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Phytochemical Screening, Partial Characterization And Antioxidant Activity Of Water Lily (*Nympheae Alba*) Bulb, From Uba Local Government Area, Borno State, Nigeria,

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ABSTRACT
This work ventures into the phytochemicals, partial characterization and antioxidant properties of *Nympaeaalba*. Qualitative phytochemical analysis reveals the presence of carbohydrates, tannins, flavonoids, phenols, saponins, alkaloids and sterroids. Quantitative constituents viz; cool maceration of methanolic crude extract attested to high phenol (108.23 mg/mL) and tannins (79.93 mg/mL). Amongst the phytochemical identified, phenols and tannins were found as the major components. Using Agar Diffusion method to access its activity against isolates depicts that, at 500 mg/mL, activities were maximum against *Basilus subtilis*, *Staphlococcus aureus*, *Salmonella taphii and Escherichia col*. It also portrayed that, activity decreases as the concentration decreases. DPPH, H_2O_2, and FRAP assay were employed to determine the antioxidant activity and they showed high IC_{50} activity of DPPH (26.77 μg/mL) H_2O_2 (32.01 μg/mL) and FRAP (97.26 μg/mL). FTIR and GCMS were used for the partial characterization of some antioxidant compounds and their possible anti-radical properties investigated. GC-MS analysis suggested a wide range of compounds, important amongst which are; phtalic acid, bis- (2-methylpropyl) ester, henoicosane, heptadecane, tricosane, 2-methyl tetracosane, 9,12-octadecanoic acid, benzenetriol, cis-vaccenic acid, butyldecyl phthalate, squalene, 11-ecocenoic acid and 9,12,14-octadecatrienoic acid. FT-IR spectroscopy was used to identify functional groups and their values to corroborate the above molecules thus; O – H (3342.1), C – H (2927.0), C = O (1711.3), N – H (1610.3), C – F (1328.3), & C – O (1034.0). These functional groups and the phytochemicals present in the molecules are obviously responsible for the antimicrobial, as well as the antioxidant activities of the extracts, since some of them inhibited oxidation.From the aforementioned, the plant bulb could be a good antioxidant / medicinal source, after nutritional and toxicology studies.

Keywords: Qualitative & quantitative phytochemicals, antioxidant, characterization,

Nymphaea alba and isolates

INTRODUCTION

Medicinal plant parts are rich in phenolics, flavonoids, tannins, alkaloids, proteins and amino acids. These compounds have multiple biological effects including antioxidant activity. The therapeutic

potential of natural medicinal plants as antioxidants in reducing free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful. Phenols contain hydroxyls that are responsible for the radical scavenging effect, mainly due to their properties. Flavonoids are a group of polyphenolic substances which exert antioxidant activities via radical scavenging, metal ion chelation, and membrane protective efficacy (Kumar *et al.,* 2013; Kumar and Pandey, 2014).

Nymphaeaalba belongs to family *Nymphaecae*; commonly known as white water lily. Its name in Yoruba is '*Osibada'*, in Hausa is '*Bedo'*, in 'Marghiis' *'Leleggu'*, inKilbais *'Lekku'*, in Kamwe *(*Higgi*)* is *'Dafarambala'* (Umberto, 2000). White water lily is a vivacious water plant, with a blackish, large fleshy perennial rhizome, growing in mud, where the water is from 91.44 cm to 304.8 cm in depth. The leaves are floating, orbicular; heart shaped up to 39 cm in diameter. The plant grows in ponds, lagoons and slow streams in forest regions.

Medically, water lilies have been investigated for the following conditions: acne, adenopathy, bleeding, boil, burn, cancer, cold, cough, cytosis, dermatosis, diarrhea, dysentery, enterosis, flu, freckle, furuncle, gonorrhea, inflammation, Leukorrhoea, nephrosis, pain, pharyygnosis, pulmosis, scrufola, sore throat, sore, spermatorrhoea, stomatisis, swelling, toothache, tuberculosis, tumor, uterosis, vaginosis, whitlow, etc (Duke, 2002). However, the leaves of *Nymphaea alba* have been used in herbal preparations to treat inflammation, wound, tumor and boil. The flowers show anti *S. aureus* activity (Shadidi, 2004).

The medicinal value of this aquatic plant lies in those chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents include alkaloids, tannins, flavonoids, saponins and phenols. These compounds are termed secondary metabolites (Shadidi, 2004).

Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resources of cure of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk-medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.*, 2008).

Plants are potent bioactive components of phyto-medicine since times immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant viz; bark, leaves, flowers, roots, fruits, seeds, bulks etc i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories (Parekh *et al.*, 2006).

Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found (Folklore) (Parekh *et al.,* 2006). Fresh or dried plant materials can be used as a source for extraction of secondary plant components. Many researchers have reported about plant extract preparation from fresh plant tissues (Parekh *et al.,* 2006). The logic behind this came from the ethno-medicinal uses of fresh plant materials among the traditional and tribal people. But many plants are used in the dry form by traditional healers and due to differences within different plant tissues, plants are usually air dried to a constant weight before extraction (Parekh *et al.,* 2006).

Nymphae alba is the most fascinating aquatic plants being consumed as food in Uvu and recognized in traditional medicine for the treatment of various life threatening diseases; yet its components have not been scientifically analyzed. It is therefore, necessary to provide scientific insight of the potential antioxidant activity of *N. alba* as an alternative remedy of those life threatening diseases.

It is a genus of hardy and tender aquatic plants in the family *Nymphaeaceae*. The genus has a cosmopolitan distribution. Many species are cultivated as ornamental plants, and many cultivars have been bred. Some taxa occur as introduced species where they are not native (John, 2012), and some are weeds (Jepson, 2008). Plants of the genus are known commonly as water lilies (John, 2012) in the United Kingdom. The genus name is from the Greek, *nymphaia* and the Latin *nymphaea*, which mean "water lily" and were inspired by the nymphs of Greek and Latin mythology (John, 2012).

Figure 1: Water lily (*Nymphae alba*) flower and fruit. **Source**: Field Survey, 2019.

