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Comparative Anti-oxidative and Acetylcholinesterase inhibition potentials of selected tropical plants in the treatment of Alzheimer's and related cognitive complications

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ABSTRACT

Acetylcholinesterase inhibitors are important therapeutic agents in Alzheimer's and other cognitive complications. The study aimed to evaluate acetylcholinesterase inhibition, antioxidant activity and other biochemical indices of the three solvent-extracts of five plant species from South-western Nigeria. The leaves were extracted with solvents; phytochemical screening and in-vitro antioxidant properties of the extracts were determined. Rats weighing between 140 to 150g were divided into groups comprising of normal control, 2,2-dichlorovinyldimethylphosphate (DDVP), aqueous, methanol and ethanol groups for each of the five plants. The rats were induced with 3.3mg/kg body weight of DDVP for two weeks with the aim of inducing cognitive complications while the induced animals were treated with 3.3mg/kg body weight of the extracts of the five plant species for another two weeks. The rats were anaesthetized and quickly dissected individually to collect serum and organs

homogenates to evaluate the studied parameters using standard procedures. The results obtained from the study clearly demonstrated that the three extracts of the five plants show powerful in-vitro and in-vivo antioxidant properties, impressive anti-lipid peroxidation potentials and promising acetylcholinesterase inhibition activity. These plants solvents extracts could be locally utilized in drug developments to prevent and cure the consequences of Alzheimer disease (AD) and associated disorders.

Keywords: Alzheimer disease, cholinergic functions, antioxidant property, acetylcholinesterase inhibitor, drug development

1. INTRODUCTION

Alzheimer's disease is characterized by selective neuronal cell death, the presence of extra cellular amyloid deposits in the core of neurotic plaques and the formation of intra neuronal neurofibrillary tangles in the brain of afflicted individuals (Dhanasekaran *et al.,* 2015). According to the cholinergic hypothesis, the inhibition of acetylcholinesterase (AChE), an enzyme that catalyzes acetylcholine hydrolysis, increases the levels of acetylcholine in the brain, thus improving cholinergic functions in AD patients (Murraya *et al.,* 2013). The cholinergic hypothesis states that decreased cholinergic transmission plays a major role in the expression of cognitive, functional and possibly behavioral symptoms in AD (Hau et al 2008; Volpicelli-Daley *et al.,* 2003). The cholinergic hypothesis rests on pathological, biochemical and pharmacological observations. Oxidative stress is also implicated in the development of many neurodegenerative diseases including Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis and Alzheimer's disease (AD)[5]. One of the important strategies for treating of AD is to maintain the levels (Heleno *et al.,* 2012) of acetylcholine through the inhibition of acetylcholinesterase (AChE) (Lahiri *et al.,* 2002). The acetylcholinesterase enzyme (AChE) is an attractive target for the rational drug design and for the discovery of mechanism-based inhibitors because of its role in the hydrolysis of the neurotransmitter acetylcholine (ACh). AChE inhibitors are the most effective approach to treat the cognitive symptoms of Alzheimer disease (AD) (Kalauni *et al.,* 2002; Atta-ur-Rahman *et al.,* 2004) and other possible therapeutic applications in the treatment of Parkinson's disease, senile dementia, and ataxia, among others (Ahmad *et al.,* 2003). AChE inhibitors as eserine, tacrine, donepezil, rivastigmine, and galanthamine are the only drugs currently approved for the treatment of AD; however, these drugs are known to have limitations for clinical use due to their short-half-lives and/or unfavorable side-effects (Sung *et al.,* 2002). Dichlorvos is a synthetic organic chemical used as an insecticide. Dichlorvos does not occur naturally in the environment but is manufactured by industry. Dichlorvos is an insecticide used on crops, animals, and in pest-strips. Acute (short-term) and chronic (long-term) exposures of humans to dichlorvos results in the inhibition of an enzyme, acetylcholinesterase, with neurotoxic effects including perspiration, vomiting, diarrhea, drowsiness, fatigue, headache, and at high concentrations, convulsions, and coma (TMI, 1989). Since the synthetic drugs have undesirable side effects or contraindications, the World Health Organization (WHO) has recommended the evaluation of traditional plants treatments for diabetes (Day, 1998). Medicinal plants are widely used worldwide to address a variety of health problems. About 25 to 50% of current pharmaceuticals are derived from plants (Cowan, 1999; Goh *et al.,* 1995). Plants are rich in a wide variety of phytochemical metabolites which are divided into two groups: Primary and Secondary metabolites. Primary metabolites consist of common sugars, amino acids, proteins and chlorophylls, while Secondary metabolites include glycosides, alkaloids, saponins, phenolic compounds, terpenes steroids and anthraquinones (Habtermariam *et al.,* 1993). These plants have been claimed to be used in the treatments of

diseases, but its AChE inhibition activity has not been studied so far. Hence the main objective of the present study is to evaluate AChE inhibition, antioxidant activity and other biochemical indices of the three solvent-extracts of five plant species [Scent leaf, ewe efirin (*Ocimum gratissimum*), Wind braker leaf, ewe igunnu (P*olyalthia longifolia* (Sonn.), Pawpaw leaf, ewe ibepe (C*arica papaya*), African mahogamy leaf, ewe apa (A*fzelia africana*) and Goat weed leaf, ewe imi-esu (*Ageratum conyzoides*)] from South-western Nigeria.

MATERIALS AND METHODS

Plants samples collection, authentication and preparation

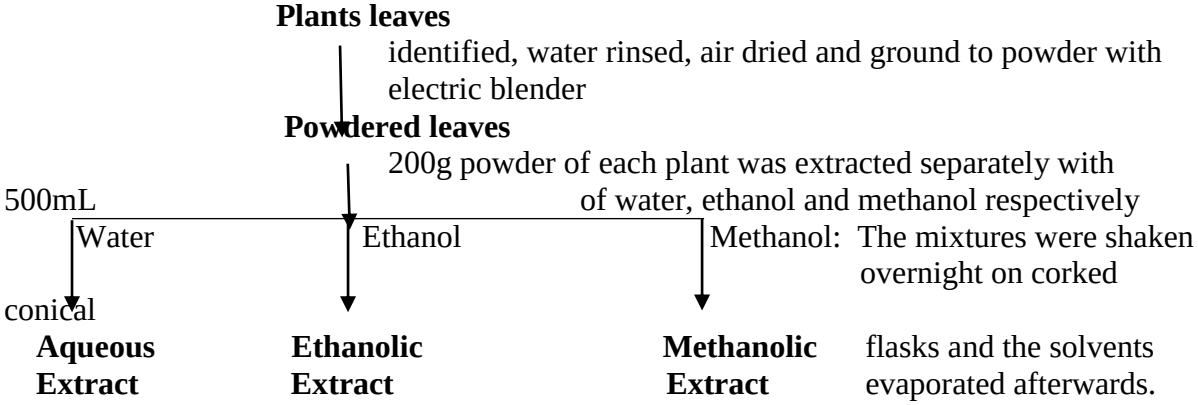
The various plants leave of *Ocimum gratissimum* (UHAE 2020051), *Polyalthia longifolia* (Sonn.) (UHAE 2020054), C*arica papaya* (UHAE 2020048), *Afzelia Africana* (UHAE 2020046) and *Ageratum conyzoides* (UHAE2020047) were sourced from various locations in Ado-Ekiti, Ifaki-Ekiti, Iworoko-Ekiti in Ekiti State and Ibadan in Oyo State Nigeria by detaching large quantities of leaves from their various trees of the plants. The leaves were however authenticated at Department of Plant Science of Ekiti State University, Ado-Ekiti, Nigeria and respective Herbarium numbers obtained.

