set point of the body temperature being raised.

The increasing prevalence of oxidative stress diseases all over the world and the extreme discomfort inherent in pyrexia from different causes poses a medical concern. The available synthetic drugs are not free from numerous side effects. However, there is need for more focus on use of natural therapy in the form of medicinal plants, which are generally considered to be safe and effective agents. Plants have been used as a source of antioxidant and antipyretic agent from time immemorial, these naturally occurring sources contain phytochemicals or secondary metabolites that may have beneficial properties (Bewaji et al., 1985), but due to discovery of chemical antioxidant and antipyretic agents they were neglected. Fortunately, due to various reasons low cost, easy access and reduced side effects, there is therefore revisitation to herbal medicines (Graz et al., 2011).

Clinical trials have shown that Silymarin exerts hepato-protective effects in acute viral hepatitis, poisoning by *Amanita phalloides*, ethanol, paracetamol, and carbon tetrachloride. Many studies have demonstrated the beneficial hepatoprotective effects when treatment with Silymarin. Also, a comparative study of plant extracts is necessary, to provide information on their efficacy independently or in combined ratio.
Ocimum gratissimum has been reported to be useful in treatment of diarrhea, hypertention, bacterial infection and diabetes. *Gongronema latifolium* has also been reported to be medically useful as an anthelmintic and antidiabetic agent. It is to this end that the ability of ethanolic leaf extracts of *Ocimum gratissimum* and *Gongronema latifolium* as an antioxidant and antipyretic agent is being investigated.

METHODS

Method of Sample Collection

The leaves of each plant (*Ocimum gratissimum* and *Gongronema* latifolium) was washed clean with distilled water, oven dried to a constant weight and then grinded into powder. The ethanol extract of the leaves of each plant was made by soaking 100g of dried powdered samples in 500ml of 70% ethanol and left for 72hours. After 72hours, the extract was filtered using Whatman no1 filter paper. The filtrate was heated in water bath at 40^0c to concentrate it better and allow the ethanol to evaporate. The crude extract was stored in the refrigerator until when ready for use.

Qualitative Determination of Certain Phytochemicals

Phytochemcial screening was carried out using standard procedures to identify the constituents as describe by Sofowara (1993); Okwu, (2005) and Ladipo, (2010). Phytochemicals such as tannins, phlobatanins, saponins, flavonoids, cardiac glycosides, terpenoids, steroids and alkaloids were determined. List of phytochemicals determined was shown in "**Table 3 and 4**".

Toxicity Test (LD_{50})

Ten animals were divided into five groups of two animals each. Each group were administered different doses (500, 1000, 2000, 4000, 5000mg/kg) of plant extract. The animals were observed for mortality after 24hours. LD_{50} was calculated as $\sqrt{(D_0 \times D_{100})}$
D_0 = highest dose that gave no mortality, D_{100}= lowest dose that produced mortality.

Antioxidant Activity

The animals were divided into seven (7) groups of four (4) rats per group. Oxidative stress biomarker activity of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA) were determined 18hours after 750mg/kg acetaminophen toxicity on the 7th day of treatments administration orally. As shown in "**Table 1**".

Antipyretic Activity

The 24hours fasted rats were divided into seven (7) groups of four rats per group. Rectal temperature of each rat was taken using a rectal thermometer, after which each animal received subcutaneous 10ml/kg of aqueous yeast suspension to elevate body temperature.
Eighteen hours post yeast injection, 150mg/kg acetaminophen and other group treatment were administered. Rectal temperature of each animal was recorded at 1hour, 2hours, 3hours, 4hours after drug and extract administration. As shown in "**Table 2**", with results in "**Table 9**" and "**Figure 5**".

Preparation of Liver Homogenate

The isolated liver was used for the preparation of liver homogenates

1. Liver homogenate in potassium chloride (0.15M)

A 10% w/v liver homogenate was prepared in 0.15M potassium chloride buffer and centrifuged at 8000rpm for 10mins. The supernatant solution was used for estimation of malondialdehyde and catalase.

2. Liver homogenate in sucrose in phosphate buffer pH (7.4)

A 10% w/v liver homogenate was prepared using 0.25% sucrose in 5M phosphate buffer, centrifuged at 8000rpm for 10mins. The supernatant solution was used for estimation of superoxide dismutase and reduced glutathione.

Estimation of Superoxide Dismutase (SOD)

SOD was assayed according to the method of Kano. The method is based on the reduction of nitroblue tetrazolium (NBT) to dark blue insoluble precipitate by the formation of nitrite in the presence of EDTA. To 100µl of 10% w/v liver homogenate in sucrose with phosphate buffer, 1ml of sodium carbonate, 400µl NBT and 200µl EDTA were added, and zero minute reading was taken at 560nm after the addition of 400µl of hydroxylamine hydrochloride. The mixture was incubated for 5mins and the reduction of NBT was measured after 5mins at 560nm. SOD was expressed as units/g of liver tissue. As shown in "**Table 6**" and "**Figure 2**".

Estimation of Reduced Glutathione (GSH)

Glutathione was assayed according to the method of Paglia. Glutathione was determined using Ellman's reagent (5,5-dithios-2-nitrobenzoic acid/DTNB). 1ml of 10% w/v liver homogenate in sucrose in phosphate buffer was treated with 1.8ml of distilled water and 2ml of phosphate buffer (pH 7) and mixed well. 5mins after adding 200µl of DTNB reagent to the reaction mixture, absorbance due to the yellow coloured complex formed by sulphydryl group of glutathione was read at 412nm. Expressed as ug/mg of liver tissue. As shown in "**Table 8**" and "**Figure 4**".

Estimation of Catalase (CAT)

Catalase was assayed according to the method of Aebi. Catalase activity was measured by decomposition of H_2O_2. 100µl of 10% liver homogenate in 0.15M potassium chloride buffer was mixed with 1.9ml of phosphate buffer (pH 7). To the mixture, 1ml of 10mM hydrogen peroxide was read at 240nm against phosphate buffer as blank. The decrease in absorbance at the end of 1min after the addition of 1ml of hydrogen peroxide was again read. The specific activity of catalase was expressed in terms of units/gram of liver tissue. As shown in "**Table 7**" and "**Figure 3**".

Estimation OF Lipid Peroxidation (TBARS)

Lipid peroxidations in liver tissues were estimated by method of Jose. The extent of lipid peroxidation was determined by the reaction of sample lipid peroxides (MDA) with thiobarbituric acid (TBA) in acidic condition to form a pink colour chromophore, which was measured at 532nm. 500µl liver homogenate in potassium chloride buffer was added to 1ml of TBA:TCA:HCL reagent (containing 0.38% TBA, 15% TCA and 0.25 HCl) and boiled for 15mins and cooled.