Description; Water lilies are aquatic rhizomatous perennial herbs, sometimes with stolons, as well. The leaves grow from the rhizome on long petioles. Most of them float on the surface of the water. The blades have smooth or spine-toothed edges, and they can be rounded or pointed. The flowers rise out of the water or float on the surface, opening during the day or at night. Many species of *Nymphaea* display protogynous flowering. The temporal separation of these female and male phases is physically reinforced by flower opening and closing, so the first flower opening displays female pistil and then closes at the end of the female phase, and reopens with male stamens (Povilus *et al.*, 2015). Each has at least eight petals in shades of white, pink, blue, or yellow. Many stamens are at the center. Water lily flowers are entomophilous, meaning they are pollinated by insects, often beetles. The fruit is berry-like and borne on a curving or coiling peduncle (John, 2012).

Cultivation; Water lilies are not only decorative, but also provide useful shade which helps reduce the growth of algae in ponds and lakes. Many of the water lilies familiar in water gardening are hybrids and cultivars. Water lilies have several edible parts. The young leaves and unopened flower buds boiled and served as a vegetable. The seeds, high in starch, protein, and oil, may be popped, parched, or ground into flour and Potato-like tubers can be collected from the species *(*Peterson, 1977).

Taxonomy: This is one of several genera of plants known commonly as lotuses. It is not related to the legume genus *Lotus* or the Chinese and Indian lotuses of genus *Nelumbo*. It is closely related to *Nuphar* lotuses, however, in *Nymphaea*, the petals are much larger than the sepals, whereas in *Nuphar*, the petals are much smaller. The process of fruit maturation also differs, with *Nymphaea* fruit sinking below the water level immediately after the flower closes, and *Nuphar* fruit remaining above the surface (Peterson, 1977, Borsch *et al*., 2007).

Taxonomical hierarchy states that it belongs to kingdom *Plantae*, Phylum *Embryophyta*, Class *Dicotyledons*, Order *Nymphaeales*, Family *Nymphaeaceae*, Genus*Nymphaea*. The floral description of the flowers states that it is actinomorphic, bisexual, calyx four, carolla five plus five, andrecium and gynecium infinity. The phylogenetic studies of the genus *Nymphaea* is based on the chloroplast trnT-trnF region and the genus divided into six subgenera such as *Anecphya*, *Ondinea*, *Brachyceras*, *Hydrocallis*, *Lotos* and *Nymphaea* (Borsch *et al*., 2007). *Nymphaea* is common in shallow lakes and ponds distributed throughout temperate and tropical Asia namely Bangladesh, India, Pakistan, Sri Lanka, Yunnan, Taiwan, Philippines, Cambodia, Laos, Myanmar, Thailand, Vietnam, Indonesia and Malaysia (Borsch *et al*., 2007).

Cultural Significance: The ancient Egyptians revered the Nile water lilies, which were known as lotuses. The lotus motif is a frequent feature of temple column architecture. In Egypt, the lotus, rising from the bottom mud to unfold its petals to the sun, suggested the glory of the sun's own emergence from the primeval slime. It was a metaphor of creation. It was a symbol of the fertility gods and goddesses as well as a symbol of the upper Nile as the giver of life (Tresidder, 1997).

The flowers of the blue Egyptian water lily (*N. caerulea*) open in the morning and then sink beneath the water at dusk, while those of the white water lily (*N. lotus*) open at night and close in the morning. Egyptians found this symbolic of the separation of deities and of death and the afterlife. Remains of both flowers have been found in the burial tomb of Ramesses II.

A Roman belief existed that drinking a liquid of crushed *Nymphaea* in vinegar for 10 consecutive days turned a boy into a eunuch (Tresidder, 1997).

Ethnopharmacology: The powdered root stock is given for dyspepsia, diarrhea, piles and urinary ailments. A decoction of the flower is given for palpitation of the heart. It is also supposed to be a blood purifier and aphrodisiac. The rhizome is prescribed for cystitis, nephritis, enteritis, fevers and insomnia (Jayaweera, 1982). The whole plant is being used for the treatment of diabetes and eye disorder. In Africa, the different species of *Nymphaea* are used in the management of cancer (Sowemimo *et al*., 2007). It is also used for the treatment of whitlow.

Phytochemicals: Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients. Other phytochemicals with physiological properties may be elements rather than complex organic molecules. For example, selenium, which is abundant in many fruits and vegetables, is a dietary mineral involved with major metabolic pathways, including thyroid hormone metabolism and immune function (Brown and Arthur, 2001). It is also an essential nutrient and cofactor for the enzymatic synthesis of glutathione, an endogenous antioxidant (Papp *et al.*, 2007).

Furthermore, due to the ample potential embedded in plants, phytochemical screening of plants have become an important requisition that cannot be over emphasized because it is a scientific process or method of evaluation that is done in order to have complete knowledge of the different chemical compounds present in a plant extract which are of biological significance (Linus, 2014).

The study is aimed at phytochemically screening and charactering water lily bulb and analyzing its antioxidant and antimicrobial activities with the mind to serve as a validating document for the folklore claims on the bulb and as a good reference material (baseline) for anyone who may want to venture into further research on *N. alba.*

MATERIALS AND METHODS

Reagents and Solvents: DPPH (2, 2-diphenyl-2-picryl hydra zyl**)** (Sigma, Chemical Company Ltd, USA), Vitamin E (tocopherol), Hydrogen peroxide, Phosphate Buffer Solution (PBS), Methanol, n-Hexane, Ethyl acetate, all reagent used were of analytical grade.

Equipment/Apparatus: General lab incubator/100, centrifuge 5308, shaker, UV/Vis CE7400, oven, FT-IR Perkin Elmer GTA-4000, Shimadzu GC-MS QP500 etc.

Plant Materials: Fresh fruits of *Nymphaea alba* were collected from Zhe pond in Uvu, Askira / Uba Local Government Area of Borno State on the 8th of November, 2019. The fruits were identified by a Botanist Dr. Comfort, Head of Department, Botany, Adamawa State University, Mubi. Vouchers specimen was kept in the Department.