The leaves were rinsed in water to remove any form of dirt's. The rinsed leaves were spread in a room for some days till they were completely air dried. The air-dried leaves were pulverized with electric blender to increase the surface area and to hasten the process of extraction.

Extraction with water, methanol and ethanol

The individual powered leaves were divided and measured into three parts using standard weighing balance. 200g each of the powdered leaves were extracted with 500mL each of water, methanol and ethanol inside stoppered glass bottles with continuous shaking overnight. The extracted solutions were evaporated to obtain the respective extracts. The resulting extracts were weighed and the percentage solvents extracts yields recorded.

The flow chart for the solvent's extraction of the ten different plants is as follows.



Preliminary phytochemical screening of the leaves' extracts

The qualitative phytochemical screening [flavonoids, saponin, phlobatannins, terpenoids, Salkowski test for cardiac glycosides (steroidal ring or terpenoids), Keller-Killani test for cardiac glycosides (deoxysugar), Lieberman's test for steroidal nucleus and test for tannins] of aqueous, methanol and ethanol extract fractions of the leaves were carried out according to the methods of (Trease, Evans 2002; Sofowora, 1993) to identify the active constituents

In-vitro antioxidants analyses of the leaves' extracts

The quantitative in-vitro scavenging activities [% H_20_2; 2,2'-azino-bis- 3-ethyl benzthiazoline-6-sulphonic acid (ABTS); Nitric oxide; 2,2-diphenyl-2-picryl hydrazyl hydrate (DPPH);; Superoxide radicals] activities; total phenol and flavonoids contents were determined respectively by the methods of (Ruch et al., 1989; Shirwaikar *et al.*, 2006; Green et al., 1982; Brand-Williams et al., 1995; Winterbourne et al., 1975; Singleton, Rossi, 1965; Zhishen *et al*., 1999).

Experimental animals

The Wistar albino rats weighing between 140g to 150g were obtained from Animal House of College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria.

Animal Care and Management

Eighty-five (85) Wistar albino rats weighing between 140-150g obtained from Animal House of Ekiti State University College of Medicine, Ado-Ekiti, Nigeria were grouped into five groups of five animals each. They were housed in plastic cages under standard laboratory conditions of natural light/dark cycle at room temperature and humidity. The rats were fed on standard rat pellets which were procured once from (Top feeds, Nigeria) and given drinking water *ad libitum*.

All animals were handled in accordance with the Guide for the care and use of laboratory animals as detailed in the International Animal Care and Use Committee (IACUC, 2010).

Experimental design and animal assay

The rats were induced with 3.3mg/kg body weight of 2,2-Dichlorovinyldimethylphosphate (DDVP) solution for fourteen days except group 1. The induced rats were then treated with 3.3mg/kg body weight solution of aqueous, methanolic and ethanolic extracts of the various plants for the next fourteen days.

Group1 Normal Control (animals given water and rats feed *ad libitum*)

Group2 DDVP induced control (animals were administered orally with 0.5mL of 3.3mg/kg body weight of DDVP solution)

Group3 (3i-3v) DDVP induced animals + 0.5mL of 3.3mg/kg body weight of aqueous solution of each extract of the plants.

Group4 (4i-4v) DDVP induced animals + 0.5mL of 3.3mg/kg body weight of methanolic solution of each extract of the plants.

Group5 (5i-5v) DDVP induced animals + 0.5mL of 3.3mg/kg body weight of ethanolic solution of each extract of the plants.

After the experiment, the rats in all the groups were anaesthetized with chloroform individually and quickly dissected to collect blood into the sample bottles and latter centrifuged at 3000rpm to obtain serum; while the organs (brain, liver and heart) were removed, weighed and placed on ice-bath. 10% of each organ homogenate was then prepared respectively in 6.7mM potassium phosphate buffer, (pH 7.4) using the electrically top driven homogenizer.

The individual organ homogenates were centrifuged at 3,000rpm for 10 minutes at 4^0C to obtain clear supernatants which were stored at 8^0C and used for measurement of the studied biochemical parameters.

The antioxidants enzymes activities [Catalase (CAT), superoxide dismutase (SOD), Glutathione-S-transferase (GST), Glutathione reductase (GR) and Glutathione peroxidase

(GPx)]; reduced glutathione (GSH) were determined by the methods described by (Chance, Maehly, 1955; Von Euler, Josephson, 1972; Misra, Fridovich, 1972; Habig *et al.*, 1974; Carlberg, Mannervik, 1985; Mohandas *et al.*, 1984; Jollow *et al.*, 1974) respectively. The lipid peroxidation was done by measuring the TBARS in accordance with the modified method of Utley et al., (1967). The Sigma-Aldrich, (2013) assay Kit was employed to determine the acetylcholinesterase activity, where one unit of AChE is the amount of enzyme that catalyzes the production of 1.0 µmole of thiocholine per minute at room temperature at pH 7.5 with the aim of evaluating the inhibition of acetylcholinesterase activity. This kit has a linear range of 10–600units/L of AChE activity.

Statistical Analysis

The mean triplicate results were determined and represented in charts as presented in the various figures.

RESULTS

The phytochemical compositional screening can be seen in Figure 1showing the leaves of the five plants containing important compounds of medicinal significant at varying intensity in all the solvents extracts as studied.

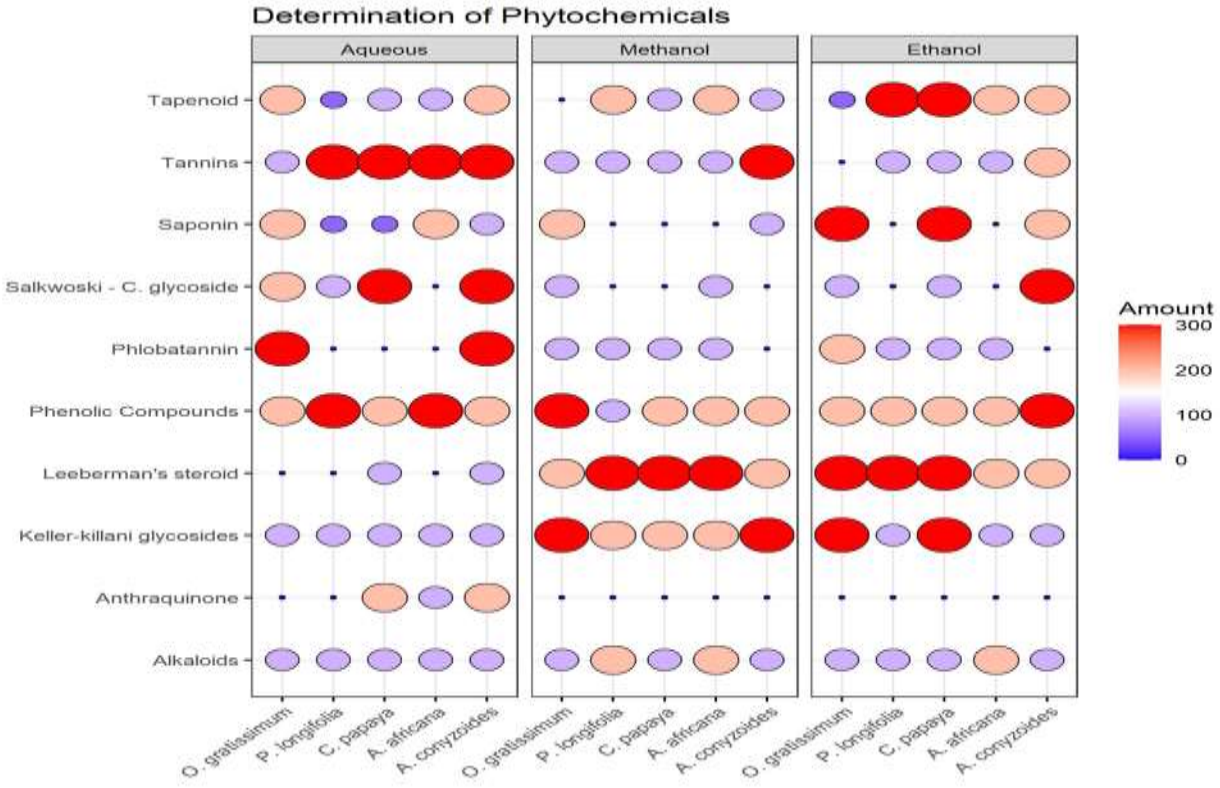


Figure 1- Phytochemical compositional evaluation of leaves extracts of five Nigerian medicinal plants. Less than 100 implies traces, 100 = "+", 200 = "++", 300 = "+++"

