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ANTIOXIDANT CAPACITY AND CYTOTOXIC EFFECTS OF *Cymbopogon citratus* (DC.) Stapf AND *Azadirachta indica* L.

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ABSTRACT. *Cymbopogon citratus* (DC.) Stapf and *Azadirachta indica* L. leaves are traditionally used for the treatment of many diseases. Although they contain antioxidant compounds with free radical scavenging ability, their safety also needs to be considered. This study aims to evaluate and compare the antioxidant capacity and potential toxicity of their leaves' aqueous extracts to determine their preference for therapeutic use. Antioxidant properties were evaluated by determining the DPPH assay, nitric oxide scavenging activity (NSA), ferric reducing antioxidant power (FRAP), ascorbic acid content (AAC), total flavonoid content (TFC), total phenolic content (TPC) and total antioxidant capacity (TAC). Their potential cytotoxic effects were evaluated using *Artemia salina* larvae. *Cymbopogon citratus* exhibited higher TFC, TAC and antioxidant activities, while *A. indica* had a higher TPC. LC_{50} of *C. citratus* was lower than that observed for *A. indica*. The results showed that *C. citratus* possesses higher antioxidant properties and it is less toxic than *A. indica*.

Keywords: *Cymbopogon citratus*, *Azadirachta indica*, Nigeria, leaves' aqueous extract, antioxidants, cytotoxicity.

INTRODUCTION

The underlying mechanism for several diseases is the excessive free radical production that results in oxidative stress (FORMAN *et al*., 2021). Antioxidants prevent many diseases by delaying or preventing substrate oxidation by pro-oxidant, thereby preventing oxidative stress and thus disease state (RAHAL *et al.,* 2014, OSMAN *et al.,* 2017). The medicinal plant contains compounds with antioxidant activities and pharmacological importance (UGBOGU *et al*., 2021) therefore, it has been used in folk medicine and in the synthesis of drugs (SAOSOONG *et al.*, 2016). Secondary metabolites contained in plants are polyphenols such as vitamin E, flavonoids and tannins, and non-phenolics such as melatonin, carotenoids, retinal and thiols (KUNWAR and PRIYADARSINI, 2011; TIBENDA *et al*, 2022).

Cymbopogon citratus (DC.) Stapf (West Indian lemongrass) is grown in the tropical. This plant species belongs to the family Poace. It is common in maritime South Asia and Southeast Asia (SHAH *et al.,* 2011; TIBENDA *et al*, 2022). *C. citratus* referred to as fever grass, is commonly used to make teas (TOUNGOS, 2019; ONYEDIKACHI *et al.,* 2021), because it has many health benefits such as antimalarial, antimicrobial effects (KHAN, 2020; TIBENDA *et al*, 2022) and hepatoprotective effects in rats (ARHOGHRO *et al.,* 2012). It contains sugars, tannins, alkaloids and phenolics such as flavonoids. These phenolic compounds possess antioxidant properties (BASERA *et al.,* 2019). This plant is also toxic, and possesses an insecticidal property (TIBENDA *et al*, 2022). The nephrotoxic, hepatotoxic and cytotoxic properties of this plant have been reported by SOUSA *et al.* (2010).

Azadirachta indica L. (called neem), is a semitropical and tropical plant, belonging to the family Meliaceae. It contains amino acids (such as tyrosine, cysteine and glutamic acid: YADAV, *et al.,* 2016) and polyphenolic flavonoids (such as quercetin and ß-sitosterol), as well as sulphurous compounds which possess antifungal, antibacterial, and antioxidant activities. (ALZOHAIRY, 2016; BABY *et al*., 2022). This plant exhibit hepatoprotective, antifungal, antibacterial, antitumor, antimalarial and antipyretic activities (YADAV, *et al.,* 2016; OLADEJI *et al.,* 2020). The therapeutic effects of *A. indica* are due to the presence of antioxidants such as quercetin and its active components which include nimbidin and azadirachtin (Baby *et al*., 2022). However, similarly to *C. citratus* leaves, *A. indica* leaves posses cytotoxic properties. Nimbolide, found in *A. indica*, contributes to the cytotoxicity of this plant (ALZOHAIRY, 2016). Additionally, azadirachtin possesses a genotoxic carcinogenic potential and results in loss of mobility in insects (AKANEME and AMAEFULE, 2012; YADAV, *et al.,* 2016).