Preparation of Fruit Samples: The fruits of *Nymphaea alba* were plucked from their stems, washed with tap water and rinsed with distil water. The bark was peeled off and fruits were dried under-shade to a constant weight and pulverized into powder using clean pestle and mortar. The powdered sample was stored in a close container and kept in the dark, at room temperature until it required for use (Doughari *et al.*, 2008).

Extraction of Fruit Materials: The powdered material, 100g was macerated in 98% methanol for 48 hrs. The liquid filtered using a clean dry cotton wool and re-filtered using Whatman's No 1. The liquid filtrate was distilled and concentrated in vacuum (oven) at 37^0C and percentage yield calculated.

The extracts were chemically tested both qualitatively and quantitatively for the presence of different phytochemicals using standard methods and for antioxidant activity.

Organisms: *Bacillus subtilis, Salmonella typhi, Staphlocuccocus spp* and *Escherichia coli*

Antioxidant: Molecular oxygen (O=O) or (O_2) is required to sustain life, but it can be toxic through the formation of reactive oxygen species (ROS). ROS includes superoxide radical, hydroxyl radical, singlet oxygen and H_2O_2 which have been found to play an important role in the initiation and /or progression of various diseases such as atherosclerosis, inflammatory injury, cancer and cardiovascular diseases (Sreeramulu *et al.*, 2013). Oxidative stress, initiated by these free radicals, seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid per-oxidation. But organisms have multiple mechanisms to protect cellular molecules (DNA, RNA and proteins) against ROS induced damage. These include repair enzymes (DNA glycosylases, AP endonucleases *etc*), antioxidant enzymes (SOD, catalase, and glutathione peroxidase), and intra as well as extracellular antioxidants (glutathione, uric acid, ergothioneine, vitamin E, vitamin C and phenolic compounds (Hamid *et al.*, 2010). However, this natural antioxidant mechanism can be inefficient for severe and/or continued oxidative stress.

Based on this idea, there has been a strong demand of therapeutic and chemo preventive antioxidant agents with limited cyto-toxicity to enhance the antioxidant capacity of the body and help attenuate the damage induced by ROS. Antioxidants are a loosely defined group of compounds characterized by their ability to be oxidized in place of other compounds present, (Gama-Castrol, *et al*., 2010).There

are mainly two strategies proposed for this, one would be to use the antioxidants with direct radical scavenging activity, and the other approach is to identify antioxidants that would increase the expression of antioxidant enzymes (Talalay, 2000). There are some synthetic antioxidants like butylated hydroxyl toluene, butylated hydroxyl anisole and tetra butyl hydroquinone that have been commonly used. However, it has been suggested that these compounds are carcinogens (Branen, 1975). This led to an increased interest in natural antioxidants from plant sources.

Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. These facts have inspired widespread screening of plants for possible medicinal and antioxidant properties; the isolation and characterization of diverse phytochemicals and the utilization of antioxidants of natural origin to prevent diseases (Mahantesh *et al.*, 2012).

Reactive oxygen species (ROS), otherwise known as free radicals, are highly reactive chemical substances that move around in the body and have deleterious effect on body cells. They include different types of radicals such as superoxide, hydroxyl radical, or singlet oxygen (Alia *et al*., 2003). They have been implicated in the aetiology of many diseases like cancer, diabetes, cataract, cardiovascular and neurodegenerative diseases (Okochi and Okpuzor, 2005). The natural defense mechanism of animals detoxifies these free radicals with the aid of its antioxidant molecules and enzymes. These antioxidants play the role of free radical scavengers by preventing and mitigating damages that may result from ROS (James *et al*., 2011). However, oxidative stress results when the balance of free radicals and natural cellular antioxidants shifts in favour of the free radicals. This leads to the requirement for supplementary exogenous antioxidants compounds. There are several reports on the antioxidant potentials of medicinal plants such as *Diospyros abyssinica*, *Pistacia lentiscus* (Krishnaiah *et al*., 2011), *Allium sativum* and *Origanum syriacum* (Al-Jaber *et al*., 2011).

Antioxidants can be defined as bioactive compounds that inhibit or delay the oxidation of molecules (Halliwell *et al.,* 1995). Many scientists have concerns about safety because synthetic antioxidants have recently been shown to cause health problems such as liver damage, due to their toxicity and carcinogenicity. Therefore, the development of safer antioxidants from natural sources has increased, and plants have been used as a good source of traditional medicines to treat different diseases. Many of these medicinal plants are indeed good sources of phytochemicals that possess antioxidant activities. Some typical examples of common ingredients that have been used in ethnic foods are tamarind, cardamom, lemon grass, and galangal basil. These spices or herbs have been shown to contain antioxidants (Javanmardi *et al.*, 2003).

Deterioration of food due to either bacterial or fungal infection has always been a major concern, causing huge losses to food industries and societies throughout the world (Javanmardi *et al.*, 2003). Moreover, the spread of food pathogens has become a major public health concern. With an increasing awareness of the negative effects of synthetic preservatives, there has been increased

demand for the use of nontoxic, natural preservatives, many of which are likely to have either antioxidant or antimicrobial activities (Negi *et al.*, 2005; Baharlouei *et al.*, 2010).

Herbs have always been used for flavor and fragrance in the food industry, and some of them have been found to exhibit antimicrobial properties (Baharlouei *et al.*, 2010). Therefore, the call for screening and using plant materials for their antioxidant and antimicrobial properties has increased. Approximately 20% of all plant species have been tested in both pharmacological and biological applications to confirm their safety and advantages (Suffredini *et al.*, 2004).

Consumption of vegetables has been linked to a reduction in the risk of many diseases, such as cancer and cardiovascular diseases, in epidemiological studies (Zahin *et al.*, 2007). Numerous studies have attempted to screen vegetables for antioxidant activities by using different oxidation systems.

These vegetables include carrots, Irish potatoes, sweet potatoes, red beets, cabbage, Brussels sprouts, broccoli, lettuce, spinach, onions, and tomatoes. In addition to the concise studies, which have used different methodologies to release bioactive compounds, it is becoming increasingly difficult to ignore advanced extraction methods, which have paved the way to extract bioactive compounds rapidly.