Similarly, Figures 2 represents the (% ABTS; % DPPH; % H_20_2; % Nitric oxide and % Super oxide) scavenging activities and concentrations of Total flavonoids and Total phenol of leaves extracts of plants, the scavenging activities of each plant extract increased in dose dependent manner.

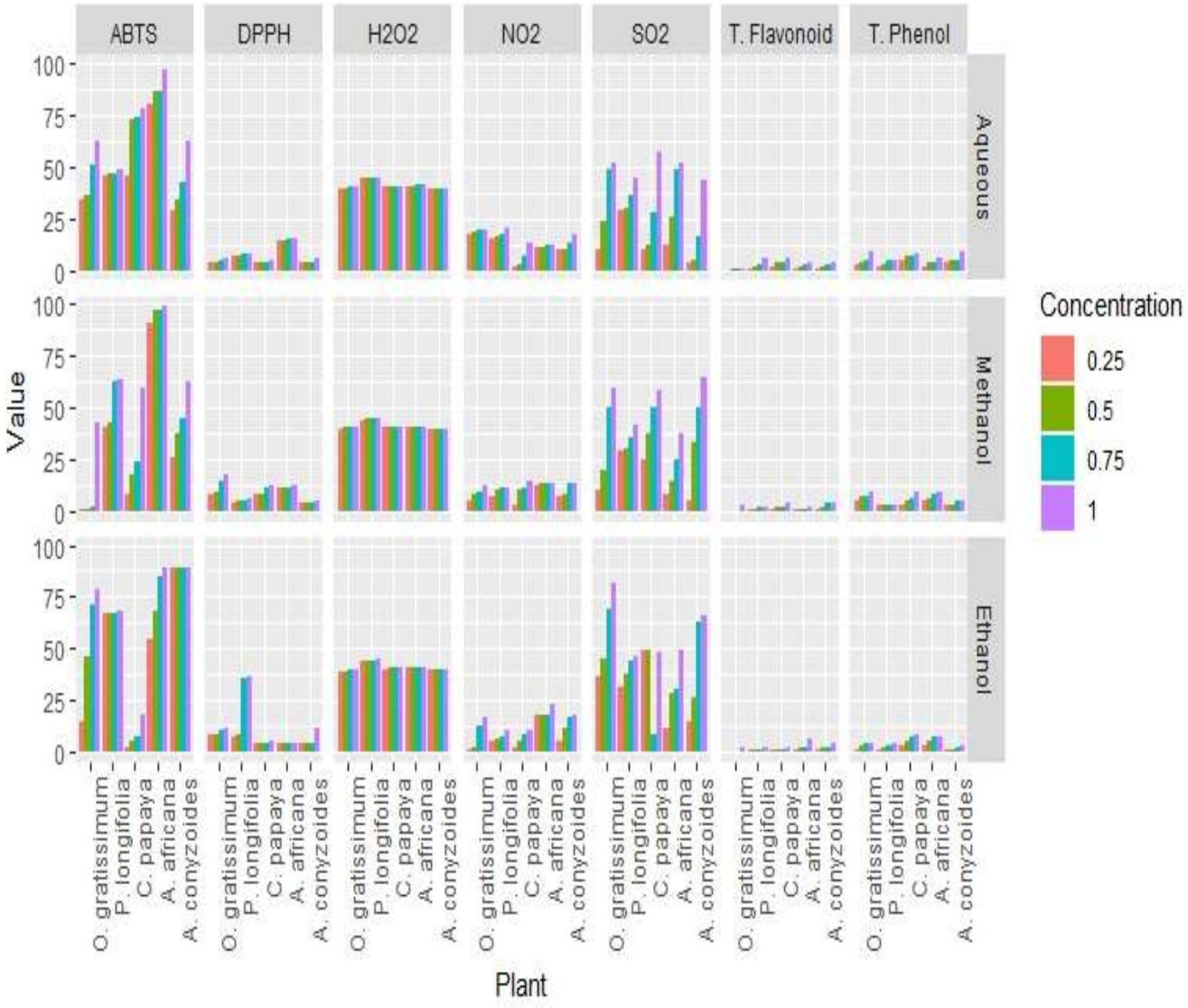


Figure 2- Some in-vitro antioxidants activities of leaves extracts of five Nigerian medicinal plants

The effects of various extracts of the plants on the activity of Catalase as seen in Figure 3, the serum, liver, heart and brain tissues of induced and treated animals showing all the plants causing restored activity of the enzyme after treatments.