The mixture was then centrifuged at 10,000rpm for 5mins. Absorbance of the supernatant solution was measured at 532nm against reagent blank. The levels of lipid peroxides were expressed as millimoles of thiobarbituric acid reactive substances (TBARS)/100gram of liver tissue. As shown in "**Table 5**" and **"Figure 1**"

Table 1: Antioxidant Determination Groupings

Groups	Administration
1	Normal feed and clean water (control)
2	Silymarin 25mg/kg only for 7days
3	250mg/kg of treatment of *Ocimum gratissimum* for 7days
4	250mg/kg treatment of *Gongronema latifolium* for 7days
5	500mg/kg treatment of *Ocimum gratissimum* for 7days
6	500mg/kg treatment of *Gongronema latifolium* for 7days
7	500mg/kg treatment of *Ocimum gratissimum* and *Gongronema latifolium* for 7days

Table 2: Antipyretic determination groupings

Groups	Administration
1	Normal saline (control)
2	150mg/kg acetaminophen
3	200mg/kg of treatment of *Ocimum gratissimum*
4	200mg/kg treatment of *Gongronema latifolium*
5	400mg/kg treatment of *Ocimum gratissimum*
6	400mg/kg treatment of *Gongronema latifolium*
7	400mg/kg treatment of *Ocimum gratissimum*and *Gongronema latifolium*

Table 3: Qualitative result of phytochemicals found in ethanolic leaf extract of *Ocimum gratissimum*.

Phytochemicals	*Ocimum gratissimum*
Alkaloids	+
Flavonoids	++
Saponins	+
Tanins	+
Phlobatanin	+
Glycosides	+
Steroids	+
Terpenoids	-

Strong presence, +: Present, -: Absent.

Table 4: Qualitative result of phytochemicals found in ethanolic leaf extract of *Gongronema latifolium*.

Phytochemicals	*Gongronema latifolium*
Alkaloids	++
Flavonoids	+
Saponins	+
Tanins	+
Phobatanin	+
Glycosides	+
Steroids	++
Terpenoids	-

++: Strong presence, +: Present, -: Absent.

Values are mean ± standard deviation.
Values with different superscripts belong to different subsets and are therefore significantly different ($p<0.05$) from one another.

Table 5: Effect of leaf extracts of Ocimum gratissimum and Gongronema latifolium on Malondialdehyde level.

Groups and treatment	Dose	MDA (mM/100gm of liver)
Group1- Feed and clean water		2.51 ± 0.03^{g}
Group2- silymarin (standard)	25mg/kg	0.71 ± 0.03^{a}
Group3- *Ocimum gratissimum*	250mg/kg	1.56 ± 0.05^{de}
Group4- *Gongronema latifolium*	250mg/kg	1.64 ± 0.04^{e}
Group5- *Ocimum gratissimum*	500mg/kg	1.20 ± 0.04^{c}
Group6- *Gongronema latifolium*	500mg/kg	1.46 ± 0.04^{d}
Group7- *Ocimum gratissimum*+ *Gongronema latifolium*	500mg/kg	1.11 ± 0.03^{b}

Values are mean ± standard deviation.
Values with different superscripts belong to different subsets and are therefore significantly different ($p<0.05$) from one another.

Table 6: Effect of leaf extracts of Ocimum gratissimum and Gongonema latifolium on superoxide dismutase level.

Groups and treatment	Dose	SOD (U/g of liver tissue)
Group1- Feed and clean water		12.29 ± 0.34^{f}
Group2- silymarin (standard)	25mg/kg	74.40 ± 2.23^{a}
Group3- *Ocimum gratissimum*	250mg/kg	30.06 ± 1.41^{e}
Group4- *Gongronema latifolium*	250mg/kg	28.34 ± 1.08^{e}
Group5- *Ocimum gratissimum*	500mg/kg	54.47 ± 1.49^{c}
Group6- *Gongronema latifolium*	500mg/kg	51.02 ± 0.85^{d}
Group7- *Ocimum gratissimum*+ *Gongronema latifolium*	500mg/kg	58.14 ± 1.16^{b}

Values are mean ± standard deviation.
Values with different superscripts belong to different subsets and are therefore significantly different ($p<0.05$) from one another.

Table 7: Effect of leaf extracts of *Ocimum* gratissimum and *Gongonema latifolium* on catalase level.

Groups and treatment	Dose	CAT (U/g of liver tissue)
Group1- Feed and clean water		48.05±1.19[a]
Group2- silymarin (standard)	25mg/kg	78.99 ±2.01[e]
Group3- *Ocimum gratissimum*	250mg/kg	61.99 ± 1.13[bc]
Group4- *Gongronema latifolium*	250mg/kg	59.10 ± 0.98[b]
Group5- *Ocimum gratissimum*	500mg/kg	66.72 ± 2.09[d]
Group6- *Gongronema latifolium*	500mg/kg	64.36 ± 1.55[d]
Group7- *Ocimum gratissimum*+ *Gongronema latifolium*	500mg/kg	68.01 ± 0.47[c]

Values are mean ± standard deviation.
Values with different superscripts belong to different subsets and are therefore significantly different ($p<0.05$) from one another.

Table 8: Effect of leaf extracts of *Ocimum gratissimum* and *Gongonema latifolium* on Glutathione level.

Groups and treatment	Dose	GSH (ug/mg of liver tissue)
Group1- Feed and clean water		15.07 ± 0.88a
Group2- silymarin (standard)	25mg/kg	43.44 ± 0.41^{b}
Group3- *Ocimum gratissimum*	250mg/kg	34.19 ± 1.03^{c}
Group4- *Gongronema latifolium*	250mg/kg	32.42 ± 0.45^{d}
Group5- *Ocimum gratissimum*	500mg/kg	39.00 ± 0.22^{e}
Group6- *Gongronema latifolium*	500mg/kg	36.75 ± 0.77^{f}
Group7- *Ocimum gratissimum*+ *Gongronema latifolium*	500mg/kg	41.05 ± 1.15^{g}

Values are mean ± standard deviation.
Values with different superscripts belong to different subsets and are therefore significantly different ($p<0.05$) from one another.