Despite scientists' successes in showing the activity of vegetables' bioactive compounds, there is little known about the activity of the antioxidant components that have been isolated from these vegetables. Researchers have tended to focus on advanced methods to isolate and measure the activity of antioxidant compounds such as flavonoids, phenolic acids, tocopherols, carotenoids, and ascorbic acid (Block *et al.*, 1992).

Fruit consumption has also been linked to a reduction in the risk of many diseases, (*Prunus persica* L.) are an economically important fruit in many countries. Studies have shown that phenolic compounds found within various peach genotypes are a major source of potential antioxidants (Block *et al.*, 1992). Interestingly, peaches have shown a great inhibition of low density lipoprotein (LDL) oxidation with a percentage of antioxidant activity of 56–87%. This antioxidant activity can be attributed to its essential compound content including hydroxycinnamic acids, chlorogenic, and neochlorogenic acids, but not to carotenoids such as β-carotene and β-cryptoxanthin. Moreover, low antioxidant activity was found in peach peel. In contrast, Plumb and others pointed out that hydroxycinnamic acids do not contribute to the inhibition of lipid peroxidation of the liver using plums and peaches because hydroxycinnamic acids has weak ability to scavenge hydroxyl radicals (Plumb *et al.*, 1999). Grape (*Vitisvini fera* L.) is a fruit crop grown throughout the world. Grapes and its juices have been recently studied by (Plumb *et al.*, 1999).

Phenolic compounds are high in both fresh grapes and commercial grape juices. The percentage of inhibition LDL oxidation was about 22% to 60% for fresh grapes, while it was approximately 68% to 75% for commercial grape juices, when standardized at 10 mg gallic acid equivalents (GAE).

According to (Frankel and Meyer, 1998), both grapes and its juices exhibited high oxygen radical absorbance capacity (ORAC), and the anthocyanin pigment malvidin-3, 5-diglucoside was a major compound isolated in grapes.

Antioxidant and antibacterial activities of various solvent (ethyl acetate, acetone, methanol, and water) extracts of *Punica granatum* peels were examined by applying the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. The results obtained showed a significantly higher decreasing power in the methanol extracts and a significantly higher antibacterial activity in the acetone extracts. Soong and Barlow investigated the antioxidant activity and phenolic content of various fruit seeds (Soong and Barlow, 2004). Petroleum ether was used to get rid of the excess fat from the seeds and extraction has been carried out with methanol. The 2, 2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), DPPH, and the ferric reducing ability of plasma (FRAP) methods were used to investigate the antioxidant activity. Abdille *et al*. (2005), examined the antioxidant activity of *Dillenia indica* fruit using different kinds of solvents viz DPPH, phospho-molybdenum, and β carotene bleaching methods (Abdille *et al.,* 2005). The methanol extracts showed the highest antioxidant activity, followed by the ethyl acetate and water extracts.

Antioxidant activity of *Syzygium cumini* fruit *in vitro* has been investigated (Abdille *et al.,* 2005). Antioxidant activity was measured by DPPH, superoxide, lipid per-oxidation, and hydroxyl radical scavenging activity methods. The results brought to light a significant correlation between the concentration of the extract and the percentage of inhibition of free radicals. The antioxidant property of the fruit might be from the presence of antioxidant vitamins, anthocyanins, phenolics, and tannins.

Juntachote and Berghofer, measured the stability of the antioxidant activity of ethanolic extracts for Holy basil and galangal using DPPH, superoxide, β carotene bleaching, reducing power, and iron chelation methods (Juntachote and Berghofer, 2005). They found higher antioxidant activity at neutral pH compared to an acidic pH. Holy basil and galangal extracts provided strong iron chelation activity, superoxide anion scavenging activity, and reducing power were proportional to the concentration of the extracts. Employing *in vitro* methods such as DPPH and superoxide scavenging activity, Orhan *et al*., (2007) measured the antioxidant activity of *Arnebiadensi flora* and observed that polar extracts had a higher antioxidant activity compared to non-polar extracts. Rathee *et al*., (2006) studied the antioxidant activity of *Mammea longifolia* buds extracted in both methanol and aqueous ethanol. The results found a significant antioxidant activity, and the activity of aqueous ethanol was higher than methanol (Rathee *et al.,* 2006).

Vitamins` Role in Cancer Prevention

Cancer has been increasing throughout the world. It is one of the causes of yearly mortality. There were 10.4 million new cancer cases registered in 2015, and scientists predict that the number of cancer cases per year will double by 2030 (Vermerris and Nicholson, 2006).

Recently, many studies have shown rigorous evidence that hydroxyl radicals (OH•) and the superoxide anion (O_2-•) are involved in the development of cancer because they are biologically reactive oxygen species. Compounds with high reactive oxygen species and reduction activity, are likely to prevent cancer's occurrence (Hursting *et al.,* 1999). As shown previously, fruits and vegetables are the primary sources of natural antioxidants, consisting of different kinds of antioxidant compounds such as Vitamin C, ascorbic acid, Vitamin E, carotenoids, lutein, and lycopene. Some researchers have confirmed that phenolic compounds and polyphenols are secondary plant metabolites, which are considered the best scavengers to prevent the production of free radicals. The United States has an amazing diversity of plant species. Some of them have been used for traditional medicines for a long period of time because of their various desirable activities. The mixtures of the plant natural products have been screened in order to study their effect on human leukemia cells (Wangensteen *et al*., 2006). The finding confirmed that mixtures of natural products are a good source for human leukemia cell inhibition. Nassr-allah *et al.,* (2009) investigated the chemical diversity of natural products from plants in order to test their ability to work as anticancer and antioxidant agents (Nassr-allah *et al.,* 2009). DPPH assay was used to measure the antioxidant activity for plant extraction while using *in vivo* and *in vitro* methods in order to measure the anticancer activity. The results confirmed that some natural products from Egyptian flora have the potential for use as therapeutics for diseases such as cancer (Khan *et al.,* 2005).