The toxic effects of both plants might be a result of the presence of other components in the plants that may have undesired effects, although these can be reduced by processing.

Synthetic antioxidants are thought to have toxicological and carcinogenic effects thus, their uses should become limited (SAOSOONG *et al*, 2016; ZAKI *et al.,* 2018). This gives rise to the increasing use of plants' active components for the treatment of diseases (ZAKI *et al.,* 2018). However, plant material also contains a variety of other compounds with side effects that can lead to toxicity to organs such as the kidneys and liver (POSADZKI *et al.,* 2013; FLAMINIA *et al.,* 2016). Although *C. citratus* and *A. indica* are both used in the treatment of common diseases like malaria, they are also used as insecticides. Their cytotoxic and genotoxic properties were also reported. It is, therefore, worthwhile to determine and compare the toxicity and pharmacological properties of these plants to define their safety and efficacy as a cure for pathological conditions. Since most of the pharmacological properties of plants are met by the bioactive compounds they possess, this study, therefore, is aimed to determine and compare the toxicity and antioxidant properties of *C. citratus* and *A. indica* leaves.

MATERIALS AND METHOD

Preparation of plant extracts

Neem (*Azadirachta indica*) and lemongrass (*Cymbopogon citratus*) leaves were collected locally from the Itoku market, Abeokuta, Ogun-State, Nigeria, dried, ground into powder. *A. indica* leaves aqueous extract (ALAE) and *C. citratus* aqueous extract (CLAE) were prepared as follows. To 50 g of each of the ground plants were added 250 mL of distilled water at 80°C for 30 minutes. Filtration was carried out using a sterile muslin cloth and the filtrate obtained was evaporated in the rotary evaporator. Then, the solutions obtained were air-dried to obtain the crude extracts.

Chemicals

L-Ascorbic acid (AA), sodium hydroxide (NaOH), distilled water, seawater, gallic acid, sodium trioxonitrate ($NaNO_3$), sodium trioxonitratecarbonate (Na_2CO_3), trichloroacetic acid, ferric chloride ($FeCl_3$), folin-ciocalteu reagent, Fehling A and B solution, aluminium chloride ($AlCl_3$), α-α diphenyl β picryl hydrazyl (DPPH), methanol, sodium nitroprusside, phosphate buffer saline, sulphanilamide, phosphoric acid (H_3PO_4), N-1-napthylethylenediamine dihydrochloride (NED), tetraoxosulphate(xi) acid (H_2SO_4), sodium phosphate (Na_3PO_4), and ammonium molybdate were bought from Sigma-Aldrich Chemical, United Kingdom.

DPPH assay

The radical scavenging ability of the plant extracts was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) by the method of BRAND-WILLIAMS *et al.* (1995). DPPH is reduced by the withdrawal of a hydrogen atom from an antioxidant compound that donates hydrogen atoms (KEDARE *et al*, 2011). The yellow color produced was measured using a spectrophotometer at 517 nm and the percent of inhibition was calculated as shown below:

$$I\% = \frac{(A_{blank} - A_{sample})}{A_{blank}} x\ 100 \quad (1)$$

where A_{blank} indicates the absorbance of the control reaction, A_{sample} indicates the absorbance of the reacting mixture containing the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plot of percentage inhibition against extract concentration.