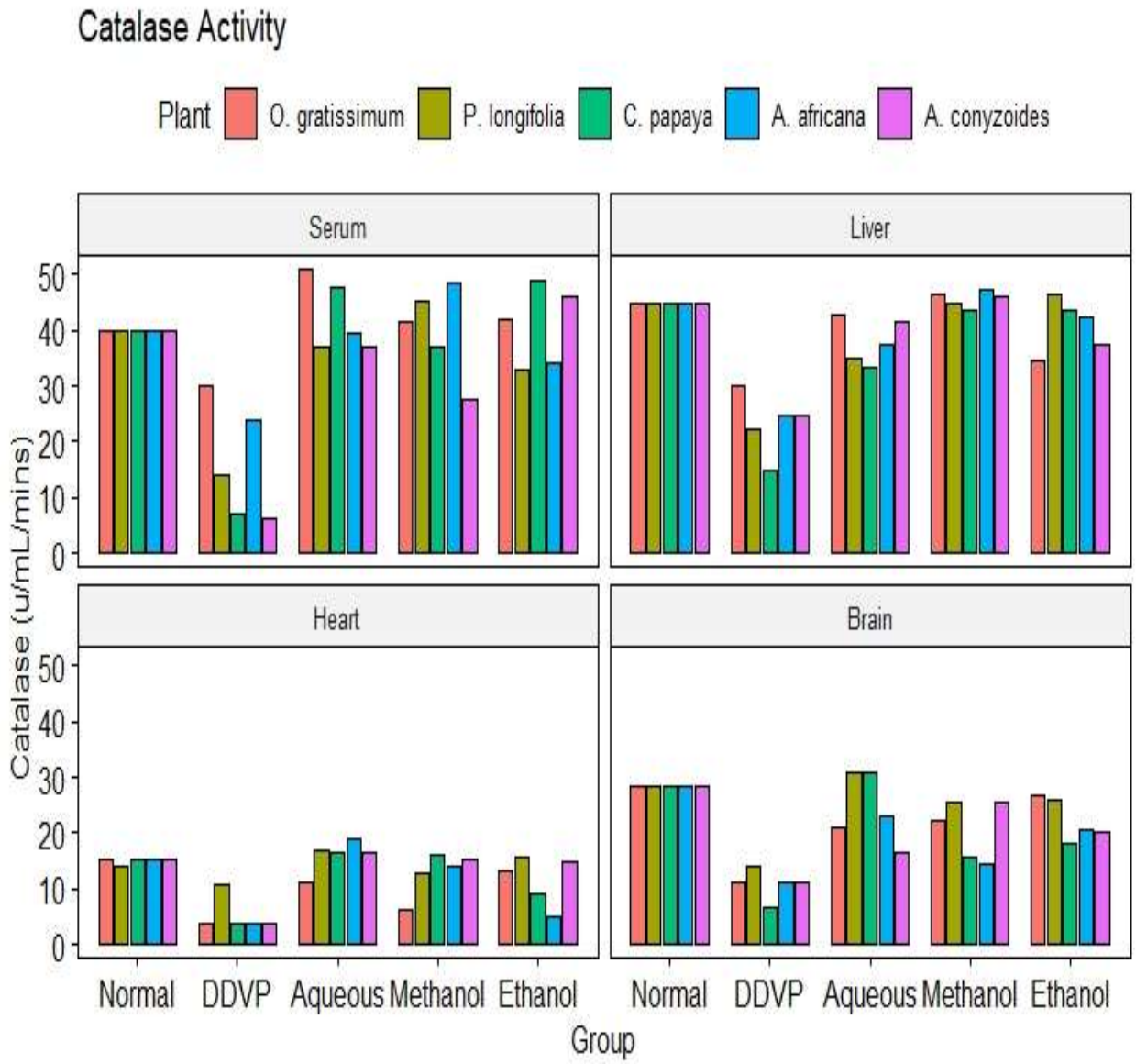


Figure 3- Effects of various extracts of some Nigerian medicinal plants on the activity of Catalase (u/ml/mins) enzyme in 2,2-dichlorovinyldimethylphosphate induced Wistar albino rats

The percentage superoxide dismutase activity as presented in Figure 4 showed the effects of various extracts of the plants indicating all the solvents extracts display increased SOD percentage activity when compared with the DDVP induced group.

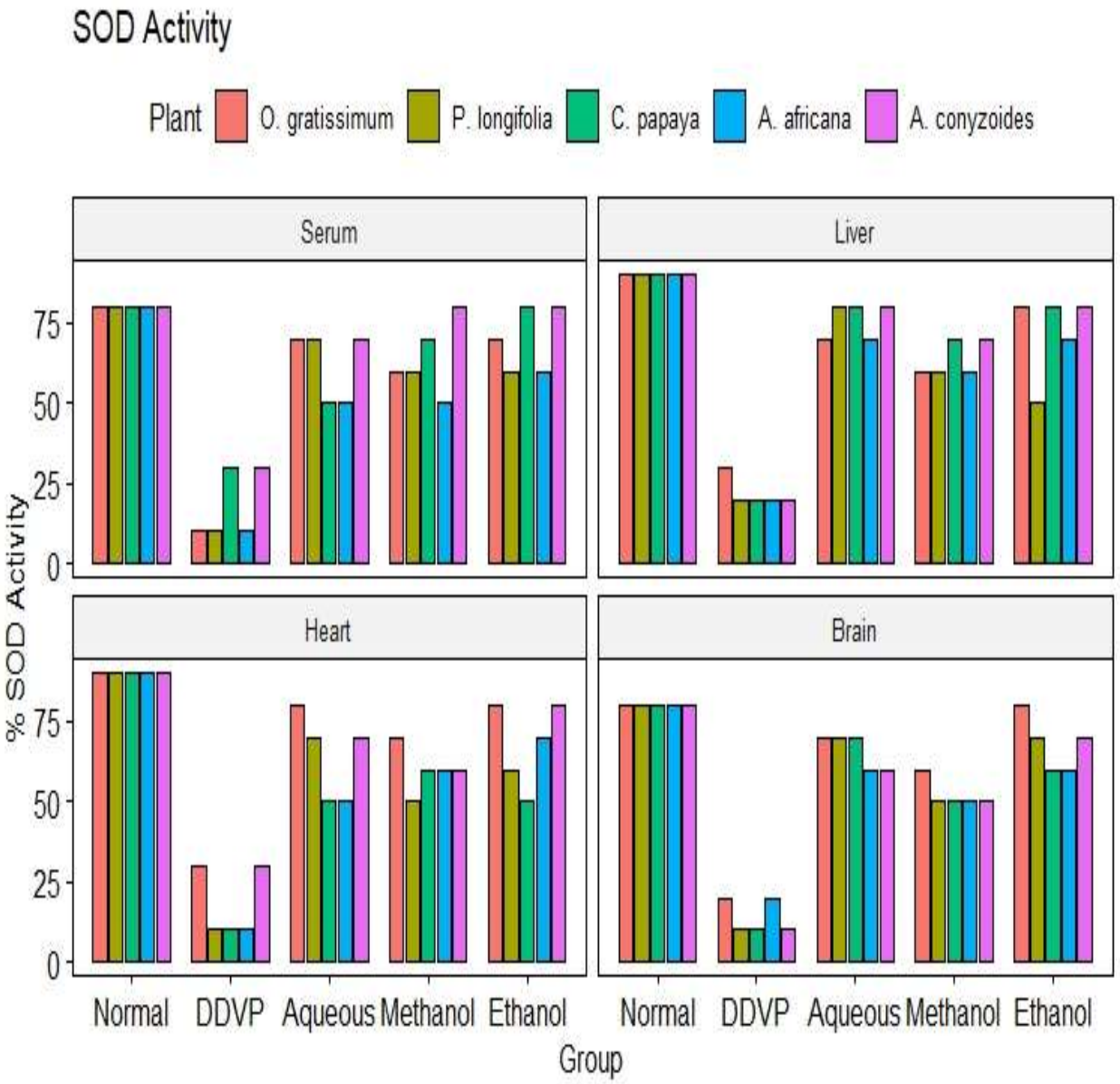


Figure 4- Effects of various extracts of some Nigerian medicinal plants on the activity of Superoxide Dismutase (% SOD activity) in 2,2-dichlorovinyldimethylphosphate induced Wistar albino rats

The glutathione peroxidase activity of various extracts of plants from Figure 5 showed that the extracts caused a reversal effect to normal control group when compared with the DDVP induced group.