The effectiveness of an aqueous extract from willow leaves (*Salix safsaf*, *Salicaceae*) against human carcinoma cells has been tested *in vivo* and *in vitro* (Nassr-allah *et al.,* 2009). The findings mentioned that the metabolites for the willow extract could inhibit tumors, thereby enhancing apoptosis and causing DNA damage. The anticancer activity of different extracts from the leaves of the drumstick tree (*Moringa oleifera*) was screened in order to test against leukemia and hepatocarcinoma cells *in vitro.* Primary cells harvested from 10 patients with acute lymphoblastic leukemia (ALL) and 15 with acute myeloid leukemia (AML) were significantly killed by hot water and ethanol extracts. Thus, *Moringa oleifera* may have the potential for use as a natural treatment for diseases such as cancer (El-Shemy *et al.,* 2007). Altemimi reported that the phenolic extracts from the olive leaf extract could be used as a source of potential antioxidant and antimicrobial agents (Altemimi, 2017).

Determination of 2, 2-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging capacity

DPPH radical scavenging capacity of the extracts of the plant materials was determined according to the method of Vijayabaskaran *et al.,* (2010). DPPH (2, 2-diphenyl-2-picryl hydrazyl), is a stable free radical, which when acted upon by an antioxidant, will be converted into diphenyl-picryl hydrazine with a colour change from deep violet to light yellow colour. This can be quantified spectrophotometrically at 517 nm to indicate the extent of DPPH scavenging activity by the plant extracts.

The effect of the methanol extract of the fruits on DPPH radical was estimated using the method of Vijayabaskaran *et al.*, (2010). The plant parts (fruits) methanolic, ethyl acetate and hexane extracts were treated with different concentrations ranging from 100µg/mL to 1000 µg/mL in 95% (v/v) ethanol. One (1.00) mL of freshly prepared DPPH solution was added into the test tube and shaken and incubated for 25mins at room temperature. The absorbance of the mixture was measured spectrophotometrically at 517nm. Vitamin E (Tocopherol) was used as standard. Control sample was prepared without any extract or vitamin E and 95% ethanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation (Koleva *et al.*, 2002).

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} x\ 100 \text{ ----- (1)}$$

Where:

Absorbance of control = Absorbance of DPPH radical + ethanol

Absorbance of sample = Absorbance of DPPH radical + sample extract/standard.

Ferric Reducing power assay: The reducing power of methanol, ethyl acetate and hexane extracts of *N. alba* were evaluated according to the method of Ganie *et al.*, (2011). Different concentrations (0.025 – 0.5 mg mL^{-1}) of the extracts and standard drugs were mixed with 2.5 mL of 1% potassium hexacyanoferrate (II). The mixture was incubated at 50^{o}C for 20 min, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min. The supernatant was mixed with 2.5 mL distilled water 0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at 700 nm. The increase absorbance of the reaction mixture indicated stronger reducing power.

Scavenging of hydrogen peroxide; The ability of extracts to scavenge hydrogen peroxide was determined according to the method by Nabavi *et al*, (2008). A solution of hydrogen peroxide 2 mM was prepared in phosphate buffer (pH. 7.4). The concentration of hydrogen peroxide was determined by absorption at 285 nm using a UV/Vis spectrophotometer. The samples of all the bulb extracts of *N. alba* and vitamin E at '1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.0625 mg/mL' was used against H_2O_2. The decrease in absorbance of H_2O_2 at 285 nm was measured spectrophotometrically after ten min (10 min) against a blank solution containing the test sample in phosphate buffer solution (PBS) without H_2O_2 and blank solution containing phosphate buffer with hydrogen peroxide (control). All these tests were performed in triplicates. The percentage of hydrogen peroxide scavenged by the extracts was calculated as follows:

$$\%\ \text{scavenged}\ [H_2O_2] = (A_c - A_s)/A_c] \times 100 \text{ ---------- (2)}$$

Where $A_{C;}$ is the absorbance of the control and A_S the absorbance in the presence of the sample of extract and standard (Nabavi *et al.*, 2008). The values of % inhibition were obtained from Eq (2). For the 50% Inhibitory Concentration (IC_{50}) evaluation of the extract, graphs showing the concentration

of the test samples and the alpha tocopherol versus % Inhibition (% H_2O_2 reduction) was plotted (Singleton *et al.*, 1999).

Antimicrobial Tests

Media Preparation (Nutrient Agar): Twenty eight (28) g of nutrient agar powder was dissolved in 1000 mL flask with distilled water; and sterilized in an autoclave machine for 15 min. at 121^0C. It was then allowed to cool so that the nutrient agar condense and was put into an oven to dry at a temperature of about 40^0C.

Preparation of Normal Saline: Sodium Chloride (NaCl) 8.5g was weighed accurately using a digital balance, and dissolved in 1 liter of distilled water and then sterilized for 15 min at 121^0C.

Disc Diffusion Sensitivity Tests: This procedure was used to test routinely for antimicrobial sensitivity: - 400 mg/mL, 200 mg/mL and 100 mg/mL concentrations were made by dissolving 0.4 g, 0.2 g and 0.1 g of extract respectively in 1.00 mL of methanol for each on a clean culture plates and the methanol allowed to evaporate under room temperature.

A disc of blotting paper was impregnated with known volume of appropriate concentration of 400 mg/mL, 200 mg/mL and 100 mg/mL of the extracts, and placed on a plate of sensitivity testing agar uniformly inoculated with the test organisms. The crude extract diffused from the disc into the medium and was incubated for 18 – 24 at 37^0C, and examined for growth, whereas resistant strains had smaller zones of inhibition or grow up to the edge of the disc.

Statistical Analysis: The data obtained, was analyzed statistically using ANOVA version 23, followed by a post test (Turkey-kramer multiple comparison test). Differences between means were considered significant at 1% and 5% level of significance, $p < 0.01$ and 0.05.

RESULTS AND DISCUSSION

Qualitative and quantitative phytochemical analyses for the presence or absence of alkaloids, carbohydrates, flavonoids, phenols, saponins, steroids, tannins and terpenoids were investigated and the result shown in **tables 1 & 2** respectively**.**

The result of the antimicrobial sensitivity test in the agar (disc) diffusion method is shown in **table 3.**

Table 4, showed results of the inhibition of antioxidant on different models (DPPH, H_2O_2, FRAP of the plant sample while **Fig 2,** gave its percentage graphical representation) and **Table 5,** the Ascorbic acid effect on the different models and **Fig 3**, its percentage graphical representation. **Fig 6** is the IC_{50}, results. **Tables 7 and 8** are the FTIR and the chemical constituents of the identified molecules from the plant sample respectively.