Nitric oxide scavenging activity (NSA)

NSA was measured by the method described by MARCOCCI *et al*. (1994). Sodium nitroprusside generates nitric oxide at physiological pH. This involves the reaction of nitric oxide with oxygen to produce nitrite ions. The coloured compound produced was measured using a spectrophotometer at 546 nm. The formation of nitric oxide radical percentage inhibition was calculated similarly to the DPPH assay shown above.

Ferric reducing antioxidant power (FRAP)

In the FRAP assay antioxidants are the reducing agents in the redox- reaction measured colorimetrically. The Frap was carried out by the method of BENZIE and STRAIN (1996). In this reaction, ferric-tripyridyltriazine complex is reduced to ferrous electrons with a change in color from colorless to blue due to the ability of the antioxidants to donate electrons. The absorbance of the reacting mixture was taken at 593 nm. The reducing power was expressed as mg of ascorbic acid equivalent per gram of extracts (mg AAE/g extract).

Ascorbic acid content (AAC)

AAC assay involves an oxidation-reduction reaction due to its reducing properties. Iron (111)- phenanthroline reagent (vitamin C reagent) was used for the determination of Vitamin C (LAU and LUK, 1987). The absorbance of the reacting mixture was measured spectrophotometrically at 546 nm. The Vitamin C contents of the extracts were expressed as mg AAE/g extract.

Determination of total flavonoid content (TFC)

The $AlCl_3$ colorimetric method, described by MILIAUSKAS *et al.* (2004) was used for TFC assay. The absorbance of the reacting mixture was measured spectrophotometrically at 510 nm. The TFC of the extract was expressed as mg quercetin equivalents per gram (mg QUE/g).

Total phenolic content (TPC)

The method of GÜLÇIN (2006), was adopted for the determination of TPC using Folin-Ciocalteu's phenol reagent as the oxidizing agent. The absorbance of the reacting mixture was measured spectrophotometrically at 750 nm. Gallic acid Equivalent (GAE) per gram dry weight extract (mg GAE/g extract) was obtained for each sample from the calibration curve.

Determination of total antioxidant capacity (TAC)

The method of PRIETO *et al.*, (1999,) was adopted for the determination of TAC. It involves a reductive reaction in which the extract reduces Molybdenum (VI) to give Molybdenum (V) with the formation of a phosphate/Molybdenum (V) complex with a green colour. The absorbance was read at 630 nm. The TAC of the extracts was expressed as mg AAE/g extract.

Cytotoxicity test

Cytotoxicity of the investigated plant extracts was evaluated by the brine shrimp lethality test (BSLT) which was developed by MEYER *et al*., (1982). *Artemia salina* is the most suitable test organism for toxicity studies because of its easy hatching from dry cysts. BSLT was carried out by the modified method of SOLIS *et al.* (1993) using brine shrimp eggs sprinkled on the darkened side in a chamber filled with sterile seawater containing 2% DMSO. The eggs hatched to produce active nauplii larvae. The number of dead larvae in varying concentrations of standard and extracts (100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL and 6.25 µg/mL) was determined after 24 hours. The dead larvae of the control and test were compared and used for the determination of the percentage lethality. The acute toxicity of the extract is expressed as LC_{50} (the median lethal concentration of a substance in water or air predicted to kill 50% of the test population).

LC_{50} was calculated by the transformation of the percentage death of the larvae at each concentration to the probit values which were plotted against log doses. The dose corresponding to probit 5, i.e., 50% was considered as the LC_{50}.

All results are expressed as mean ± standard deviation of at least three independent experiments.

Statistical analysis

Results are expressed as the mean ± standard error of the mean. GraphPad Prism Version 5.0 for Windows (GraphPad®) Software, San Diego, CA, USA) was used for the statistical analysis. The mean was compared using independent Student T-test at $p < 0.05$.