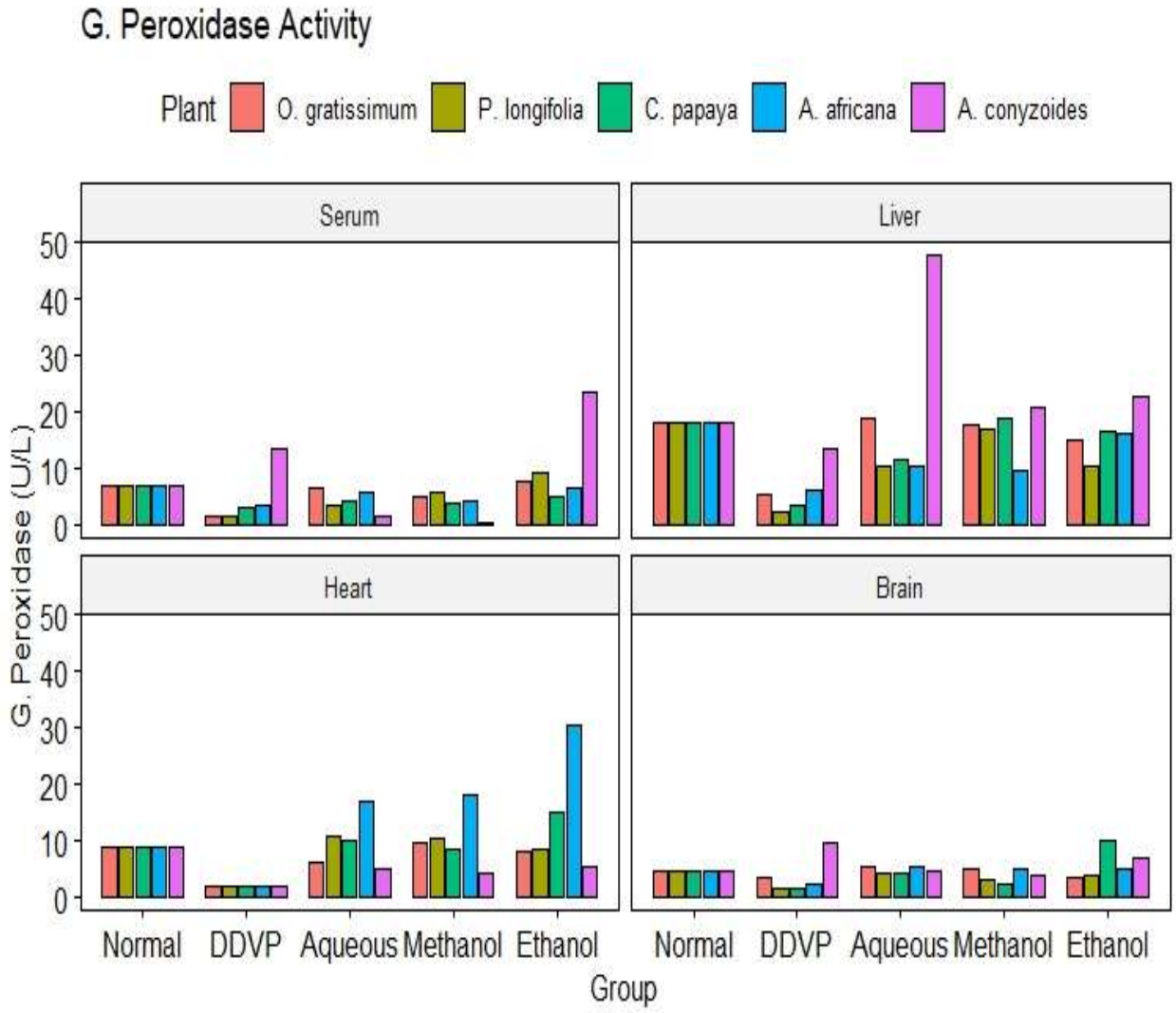


Figure 5- Effects of various extracts of some Nigerian medicinal plants on the activity of Glutathione peroxidase (Gpx U/L) in 2,2-dichlorovinyldimethylphosphate induced Wistar albino rats.

Figure 6 showed the Glutathione transferase activity in DDVP induction and treatment with various extracts of the plants, the extracts caused enhanced activity of the enzyme on the treated groups

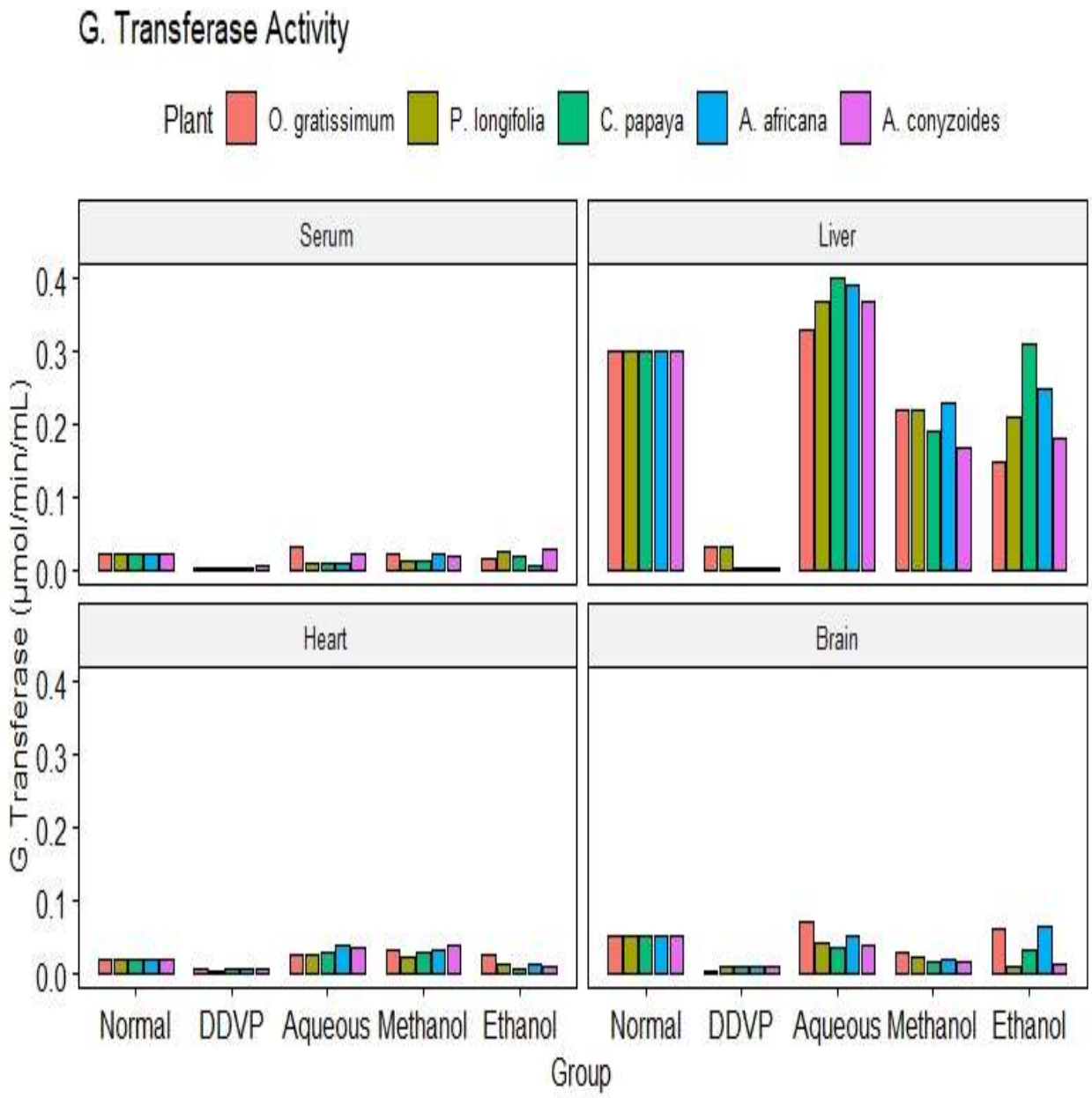


Figure 6- Effects of various extracts of some Nigerian medicinal plants on the activity of Glutathione transferase (µmol/min/ml) in 2,2-dichlorovinyldimethylphosphate induced Wistar albino rats

The activity of glutathione reductase in Figure 7 revealed the improved activity on treatment with the various extracts of the plants.