The GC-MS analysis of *N. alba* fruit extracts showed a wide range of poly phenolic compounds. Using the reference standard and comparing the obtained mass spectra with literature data, has led to identification of different fragments of poly phenolic compounds; phenolic acid, flavonoids, tannins

and other compounds in total of nine compounds, their retention time (TR), chemical formulae, molecular weight, area and peak number as presented in **table 8.**

Table 1: Qualitative Phytochemical of *Nymphaea alba* fruit

S/N	Phytochemical	Relative Abundance Methanolic Extract
1.	Carbohydrates	+
2.	Saponins	+
3.	Phenol	+
4.	Tannin	+
5.	Alkaloid	+
6.	Flavonoid	+
7.	Terpenoid	_
8.	Steroid	+

Key: −Below Detection limit (BDL) + presence

Table 2: Quantitative Phytochemical of *Nymphaea alba* bulb

S/N	Phytochemical	Relative Abundance Methanolic Extract
1.	Phenols	108.23 mg/Ml
2.	Saponins	18.51%
3.	Tannins	79.93 mg/mL
4.	Alkaloids	1.85 %
5.	Flavonoids	9.34 mg/mL

Table 3: Antimicrobial sensitivity test for methanolic extract with different concentration in mg/mL

Conc. mg/mL	**Microorganisms**	**ME**
500	*Bacillus subtilis*	+++
	Salmonella taphi	+++
	Staphlacoccusaureas	+++
	Escherichia coli	++
250	*Bacillus subtilis*	+++
	Salmonella taphi	++
	Staphlacoccusaureas	+++
	Escherichia coli	+
125	*Bacillus subtilis*	++
	Salmonella taphi	+
	Staphlacoccusaureas	++
	Escherichia coli	+
62.5	*Bacillus subtilis*	+
	Salmonella taphi	R
	Staphlacoccusaureas	++
	Escherichia coli	R
Aug. 30 mg/mL	*Bacillussubtilis*	+++
	Salmonella taphi	+++
	Staphlacoccusaureas	+++
	Escherichia coli	+++

Key: R = 1 – 6, + = 7 – 10, ++ = 11 – 15, +++ = 15 above

Table 4: Effects of methanolic extract of *N. alba* fruit on different antioxidant models

S/N	Concentration μg/mL	% Inhibition DPPH	H_2O_2	FRAP
1.	10	39.73±1.000^{e}	39.54±0.095^{a}	16.33±1.000^{a}
2.	20	50.33±1.000^{a}	46.94±0.095^{a}	20.33±1.000^{b}
3.	30	60.92±1.000^{b}	52.41±1.000^{b}	24.66±1.000^{c}
4.	40	71.52±1.000^{c}	60.12±1.000^{c}	31.00±1.000^{d}
5.	50	84.10±1.000^{d}	65.59±1.000^{d}	35.00±1.000^{e}
6.	IC_{50}	26.77	32.01	97.26

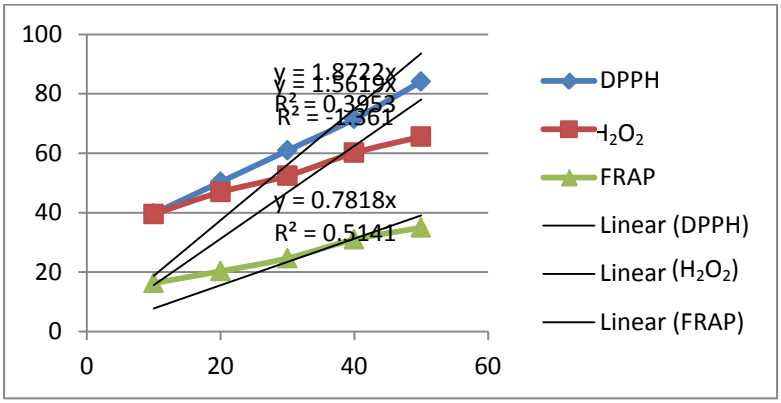

Figure 2: DPPH, H_2O_2 and FRAP inhibition percentage of the methanolic extract of *N. alba* fruit.

Table 5: Effects of Ascorbic acid on *N. alba* fruit on different antioxidant models

S/N	Concentration μg/mL	% Inhibition DPPH	H_2O_2	FRAP
1.	10	45.03±1.000^{a}	51.56±1.000^{a}	22.0±1.000^{a}
2.	20	55.62±1.000^{b}	65.62±1.000^{b}	27.33±1.000^{b}
3.	30	70.19±1.000^{c}	76.56±1.000^{c}	32.66±1.000^{c}
4.	40	80.79±1.000^{d}	85.15±1.000^{d}	37.66±1.000^{d}
5.	50	90.06±1.000^{e}	90.62±1.000^{e}	44.67±1.000^{e}
6.	IC_{50}	24.12	22.80	50.13

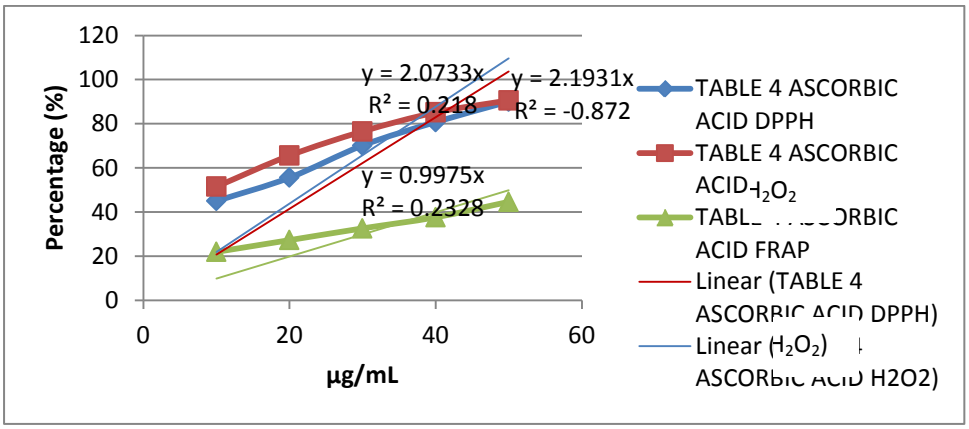

Figure 3: DPPH, H_2O_2 and FRAP inhibition percentage of the ascorbic acid extract of *N. alba* fruit.