RESULTS AND DISCUSSION

DPPH assay

As indicated in Table 1, the IC_{50} value obtained for CLAE was significantly lower relative to that of *A. indica*, but significantly higher relative to that of the standard. This

indicates that CLAE has a higher ability than ALAE to scavenge free radicals by the donation of hydrogen atoms. It has also been reported that the extract and fractions of *C. citratus* possess high DPPH scavenging activity which is a measure of its antioxidant activity (JAMUNA *et al.* 2017; UNUIGBE *et. al.,* 2019), while NAGANO and BATALINI (2021) revealed the presence of only moderate DPPH scavenging activity of the *A. indica* leaves.

Table 1. DPPH assay and nitric oxide scavenging of CLAE and ALAE.

Sample	DPPH activity IC_{50} (mg/ml)	Nitric oxide scavenging activity IC_{50} (mg/ml)
Standard	0.07±0.004	0.09±0.032
CLAE	1.45±0.213$^{\#}$	0.21±0.022$^{\#}$
ALAE	5.63±0.414$^{\#}$*	0.66±0.014$^{\#}$*

* $p < 0.05$ significantly different from *C. citratus;*
$^{\#}$ $p < 0.05$ significantly different from the standard.

Nitric oxide scavenging activity (NSA)

As shown in Table 1, CLAE exhibited a significantly lower IC_{50} value with respect to ALAE, indicating a higher scavenging ability, although both leaf extracts have significantly higher values of IC_{50} than the standard. In line with this study KIM *et al.* (2022), showed that *C. citratus* scavenge NO while DIATTA *et al.* (2019) revealed that *A. indica* possesses low antioxidant activity. Earlier studies have shown that NO is inhibited by natural antioxidants (CHEN *et al.*, 2017). It was observed in this study that CLAE extract inhibits NO production more effectively than ALAE and thus acts as a better antioxidant. These results are also consistent with that of DPPH scavenging activity. Therefore, antioxidant activities as expressed by nitric oxide scavenging activity and DPPH scavenging activity are lower in ALAE in relation to CLAE.

Ferric reducing antioxidant power (FRAP) assay

Table 2 shows that CLAE exhibited a significantly higher FRAP value in relation to ALAE, indicating a higher antioxidant power of the CLAE regarding its ability to donate an electron. Thus, as with the other assays above CLAE exhibited a higher reducing power than ALAE. KUSMARDIYANI *et al.* (2016) have also shown that *C. citratus* extract possesses the highest FRAP capacity compared to other species of lemongrass. The FRAP value of its essential oil was also similar to the value obtained for AA (JAMUNA *et al.,* 2017). The lower FRAP capacity obtained for ALAE in this study was supported by KIRANMAI *et al.* (2012), who indicated that *A. indica* leaves contain low FRAP capacity compared to the standard and to other parts of the same plant.

Table 2. Ferric reducing antioxidant power (FRAP) and ascorbic acid content of CLAE and ALAE.

Sample	FRAP assay (mgAAE/g)	Ascorbic acid content (mgAAE/g)
CLAE	1.76±0.002	4.07±0.014
ALAE	0.45±0.002*	3.95±0.021

*$p < 0.05$ significantly different from *C. citratus.*

Ascorbic acid content (AAC) assay

As shown in Table 2, there was no significant difference between the AAC of CLAE and ALAE. An earlier study also detects the presence of AA in *C. citratus* tea (LONKAR *et al.,*

2013) and in *A. indica* leaves (BHAGWAT *et al.,* 2020). AA reduces the occurrence of chronic diseases caused by toxic compounds by scavenging free radicals (BURGER *et al.,* 2017). Thus, the presence of AA in these leaves contributes to their antioxidant potential.

Total flavonoid content (TFC)

As indicated in table 3, the TFC of ALAE was significantly lower in relation to CLAE. Flavonoid is one of the most important antioxidant components in plants and it has a significant correlation with total antioxidant capacity (KIRANMAI *et al.,* 2012), thus, the lower total flavonoid content of ALAE might account for the lower antioxidant activity of ALAE in relation to CLAE as observed for NSA, DPPH assay and FRAP capacity. A moderate amount of flavonoid has been shown to be present in *C. citratus* leaves (KIM *et al.,* 2022) and in *A. indica* leaves (KIRANMAI *et al.,* 2012). However, NAGANO and BATALINI (2021) reported the absence of flavonoids in *A. indica* leaves.