Table 9: Antipyretic effect of leaf extracts of Ocimum gratissimum and Gongronema latifolium

Groups and treatment	**Dose**	**Basal rectal temperature (ºC)**	**1hour (ºC)**	**2hours (ºC)**	**3hours (ºC)**	**4 hour (ºC)**
Group1- Feed and clean water		37.07 ± 0.17[a]	38.04 ± 0.18[ab]	38.30 ± 0.09[a]	38.85 ± 0.15[a]	38.93 ± 0.17[a]
Group2 – Acetaminophen	150mg/kg	37.03 ± 0.2[a]	37.75 ± 0.15[c]	37.64 ± 0.22[cd]	37.45 ± 0.07[b]	37.05 ± 0.08[c]
Group3- *Ocimumgratissimum*	200mg/kg	36.95 ± 0.14[a]	37.98 ± 0.18[ab]	37.78 ± 0.14[bcd]	37.51 ± 0.19[b]	37.25 ± 0.13 [b]
Group4- *Gongronemalatifolium*	200mg/kg	37.03 ± 0.66[a]	38.17 ± 0.22[ab]	37.88 ± 0.11[b]	37.56 ± 0.24 [b]	37.32 ± 0.17 [b]

Group5- *Ocimumgratissimum*	400m g/kg	37.04 ± 0.34[a]		38.01 ± 0.30[ab]	37.77 ± 0.13[bcd]	37.41 ± 0.13 [b]	37.16 ± 0.15 [b]	
Group6- *Gongronemalatifolium*	400m g/kg	37.03 ± 0.45[a]		38.22 ± 0.12[a]	37.83 ± 0.23[bc]	37.46 ± 0.13 [b]	37.22 ± 0.25 [b]	
Group7- *Ocimumgratissimum+ Gongronemalatifolium*	400m g/kg	37.02 ± 0.12[a]		38.09 ± 0.21[ab]	37.60 ± 0.12[d]	37.31 ± 0.15 [b]	37.09 ± 0.17 [b]	

Values are mean ± standard deviation.
Values with different superscripts belong to different subsets and are therefore significantly different ($p<0.05$) from one another.

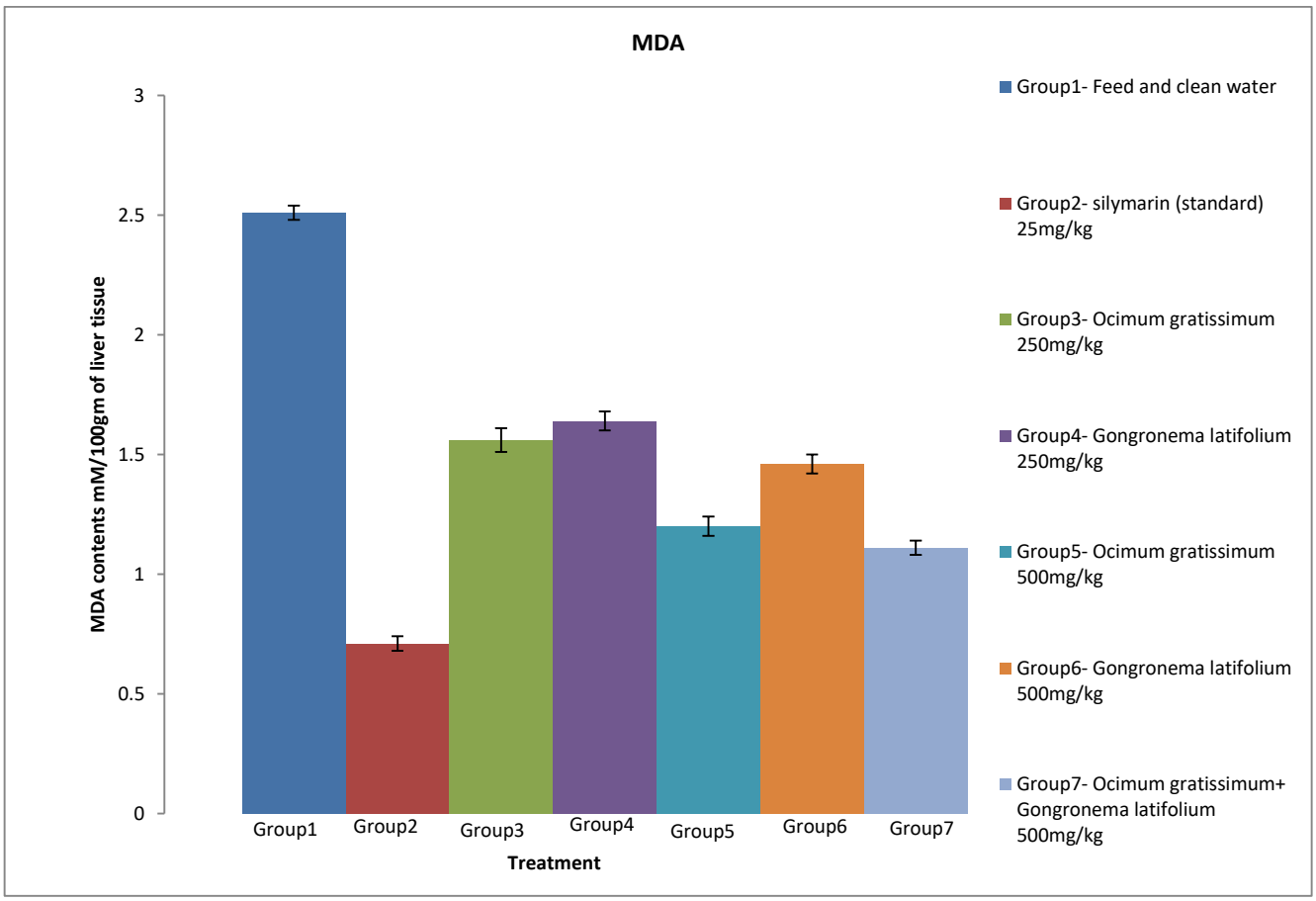

Figure 1: Effect of leaf extracts of *Ocimum gratissimum* and *Gongronema latifolium* on malondialdehyde level (Graphical presentation).