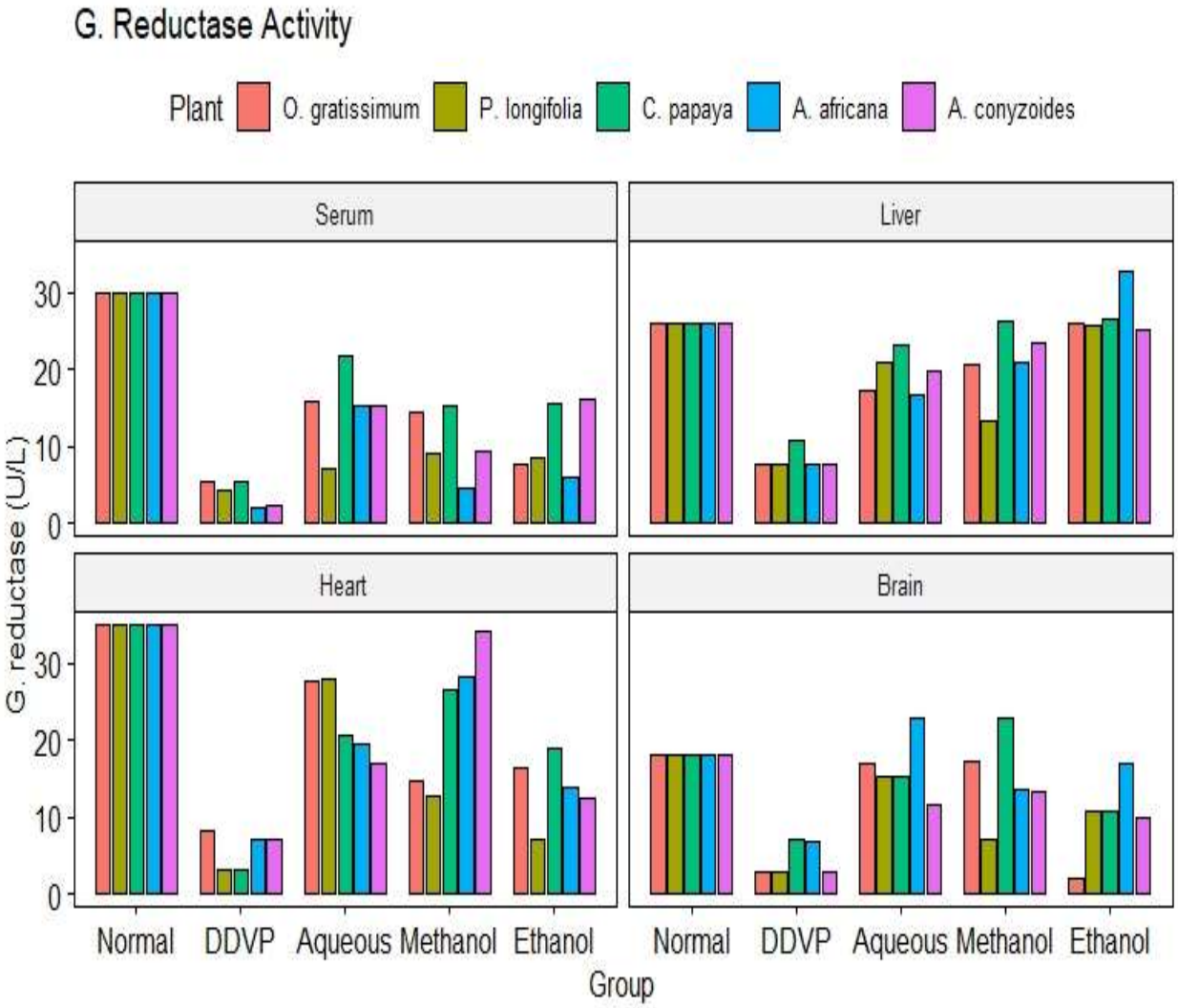


Figure 7- Effects of various extracts of some Nigerian medicinal plants on the activity of Glutathione reductase (U/L) in 2,2-dichlorovinyldimethylphosphate induced Wistar albino rats

The concentration of reduced glutathione from Figure 8 showed that the various extracts of the plants caused increased concentration of GSH in the depleted concentration of the DDVP induction group.

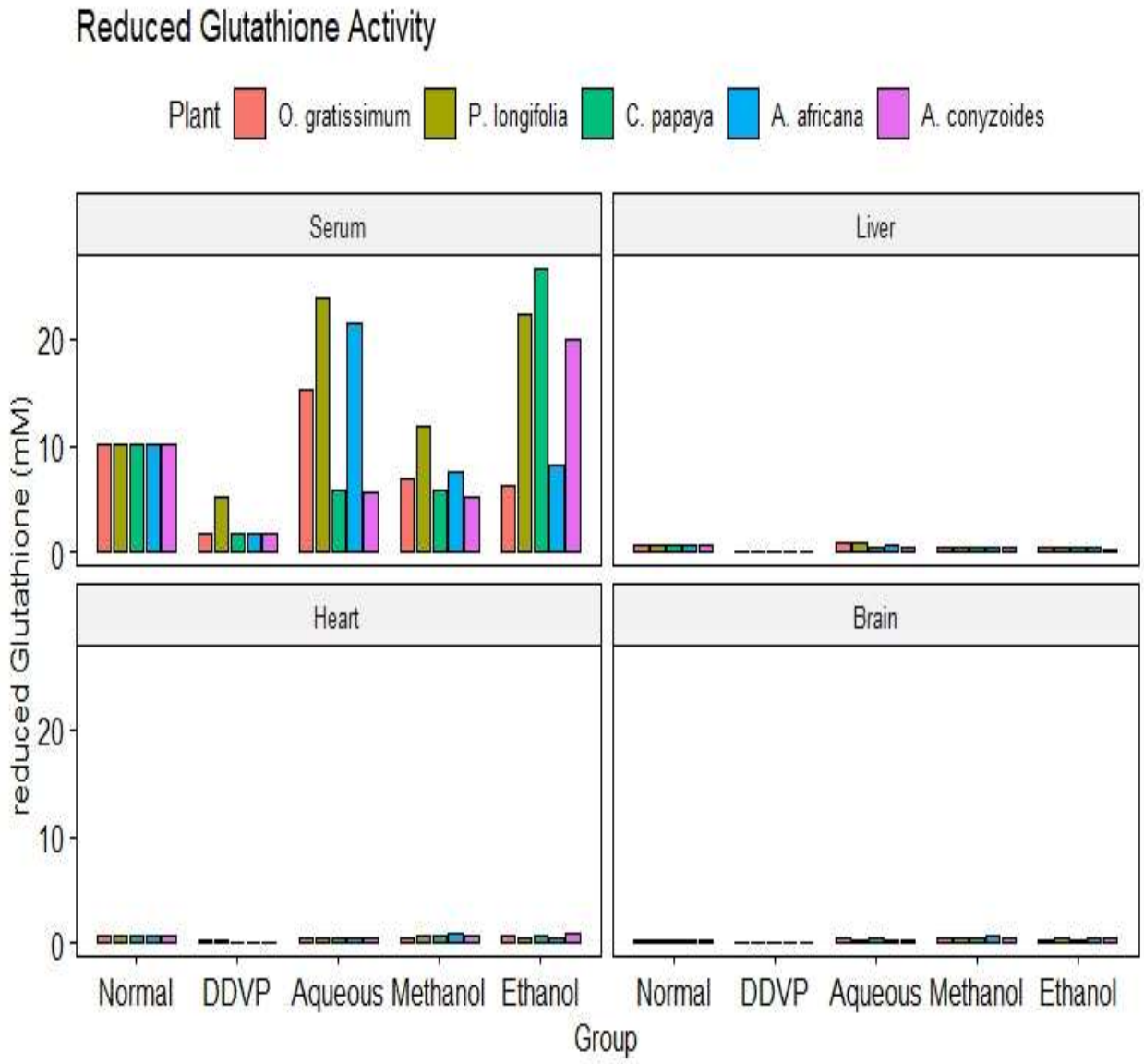


FIGURE 8- Effects of various extracts of some Nigerian medicinal plants on the concentration of Reduced glutathione (GSH mM) in 2,2-dichlorovinyldimethylphosphate induced Wistar albino rats

From Figure 9, the concentration of MDA was grossly reduced from the DDVP induced group when compared to the solvent's extracts treated groups.