Table 6: IC_{50} Result of *N. alba* fruit

Sample	IC_{50} µg/Ml DPPH	H_2O_2	FRAP
Methanolic Extract	26.77	32.01	97.26
Ascorbic Extract	24.17	22.80	50.13

Table 7: FTIR Result of Methanolic Extract

Peak Value	Functional Group	Type of Vibration	Characteristics Absorption cm^{-1}	Intensity
3342.1	O – H	(Stretch, H-bonded)	3300 – 3600	Strong
2927.0	C – H	Stretch	2850 – 3000	Strong
2577.0	O – H	Stretch	2500 – 3300	Strong, very broad
2337.4	C – H	Stretch	2300 – 2400	Strong
1812.1	C=O	Stretch	1800 – 1830	Two band
1711.3	C=O	Stretch	1700 – 1725	Strong
1610.3	N – H	Bending	1550 – 1640	Medium
1447.3	C=C	Stretch	1400 – 1600	Medium weak, multiple band
1328.3	C – F	Stretch	1000 – 1400	Strong
1204.9	C – O	Stretch	1210 – 1320	Strong
1034	C – O	Stretch	1000 – 1300	Strong
801.7	= C – H	Bending	675 – 1000	Strong

Table 8: Chemical constituents identified from *N. alba* fruit methanolic extracts and their retention time

Peak	Molecular formula	Chemical name	MW g/mol	R Time	Area %
2	$C_6H_6O_3$	1, 2, 3-benzenetriol	126.110	5.015	9.76
3	$C_6H_6O_3$	1, 2, 4-benzenetriol	126.110	5.828	1.34
7	$C_{18}H_{34}O_2$	Cis - vaccenic acid	282.461	8.054	3.45
12	$C_{18}H_{32}O_2$	9, 12-octadecadionoic acid	280.445	10.697	2.07
11	$C_{17}H_{36}$	Heptadecane	240.468	9.942	17.13
13	$C_{19}H_{30}O$	12-Methyl-E,E-2,13-octadecadien-1-ol	280.489	11.493	3.93
16	$C_{13}H_{16}N_2O_2S$	3-(azepan-1-yl)-1, 2-benzothiazole 1, 1 dioxide	264.350	13.003	4.02
18	$C_{21}H_{42}O_4$	9-octadecanoic acid (z)-, 2, 3-dihydroxypropyl ester	358.600	14.439	7.79
19	$C_{27}H_{46}O_2$	2H-1-benzopyran-6-ol, 3, 4-dihydro-2-8-dimethyl-2[4R, 8R) 4, 8, 12-trimythyltridecyl]-, [2R[2R*(4R*, 8R*)]]	402.660	15.172	8.92

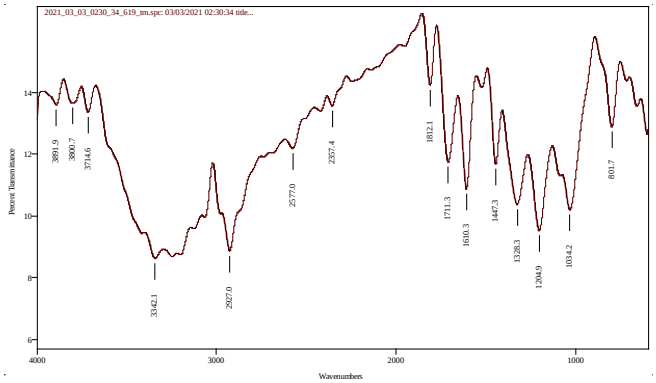

Figure 4: Methanol FTIR spectrum of *N. alba*

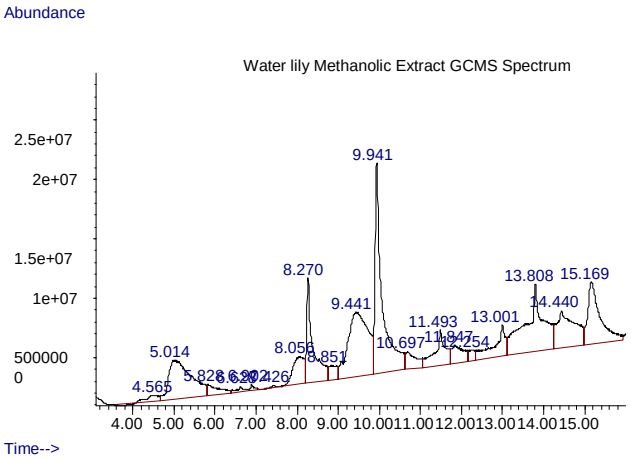

Figure 5: *N. alba* Methanol Extract

DISCUSSION

Qualitative result of *N. alba* fruit methanolic extract indicated that, carbohydrates, alkaloids, phenols, saponins, tannins, flavonoids and steroids are present in extract. Literature suggested that plants which are rich in tannins exhibit anti-diarrhea, anti-inflammatory and anti-oxidant activities. The phytochemicals present in the methanolic fruit extract are responsible for the anti-microbial activity and antioxidant property of the plant and this is corroborated by the work of Kumar *et al.*, 2014.

The quantitative phytochemical was also determined, **table 4.** The result revealed the presence of Phenol (108.22mg/g), tannin (78.93mg/g), saponin (18.5%), flavonoid (9.34mg/g) and alkaloid (1.85%), this result is confirmed by the work of Linus and friends; Linus *et al*., 2014.

The result of the antimicrobial test in the agar (disc) diffusion method for methanolic extract showed no visible growth for *salmonella taphii* and *E. coli* at concentration of 62.5 mg/mL. The activity is better at 125 mg/mL and 250 mg/mL. Maximum activity is at 500 mg/mL, thus the higher the

concentration, the higher the activity. Augumentine was used as a standard and all the test organisms showed maximum activity at concentration of 30 mg/mL.