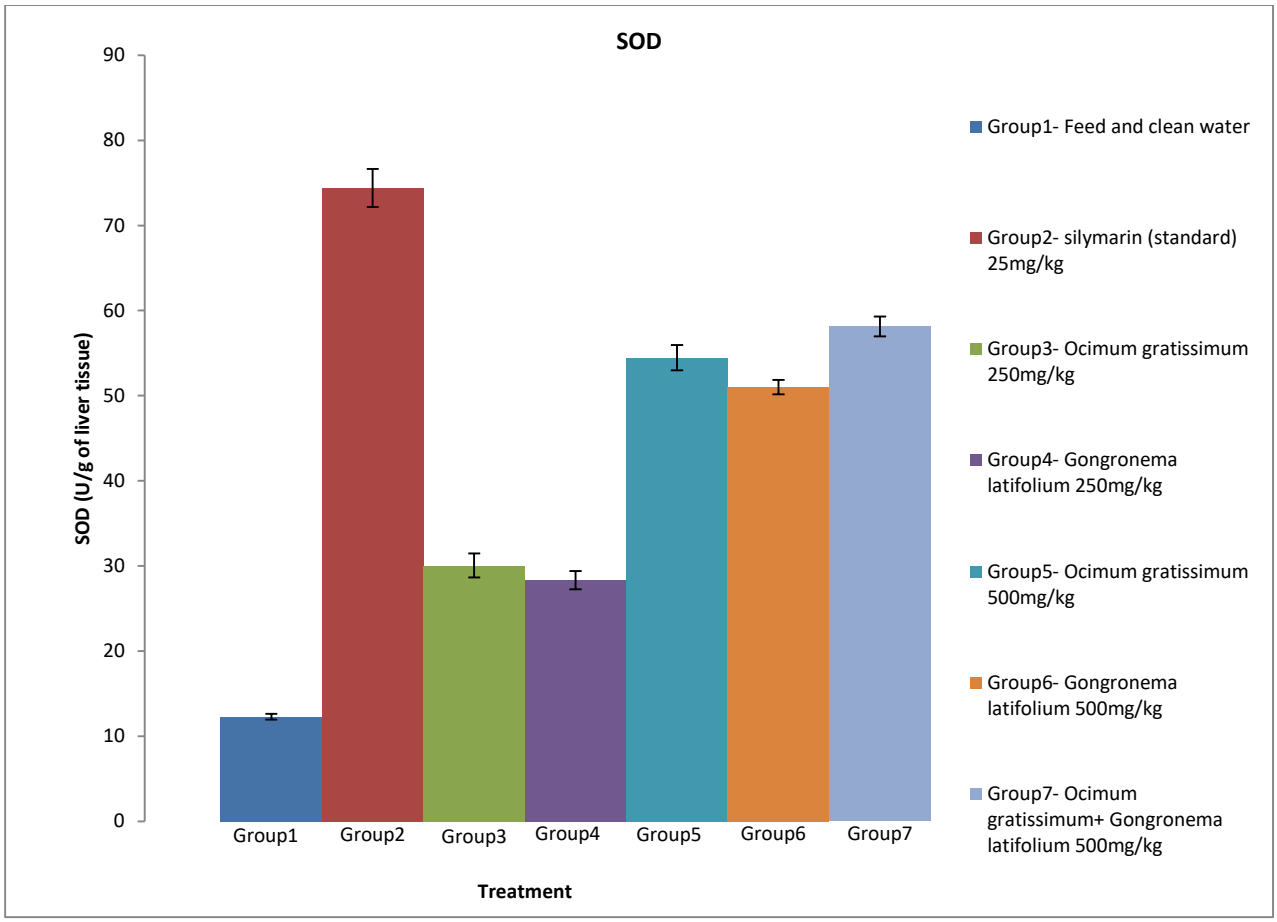

Figure 2: Effect of leaf extracts of *Ocimum gratissimum* and *Gongronema latifolium*on superoxide dismutase level (Graphical presentation).

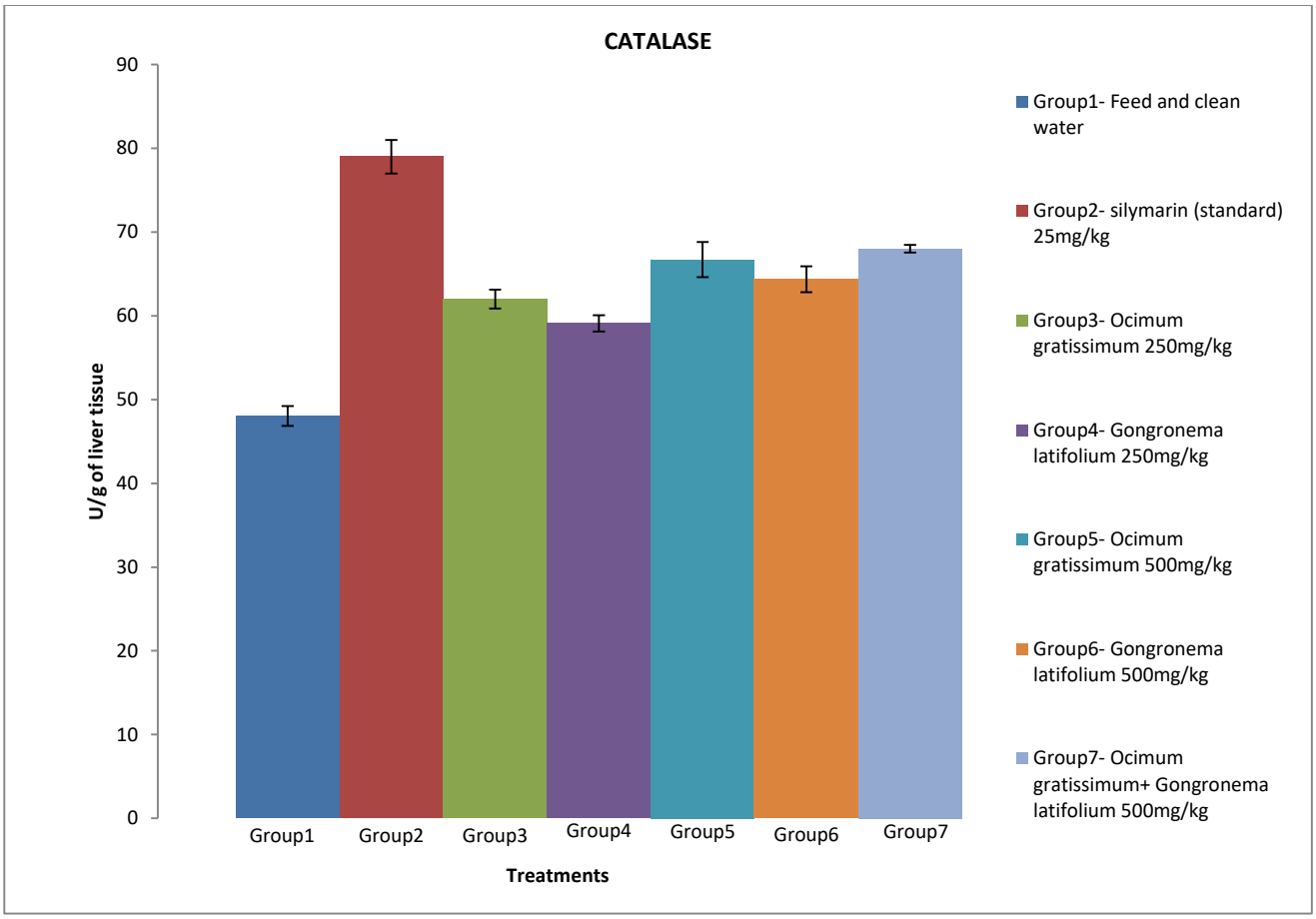

Figure 3: Effect of leaf extracts of *Ocimum gratissimum* and *Gongronema latifolium*on catalase level (Graphical presentation).