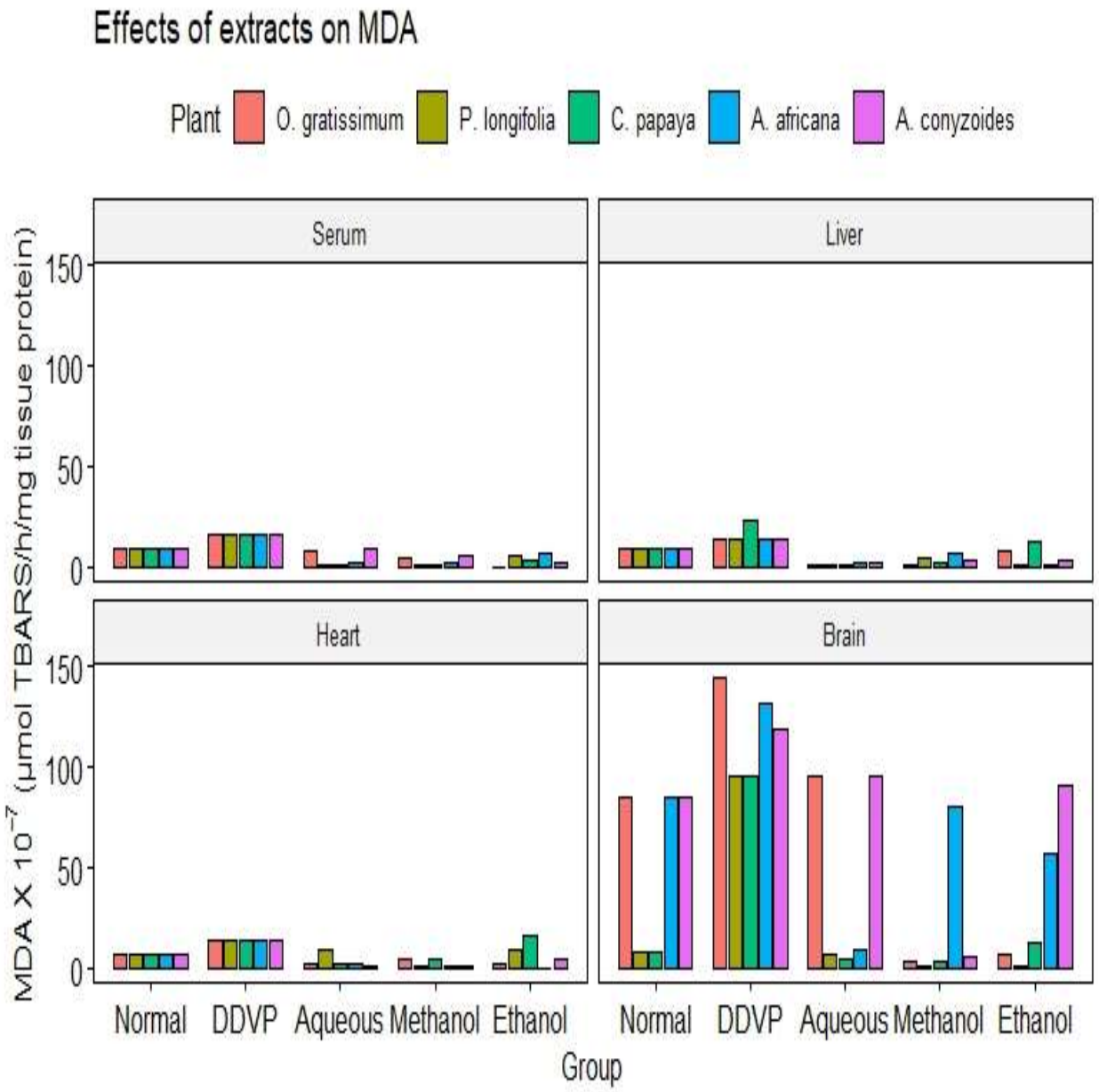


FIGURE 9- Effects of various extracts of some Nigerian medicinal plants on the MDA (nmol TBARS/h/mg tissue protein) in 2,2-dichlorovinyldimethylphosphate induced Wistar albino rats

The inhibition of the Acetylcholinesterase activity observed in Figure 10 showed that the various extracts of the plants caused substantive activity inhibition from the DDVP induction group.

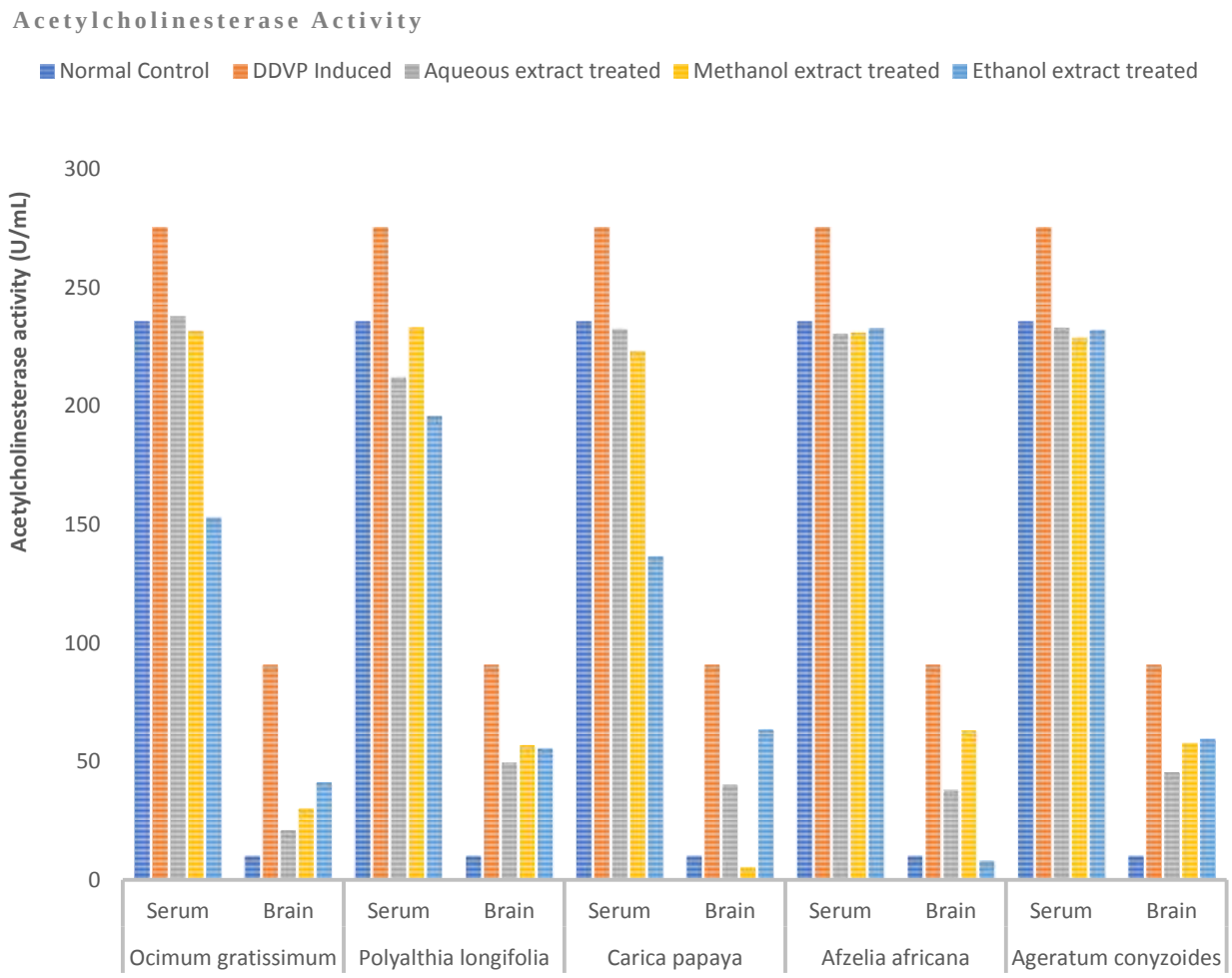


Figure 10- Effects of various extracts of some Nigerian medicinal plants on the Inhibition of Acetylcholinesterase activity (U/mL) in the Serum and Brain of 2,2-dichlorovinyldimethylphosphate induced Wistar albino rats