B*acillus subtilis* has maximum activity of all the isolates at all concentration. Methanolic extracts have high activity on all microorganisms. The reason for the high activity of the methanol extract may be due to the combination of many chemical components found in *N. alba,* as revealed by GC-MS analysis and confirmed by the work of (Seidel and Tylor, 2004, Hong-wai *et al.*, 2014).These isolated bacteria are the major causes of intestinal problems like diarrhea, typhoid, pneumonia, dysentery, etc (Google, 2021). Methanol extract showed positive microbial activity against most of the isolates.

From **Table 3**, extracts exhibited significant antimicrobial activity against the study microorganisms, and this can be linked to the presence of various bioactive molecular components in varying percentages. The antimicrobial effect of alkyl ester, phenolic and aliphatic acid constituents increase with the increased length of alkyl chain and number of hydroxyls attached (Cimolai, 2008). The extracts especially at high concentrations were more sensitive on *B. subtilis*.

Free radical scavenging activity was carried out in different *in vitro* antioxidant models. Several concentrations ranging from (10-50 µg/mL) of *nymphaea alba* methanolic fruit extracts were tested and the result of their study showed that free radicals were scavenged by the test extracts in a concentration dependent manner in all the models.

The IC_{50} values of DPPH, hydrogen peroxide and FRAP were found to be 26.77 µg/mL, 33.01 µg/mL and 97.26µg/mL respectively. While for **Table 5** and **figure 2**, were 24.12 µg/mL, 22.80 ug/mL and 50.13 µg/mL in that order. **Table 6,** and **figure 3**, showed better result in all the models, in the order DPPH > H_2O_2 > FRAP. The extracts of *N. alba* fruit exhibited marked and concentration dependent free radical scavenging effects in DPPH radical scavenging assay. The inhibition of H_2O_2 radical by the extract can be attributed to the proton donating abilities of their phyto-molecules; 1,2,3-benzenetriol, 9, 12-octadecadionoic acid, 12-Methyl-E,E-2,13-octadecadien-1-ol, 2H-1-benzopyran-6-ol, 3, 4-dihydro-2-8-dimethyl-2[4R, 8R) 4, 8, 12-trimythyltridecyl]-, [2R[2R*(4R*, 8R*)]], 3-(azepan-1-yl)-1, 2-benzothiazole 1, 1 dioxide, cis-11-ecosenoic acid, heneicosane, 9-octadecanoic acid, 9,12-octadecadienoic acid, heptadecane, as revealed by GC-MS.These results are in consonance with those of Nagulendran *et al.* (2007), Yazamparast and Johan *et al.*, (2011), who also opined that it`s because these molecules can readily donate hydrogen atom to the radicals. Javanmardi *et al.*, *(*2003), Oki *et al.*, (2002) reported that the quantity of phenolic compound in an extract is directly proportional to its free radical scavenging activity. Haung *et al*., (2012) further reported that phenolic compounds have chemo-preventive properties such as antioxidant.

The present study showed that FTIR spectroscopy is an informative technique that gives detail information about the extracts and the functional groups of the compounds present; **table 7.** Viz, (O –

H) strong, (C – H) strong, (O – H) strong, very broad, (C=O) two band, (C=O) strong, (C=C) medium weak, multiple bands, (C – O) strong, (=C – H) strong, (N – H) medium, (C – F) strong. . At finger print region, there is presence of carbonyl group (C=O) in all the extracts. Among the functional groups observed in the extracts, OH group was found to be present in methanol extract. As OH group has got the ability of forming hydrogen bonding, presence of OH group particularly in the extract probably aided the high potential of methanol extract towards inhibitory activity against microorganisms. The result is in agreement with the work of Ashokkumar and Romaswamy (2013). The GC-MS analysis of the extracts has shown a wide range of alkyls, fatty acid and poly phenolic compounds, **table 8,** which were responsible for antimicrobial and antioxidant activities as comfirmed by the work of Nagulendran and colleagues, (Nagulendran *et al.*, 2007). Using the reference standard and comparing the obtained mass spectra with the literature data has led to the authentication of the above structures. The presence of some of those major compounds contributed to the antimicrobial activity of the fruits. For instance, Heptadecane is used as anti-inflammatory and antioxidant. Phthalic acid is used as antioxidant, antimicrobial and antifouling. Butyl decyl phthalate is used as antifungal, antimicrobial, antimalarial and plasticizer and Squalene is used as antioxidant, antibacterial, anti-tumor and cancer immuno-stimulant, chemo-preventive lepoxygenase inhibitor, pesticide and diuretic, (Shaikh *et al.*, 2012, Alqasim, 2013, Kesava and Usha, 2016, Thirunavukkarasu *et al.* 2016).

CONCLUSION

To the best of my knowledge, this is the first study conducted on the phytochemical, antioxidant and characterization of *N. alba* fruit extracts from Uvu Zhe Pond of Uba LGA. The evaluation of the bioactive compounds using both quantitative and qualitative phytochemical analysis was investigated. GC-MS analysis highlighted the present of aliphatic acid, ester, straight chain alkyl and polyphenol compounds which were found to be responsible for the antimicrobial and antioxidant activities of the fruits. FT-IR analysis of the fruit extract identified many functional groups, through vibration / absorption and intensity of the compounds. The antioxidant activity of the extracts evaluated by using DPPH, H_2O_2 and FRAP assay, was comparable with literature data obtained through different techniques. From the present study, it has been proven that the cool maceration methanol extract of *N. alba* fruit from Uvu Zhe Pond possess medicinal and pharmacological properties, which either act singularly or in synergy to accomplish the aforementioned activities. Therefore, the extract of fruit has a scientific basis for its use as food, and /or traditional medicine against diseases.

RECOMMENDATION

The fruit is a good candidate for further study in term of isolation of active compound (s) and nutritional value of the fruit extracts. Toxicology study of the fruit extracts should be conducted.

CONFLICTS OF INTEREST

All the authors declare that there is no conflict of interest

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