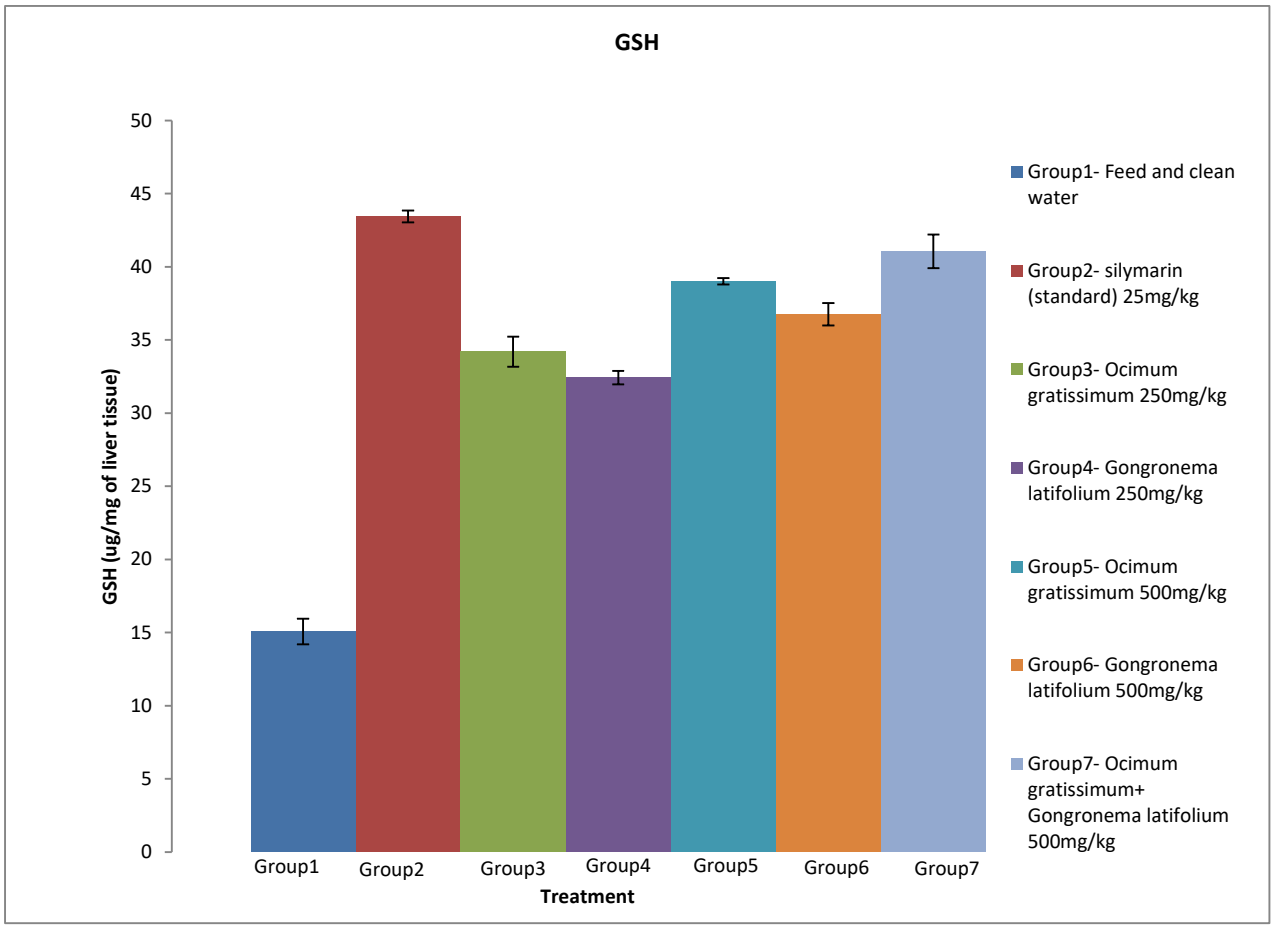

Figure 4: Effect of leaf extracts of *Ocimum gratissimum*and *Gongronema latifolium* on glutathione level (Graphical presentation)

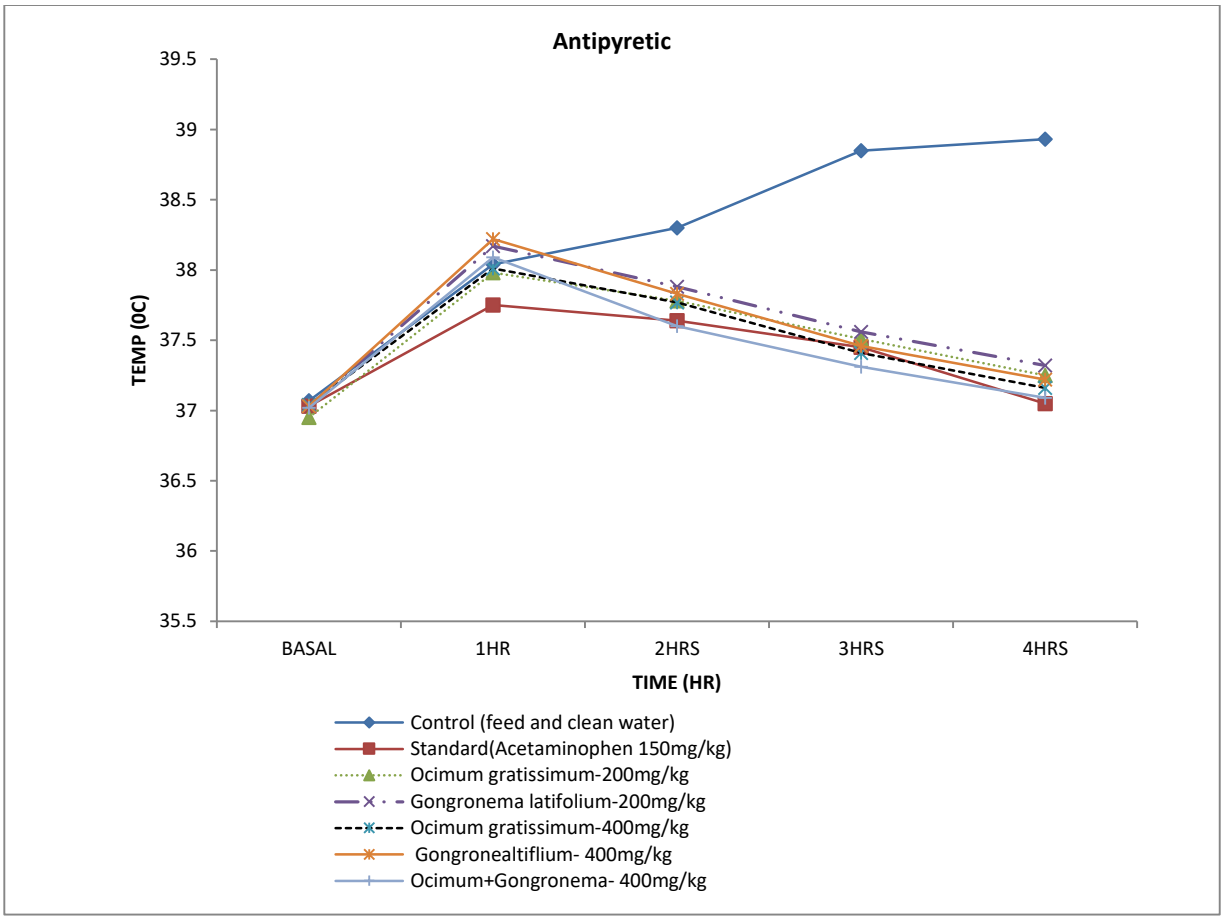

Figure 5: Antipyretic effect of leaf extracts of *Ocimum gratissimum* and *Gongronema latifolium* (Graphical presentation).