DISCUSSION

The qualitative phytochemical composition of the five plants leaves solvents extracts were evaluated to assess the presence of bioactive components. All the studied plants contained few and/or all the tested phytochemicals (alkaloids, saponins, tannins, phenolic compounds, phlobatannin, steroids, anthraquinone, terpenoids and cardiac glycosides) as presented in Figure 1. All the studied phytochemicals were present in either aqueous, methanolic, ethanolic and in all extracts of the plants except anthraquinone that was present in only aqueous extracts of *Carica papaya*, *Afzelia Africana* and *Ageratum conyzoides*. These secondary plant metabolites have been reported to contain antibacterial potency and have been actively used or in combination with antibiotics in the therapy of bacterial infections (Liu *et al.*, 2001; Deba et al., 2008). The demand in the use of natural products as antioxidants and antimicrobial compounds has led to investigate various extracts from many plants, this has recently been of great interest in both research and the food industry, because of growing tendency and possibility to replace synthetic antioxidants and antimicrobials compounds with natural ones[37]. Many other researchers have reported diverse plants and plants parts solvents extracts containing arrays of these

phytochemicals worldwide (Vaghasiya *et al.,* 2011; Oseni, Williams, 2018). The in-vitro antioxidants scavenging potentials of (%H_20_2; % ABTS; % Nitric oxide, % DPPH; % Super oxide scavenging activities and concentrations of total phenol and total flavonoids were investigated as reported in Figure 2. Reactive oxygen species (ROS) is a term that encompasses all highly reactive, oxygen containing molecules, including free radicals like hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides with capability of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage (Krishnamurthy, Wadhwani, 2012). Antioxidant compounds may function as free radical scavengers, initiator of the complexes of prooxidant metals, reducing agents and quenchers of singlet oxygen formation. The in-vitro antioxidants scavenging potentials results obtained in this study however corroborated the observations of earlier investigations by other researchers worldwide revealing plants as major sources of antioxidants (Al-Mustafa, Al-Thunibat, 2008; Nilima, Hande, 2011; Ashafa *et al.,* 2010; Ilahi *et al.,* 2013). Antioxidant's enzymes (CAT, SOD, GPx, GR, GST) and GSH were also investigated as presented in Figures 3.0-8.0. The results showed the plants extracts exhibited high antioxidant enzyme activities and promising concentration of non-enzyme antioxidant compound (GSH). Antioxidants are molecules that prevent oxidation or inactivates the reactive oxygen species and thus prevent oxidative damage to the cells and body tissues (Aliyu *et al.,* 2019). It has been reported that catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues, decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals (Nandy *et al.,* 2012). Its activity varies greatly from tissue to tissue, the highest activity is found in liver and kidney, whereas the lowest activity has been reported in the connective tissue (Naazeri *et al.,* 2014). It is evidenced in this study that the five plants and the three extracts enhanced the activity of CAT in serum and all the organs studied, this is in agreement with what was obtained by (Merghem *et al.,* 2019; Doungue *et al.,* 2018). The enhanced catalase activity could be due to the presence of bioactive compounds present in these plants extracts that would give their protons to stabilize radicals formed during stress. Administration of DDVP significantly decrease SOD activity in the studied organs while the five plants solvents extracts caused a concomitant increased activity in the treated groups. Similar tends were also observed in GPx, GR and GST activities. The concentration of reduced glutathione (GSH) was also observed to be grossly reduced by the administration of DDVP but treatments with the plants solvents extracts produced upward effects by restoring the GSH concentrations in the serum and the studied organs. In all the antioxidants investigations, the plants solvent extracts reduced the effects of DDVP and hence reversed the oxidative stress expressed by DDVP. From Figure 9 showing the effects of various extracts of plants on the MDA in DDVP induction, there was increased MDA concentrations in both serum and the studied organs indicating lipid peroxidation which implicates oxidative stress. On treatments, all the plants extract showed protective role by decreasing the lipid peroxidation level in DDVP induced animals and increased activities of enzymatic (CAT, SOD, GPx, GR and GST) antioxidants and the level of non-enzymatic (GSH) antioxidant. The results obtained in this study however corroborates those reported by other researchers in their various works on different plants extracts and effects on oxidative stress (Ojo *et al.,* 2014a; Ojo *et al.,* 2014b; Yadav *et al.,* 2012; Ahlamet *et al.,* 2014; Armelle *et al.,* 2018). Figure 10 showed the results of effects of various extracts of the five plants on the inhibition of acetylcholinesterase activity in the serum and brain of DDVP induced animals. The results obtained in this study showed that DDVP induction of 3.3mg/kg body weight for 14 days significantly elevated AChE in both serum

and brain tissues, it has also been reported by other researchers that DDVP caused increase AChE activity and oxidative stress (Ojo *et al.,* 2014a; Maysaa *et al.,* 2016; Yadav *et al.,* 2012;). It has been reported that AChE interact with amyloid β and promotes the formation of amyloid fibril through a pool of amino acids situated in proximity of the peripheral anionic site (PAS) of the enzyme resulting in neurocognitive impairment (Anand *et al.,* 2012). Several studies have revealed that AChE inhibitors not only facilitate transmission of the cholinergic system, but also interfere with the synthesis, aggregation and deposition of toxic amyloid β (Anand *et al.,* 2012). In addition, dysregulation of AChE levels has also been reported in myasthenia gravis, glaucoma, Lewy bodies and Parkinson's disease (Colovic *et al.,* 2013). The observations in this study however corroborated the earlier similar works of Doungue *et al.,* (2018) in neuroprotective effect and antioxidant activity of *Passiflora edulis* fruit flavonoid fraction, aqueous extract, and juice in aluminum chloride-induced Alzheimer's disease rats; Mahdy *et al.,* (2012) in effect of some medicinal plant extracts on the oxidative stress status in Alzheimer's disease induced in rats and Nwidu *et al.,* (2017) in anti-acetylcholinesterase activity and antioxidant properties of extracts and fractions of *Carpolobia lutea*.

CONCLUSION

The inhibition of the AChE activity by the plants solvents extracts increased the acetylcholine concentration of the serum and the brain tissues with positive impact on the cognitive function, hence this study clearly showed that these plants solvents extracts possessed AChE inhibition and antioxidant properties thus could reverse DDVP-induced cognitive dysfunction like Alzheimer's disease and other related cognitive complications.

Declarations section

Ethics approval and consent to participate

We hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

Consent for publication

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them. We also give our consents for the manuscript publication.

Availability of supporting data

All the data and materials for the study are available at authors possession.

Competing interests

The Authors declare no conflict or competing of interest exist

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Author contributions: The authors read and approved this submitted manuscript. Author OAO designed, executed and wrote the draft manuscript. Author GSA involved in the laboratory analyses.

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