DISCUSSION

Ocimum gratissimum commonly known as scent leaf or nchanwu and *Gongronema latifolium* commonly known as utazi has been found to possess various medicinal properties, such as anti-bacterial, anti-nociceptive and anti-hypertensive effects (Prabhu *et al*., 2009 and Omodamiro *et al*., 2015).

This present experiment studued the antioxidant and antipyretic effects of Ocimum gratissimum and Gongronema latifolium ethanolic leaf extracts on acetaminophen induced oxidative stress and bakers yeast induced pyrexia.

The phytochemical screening of Ocimum gratissimum and Gongronema latifolium reveals the presence of saponins, alkaloids, flavonoids, phenols, tannins, glycosides and steroids. These results were similar with

those of Alexander P., (2016) and Okoli *et al*., (2017). These metabolites are known to have varied pharmacological actions in man and animals, and their presence suggest great potentials of the plant source of useful phytomedicines (Sofowara A., 1993).

Alkaloids are also considered as nitrogenous bases that occur in plants, many of them have marked physiological effects on humans. Some alkaloids used as medicines are morphine, caffeine and coffee, in which caffeine intea and coffee are alkaloids that stimulate the central nervous system (Stanley *et al*., 2007). The presence of alkaloids suggests that it has possesses antimicrobial and anti-malarial potentials (Stanley *et al*., 2007).

Flavonoids are polyphenolics that contribute to many plant leaves and fruits colours found in nature. Plant phenolics, especially flavonoids have antioxidant properties (Bohn *et al*., 1998), anti-inflammatory effects on both acute and chronic inflammation (Boham and Kocipai, 1994). Flavonoids were found in Ocimum gratissimum and gongronema latifolium.

Saponins are a class of chemical compounds found in abundance particularly in various plant species. Normal intake of saponin is not toxic to humans. Saponins also protect against microbial attack (Sheikh *et al*., 2013). Appreciable quantities of saponins were found in *Ocimum gratissimum* and *Gongronema latifolium*.

Steroids play a role in metabolism control, inflammation, immune functions etc. Appreciable quantities of steroids were found in Ocimum gratissimum and Gongronema latifolium.

Overdosage of acetaminophen can be used to induce toxicity in mice in the form of liver and kidney damage (Placke *et al.*, 1987). The enzymatic antioxidant defence system is the natural protector against lipid peroxidation. SOD, CAT and GPx enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage (Scott et al., 1991). In this present study, it was observed that ethanolic leaf extracts of *Ocimum gratissimum* and *Gongronema latifolium* significantly increased SOD and catalase activity in a dose-dependent manner. This is in line with antioxidant study done by Deepak *et al.*, (2007) and Uzma *et al*., (2014).

Glutathione, a non-enzymatic antioxidant is one of the most abundant tripeptides present in the liver. Its functions are mainly the removal of free radical species such as hydrogen peroxide, superoxide radicals, alkoxy radicals, and maintenance of membrane protein thiols and as substrate for glutathione peroxidase and GST (Prakash *et al*., 2001). Administeration of ethanolic leaf extracts of *Ocimum gratissimum* and *Gongronema latifolium* increased the concentration of glutathione in a dose-dependent manner. This is in line with antioxidant study done by Deepak *et al*., (2007), but in disagreement with the antioxidant study done by Uzam *et al*., (2014). The decrease in glutathione activity with increased dosage from study of Uzma *et al*., (2014) could be due to a toxic effect of Euphorbia helioscopia at a higher dosage of 1200mg/kg.

Lipid peroxidation has been postulated to the destructive process of liver injury due to the acetaminophen adminiseration. Increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation,

leading to tissue damage and failure of the antioxidant defence mechanisms to prevent formation of excessive free radicals. Treatment with *Ocimum gratissimum* and *Gongronema latifolium* significantly reversed these changes, leading to a reduction in concentration of MDA in a dose-dependent manner. This is in line with the Antioxidant study done by Deepak *et al*., (2007) and Henry *et al*., (2015), but there was inconsistency with the work done by Uzam et al., (2014), which showed dose-dependent increase in MDA concentration in latex extract of *Euphorbia helioscopia*, but not in its leaf extract. This could be as a result of some toxic activity at a higher dose in its latex extract administration.

Higher antioxidant activity was observed in leaf extract of Ocimum gratissimum than Gongronema latifolium, with highest activity observed with bi-herbal extract administration (500mg/kg) of both plant extracts. It could be inferred that the bi-herbal extract administration is a more potent formulation.

Significant dose-dependent antipyretic activity was also observed in both leaf extracts in this study, with optimum antipyretic activity observed at 4hours post extract treatment. The bi-herbal extract concentration (400mg/kg) was a more potent formulation to mono-herbal concentration of *Ocimum gratissimum* and *Gongronema latifolium* respectively. This is in line with the antipyretic study done by Ukwani *et al*.,(2012) and Gege-Adebayo *et al*., (2013).

It is currently accepted that prostaglandin E_2 (PGE_2) is the final fever mediator in the brain, specifically in the preoptic area of the anterior hypothalamus (Li *et al*., 2008). Antipyretics such as acetaminophen and other non-steroidal anti-inflammatory drugs ($NSAID_S$) reduce fever by suppressing peripherial production of interleukin -1β, while consecutively lowering the thermoregulatory set point by blocking central cyclooxygenase formation of prostaglandin E_2. Thus, it can be inferred that Ocimum gratissimum and Gongronema latifolium inhibits the synthesis of prostaglandins.

CONCLUSION

The ethanolic extracts of *Ocimum gratissimum* and *Gongronema latifolium* possess biologically active compounds with antioxidant and antipyretic potentials.
The bi-herbal extract concentration of both leaves showed higher antipyretic and antioxidant efficacy on enzymatic and non enzymatic antioxidants, therefore, it is a more potent formulation. *Ocimum gratissimum* and *Gongronema latifolium* having shown antioxidant and antipyretic activities could be useful in its subsequent development for clinical applications in the management of oxidative stress related diseases, such as diabetes, cancer, Alzheimer's disease, Parkinson's disease, cardiovascular conditions etc and symptomatic management of malaria fever.

Acknowledgements

My gratitude goes to the Dean, School of Biological Science, Prof. J.N Ogbulie, The HOD, Department of Biochemistry, Prof. A.C Ene, My Supervisors, Prof. G.N Okwu and Prof. C.S Alisi, Dr Chidi Igwe for his biostats knowledge and all my lecturers in Department of Biochemistry, Federal University of technology, Owerri, for their efforts during my coursework and corrections during the stages of defence.
Most importantly, none of this could have happened without my family. Their consistent financial support

has made me come this far. I cannot thank them enough for bearing me and being beside me through highs and lows, every time I was ready to quit, they did not let me and I am forever grateful.
I also thank God for his sufficient grace and provisions every day, especially during the course of this work.

References

[1] Afolayan, A.J., Srinivas, K. and Grierson, D.S. (2007). Ethno botanical information medicinal plants used for treatment of cancer in the Eastern Cape Province. *South Africa Current Science*, **92**: 7.

[2] Akinmoladun, A.C., Ibukun, E.O., Afor, E.D., Obuotor, E.M., Farombi, E.O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimumgratissimum*. *Science Research Essay*, **2**(5): 163-166.

[3] Al-Ghamdi, M.S. (2001). Anti inflammatory, analgesics and antipyretic activities of *Nigella sativa*. *J. Ethnopharmacol.*, **76**: 45-48.

[4] Amos, S., Kolawole, E., Akah, P., Wambebe, C. and Gamaniel, K. (2001). Behavioral effects of the aqueous extract of *Guierasenegalensis* in mice and rats. *Phytomedicine*, **8**(5): 356-361.

[5] Anaso, H.U. and Onochie, C.C. (1999). A comparative study of nutrients in *Gongronemalatifolium*, *Piper Guineense* and *Piper nigrum*, to testify high acceptability in local dishes. *Journal of Science Engineering and Technology*,**6** (2):1321-183

[6] Asongalem, E.A., Foyet, H.S., Ekobo, S., Dimo, T. and Kamtchouing, P. (2004).Anti- inflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness), T. Anderson. *J. Ethnopharmacol*., **95**: 63-68.

[7]Asuzu, I.U., Onu, O.U. (1990). Anti-Ulcer activity of the ethanolic extract ofCombretumdolichopetalum root. *Int.Crude Drug Res*., **28**: 27-32.

[8] Bantoniene, D., Pukalskas, A., Venskutonis, P.R., Gruzdiene, D. (2000). Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil. *FoodRes. Int*., **33**:785–791.

[9] Bentley, G.A., Newton, S.H., Starr, J.B. (1983). Studies on the antinociceptive action of alpha agonist drugs and their interaction with opiod mechanism. *Br. J.Pharmacol.*,**79**: 125-134.

[10] Calixto, J.B. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines. (2000). *Brazil J. Med. Biology Res.*, **33**:179-189.

[11] Collier, H.O.J., Dinneen, L.C., Johnson, C.A., Schineider, C. (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br. J. Pharmacol*., **32**: 293-310.

[12] Catherine W, Hosni MH. Introduction and inactivation of catalase and superoxide dismutase of Escherichia coli by ozone. Arch BiochemBiophys 1987; **259 (2)**: 464-71

[13]Danquah, C.A., Woode, E., Gyasi, E.B., Duwiejua, M. and Ansah, C. (2011). Anti- inflammatory and antipyretic effects of an ethanolic extract of Capparis erythrocarposisert roots. *Res. J. Med. Plant*, **5**:158-168.

[13] Dejan, Z., Orcic, N.M., Mimica, D., Marina, M., Slobodan, S., and Emilija, D. J. (2011). Antioxidant activity relationship of phenolic compounds in *Hypericumperforatum.L.Chemistry Central Journal*, **5**(34):

1-8.

[14] Deraedt, R., Jougney, S., Falhout, M. (1980). Release of prostaglandin E and F in an algogenicreaction and its inhibition. *Eur. J. Pharmacol*., **51**: 17-24.

[15] Dubuisson, D., Dennis, S.G. (1977). The formalin test: a quantitative study of the analgesic effect of morphine, meperidine and brain stem stimulation in rats and cats. *Pain*, **4**: 161-174.

[16] Exarchou, V., Nenadis, N., Tsimidou, M., Gerothanassis, I.P., Troganis, A., Boskou, D. (2002). Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage, and summer savory. *J. Agric. Food Chem.,***50**: 5294–5299.

[17] Feroz, M.H., Ahmad, R.S., Sindhu, S.T.K., Shahbaz, A.M. (1993). Androgenic effect of herbs. *J.Ethnopharmacol*.,**56**: 55-57.

[18] Freeman, B.A., Crapo, J.D. (1982). Biology of disease: free radicals and tissue injury. *Lab invest*.,**47**:412-426.

[19] Germano, M.P., Sango, R., Guglielmo, M., De Pasquale, R., Crisafi, G. (1998). Effects of *Pfeleopsissubcrosa* extract on experimental gastric ulcers and Helicobacter pylori growth. *J. Ethnopharmacol*., **59**:167-172.

[20] Glavin, G.B., Szabo, S. (1992). Experimental gastric mucosal injury: laboratorymodels reveal mechanism of pathogenesis and new therapeutic strategies. *Fed. Am. Soc. Exp. Biol. J.,***42**: 111-116.

[21] Grabley, S., Theierickee, R. (1999). Drug Discovery from nature Springer: London. pp. 5-7.

[22] Halliwell, B., Guteridge, J.M.C. (1989). Free Radicals in Biology and Medicine, 2nd ed. Clarendon Press: Oxford, UK.

[23] Jain, B.B., Rathi, B.S., Thakurdesai, P.A. and Bodhankar, S.L. (2007). Antipyretic activity of aqueous extract of leaves of *Cocculushirsutus*. *Indian J. Nat. Prod*., **23**: 26-29.

[24] Jayaprakasha, G.K., Jaganmohan, R.L., Sakariah, K.K. (2004). Antioxidant activities of flavidin in different in vitro model systems. *Bioorganic and Medicinal Chemistry*, **12**: 5141-5146.

[25] Jingsheng He, Bo Huang, Xiaoquan Ban, Jun Tian, Ling Zhu, Youwei Wang. (2012). In vitro and in vivo antioxidant activity of ethanolic extract from *Meconopsisintuplinervia*. *J. Ethnopharmacol*., **141**: 104-110.

[26] Jose, M.M., Aledo, J.L., Cristina, P.G., Estebandelvalle, A., Jose, M.S. (2000).Inter relationship between oxidative damage and antioxidant enzyme activities: an easy and rapid experimental approach. *Biochem. edu*.,**28** (2): 93-95.

[27] Justesen, U., Knuthsen, P., Torben, T.J. (1998). Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high- performance liquid chromatography with photo-diode array and mass spectrometric detection. *J.Chromatogr*.,**799**: 101-110.

[28] Kokate, C.K. Practical Pharmacognosy. (1999). *New Delhi, VallabhPrakashan*, pp 107-121.

[29] Elizabeth, K., Rao, M.N.A. (1990). Oxygen radical scavenging activity of curcumin. *Int. Journal of pharmaceutal*.,**58**: 237-240.

[30] Kono, Y. (1978). Generation of Superoxide radical during autoxidation of hydroxylamine and an

assay for superoxide dismutase. *Arch Biochem. Biophys.*,**186** (1): 189-195.

[31] Koster, R., Anderson, M. De Beer, E.J. (1959). Acetic acid for analgesic screening. *Federa. Proceeding*, **18**: 412-417.

[32] Kumar, P.J., Clarke, M.L. (2002). A new approach to practical acute toxicitytesting. *Arch. Toxicol*., **54**: 275-287.

[33] Li, S., Dou, W., Tang, Y., Goorha, S., Ballou, L.R. and Blatteis, C.M. (2008). Acetaminophen: antipyretic or hypothermic in mice? In either case, PGHS- 1b (COX-3) is irrelevant. *Prostaglandins Other Lipid Mediat*.,**85**: 89-99.

[34] Ling, J. J., Maryati, M., Asmah, R. and Mohd, F.A. (2010). Phytochemicals, antioxidant properties and anticancer investigations of the different parts of several gingers species. *Journal of Medicinal Plants Research*, **4**(1): 027 -032.

[35] Magistretti, M.J., Conti, M., Cristoni, A. (1988). Anti-ulcer activity of ananthocyanidin from *Vaccinummyrfillus* and *Arzneimittleforschung*. *Drug Res.*,**38**: 686-690.

[36] Martin, M.J., Marhuenda, E., Perez-Guerrero, C., Franco, J.M. (1994). Antiulcer effect of Narinjin on gastric lesion induced by ethanol in rats. *Pharmacol*., **49**: 144-150.

[37] Maryam, Z., Farrukh A. and Iqbal A. (2009). The in vitro antioxidant activity and total phenolic content of four Indian medicinal plants. *International Journal of Pharmacy and Pharmaceutical Sciences*,**1**: 88-95.

[38] Morebise, O., Fafunso, M.A., Makinde, J.M., Olajide, O.A., Awe, E.O. (2002).Anti-inflammatory property of the leaves of *Gongronemalatifolium*. *Phytothera Res*., **16**: 75-77.

[39] Murray, C.W., Porcca, F., Cowan, A. (1998). Methodological refinements in themouse paw formalin test an animal model of tonic pain. *J. Pharmacol. Methods*, **20**: 175-186.

[40] Nagata, K.N., Taitro, T.H., Estsuji, H.B., Noboyasu, E.C., Shumuchi, M.A., Chikao, N.U. (1985). Medicinal plants in Nigeria. *Agric Biological Chemistry*, **49**:1181-1186.

[41] Nwinyi, O.C., Chinedu, H.S., Ajani, O.O. (2008). Evaluation of antibacterial activity of *Psidiumguajava* and *Gongronemalatifolium.J. Med. Plant Res*., **2** (8): 189-192.

[42] Odugbemi, T.A. A textbook of medicinal plants from Nigeria, Lagos: University of Lagos Press; 2008.

[43] Okafor JC (1975). The role of common edible (wild and semi-wild) wood plants inthe native diets inNigeria. Agricultural Information, Ministry of Agricultureand Natural Resources, Enugu. p. 40.

[44] Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucoseamine.*Japanese Journal of Nutrition*, **44**: 307-315.

[45] Scartezzini, P., Antognoni, F., Raggi, M.A., Poli, F. C. (2006).Vitamin C contentand antioxidant activity of the fruit and the Ayurvedic preparation of Emblica officinalis Gaertn. *Journal of Ethnopharmacology*, **104**:113– 118.

[46] Paglia, D.E., Velentine, W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J lab Clin Med.*,**70** (1):159-68.

[47] Panthong, A., Kanjanapothi, D., Taestikul, T., Wongcome, T. and Reutrakul, V. (2003). Antiinflammatory and antipyretic properties of *ClerodendronpetasitesJ. Ethnopharmacol*., **85**: 151-156.

[48] Proestos, C., Chorianopoulos, N., Nychas, G.J.E., Komaitis, M. (2005). RP-HPLC analysis of the phenolic compounds of plant extracts, Investigation of theirantioxidant capacity and antimicrobial activity. *J. Agric. Food Chem*., **53:** 1190– 1195.

[49] Rice-Evans, C.A., Packer, L. (1998). Flavonoids in Health and Disease, 2nd ed. Marcel Dekker Inc. New York, NY,USA.

[50] Romanovsky, A.A., Almeida, M.C., Aronoff, D.M., Ivanov A.I., Konsman, J.P., Steiner, A.A. and Turek, V.F. (2005). Fever and hypothermia in systemicinflammation: Recent discoveries and revisions. *Front. Biosci.*,**10**: 2193- 2216.

[51] Roth, J., Rummel, C., Barth, W., Gerstberger, R. and Hübschle, T. (2006). Molecular aspects of fever and hyperthermia. *Neurologic Clin.*,**24**: 421-439.

[52] Siddhuraju, P., Mohan, P.S., Becker, K. (2002). Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula*): A preliminary assessment of crude extracts from stem, bark, leaves, flowers and fruit pulp. *Food Chem*., **79**: 61-67.

[53] Slobodan, V.J., Steen, S., Mihajlo, T., Budimir, M., Michael, G.S. (1994). Flavonoids as antioxidants. Journal of American Chemical Society, **116** (11): 4846- 51.

[54] Sumanth M, Ahmed R. Antihepatotoxic and Antioxidant activity of root of TaraxacumOfficinale in CCl_4-intoxicated Rats. Phcog Mag 2008; **4 (16)**:188-194

[55] Turkmen, N., Sari, F., Velioglu, Y.S. (2006). Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined byferrous tartrate and Folin-Ciocalteu methods. Food Chem., **99**: 835–841.

[56] Ugochukwu, N.H., Babady, N.E., Cobourne, M.K. and Gasset, S.R. (2003). The effect of *Gongronemalatifolium*extract on serum lipid profile andoxidative stress in hepatocytes of diabetic rats. *J. Biosci.,***28** (1): 1 - 5.

[57] Val Vallyathan and Xianglin Shi. (1997).The role of oxygen free radicals in occupational and environmental lung diseases. *Environmental Health perspectives,***105 (1)**: 165- 177.

[58] Ye-Shih Ho, Jean-Luc Magnenat, Mary Gargano and Jin Cao. (1998). The Nature of Antioxidant Defence Mechanisms: A Lesson from Transgenic studies. Environmental Health Perspectives, **106** (5): 1219-1228.[59] Yesilada, E. and Kupeli, E. (2007). *Clematis vitalba* L. aerial part exhibits potent anti-inflammatory, antinociceptive and antipyretic effects. *J. Ethnopharmacol*., **110**: 504- 515.

[60] Zhang, J., Wider, B., Shang, H., Li, X., Ernst, E. (2012). Quality of herbal medicines: Challenges and solutions. *Complement Ther Med*., **20** (1-2): 100- 6.