Table 3. Total antioxidant capacity, total flavonoid content and total phenolic content of CLAE and ALAE.

Sample	Total antioxidant capacity (mg AAE/g)	Total flavonoid content (mg QUE/g)	Total phenolic content (mg GAE/g)
C. citratus	10.89±0.20	22.38±0.52	32.26±0.13
A. indica	7.64±0.36*	6.55±0.32*	43.49±0.67*

*$p<0.05$ significantly different from *C. citratus.*

Total phenolic content (TPC)

As shown in table 3, ALAE contained significantly higher total phenolic content when compared with CLAE. Phenolic compounds account for and correlate strongly with the antioxidant activity exhibited by plants (KUMAR *et al.,* 2018). Thus, the total antioxidant capacity obtained for ALAE in this study might be accounted for by the phenolic content of the leaves. This observation is in line with the study of NAGANO and BATALINI (2021) who concluded that phenolic groups which were considerably present in *A. indica* extract were responsible for its antioxidant capacity. Phenolic compounds have also been found in *C. citratus* (UNUIGBE *et al.,* 2019).

Total antioxidant capacity (TAC)

The TAC value indicates the flavonoid and related polyphenols content of herbs (SAEED *et al.,* 2012). From the result obtained in this study (Tab. 3), it could be seen that the CLAE exhibited a significantly higher TAC value in relation to ALAE, which may be due to its very low flavonoid content of ALAE. JAMUNA *et al.* (2017) indicated that the TAC value of *C. citratus* essential oil was similar to the value obtained for AA. Considerable value was also obtained for the total antioxidant capacity in the leaf (SANADHYA *et al.,* 2016) while (ARASH *et al.,* 2011) demonstrated that *A. indica* plant has a lower antioxidant activity compared to *C. citratus*.

As phenolic acids and flavonoids contribute mainly to the plant's antioxidant activity, the higher TAC value of CLAE and other antioxidant activity exhibited by it may be accounted for by the content of its total phenolic and flavonoid contents (SAEED *et al.,* 2012) while the TAC value of ALAE may be contributed more by its total phenolic content. Flavonoids possess many antioxidants and advantageous biochemical effects that prevent diseases such as cancer and diabetes mellitus (PANCHE *et al.,* 2016). Given that CLAE has a higher quantity of

flavonoids and higher antioxidant activity than ALAE, it is a more promising agent for preventing and treating diseases.

Cytotoxicity

Table 4 shows the number of survival and the number of death obtained for the standard, CLAE and ALAE. Table 5 shows that the LC_{50} of the standard ($K_2Cr_2O_{7s}$) was much lower than that of CLAE and ALAE, while that of ALAE was lower than that of CLAE indicating that the ALAE is less toxic than the standard, but more toxic than the CLAE.

Table 4. $LC_{50,}$ number of survival and death of *Artemia salina* larvae in different concentrations of standard, CLAE and ALAE.

Concentration (µg/mL)	Sample					
	Standard		CLAE		ALAE	
	No of Survival	No of Death	No of Survival	No of Death	No of Survival	No of Death
100	0	20	7	13	7	13
50	1	19	10	10	9	11
25	3	17	11	9	12	8
12.5	5	15	13	7	15	5
6.25	8	12	16	4	17	3

Table 5. LC_{50} of standard, CLAE and ALAE.

Sample	Standard	CLAE	ALAE
LC_{50}	5.65 µg/ml	52.16 µg/ml	43.33 µg/ml

In expressing the toxicity of plant extract, the LC_{50} can be compared using either Clarkson's or Meyer's toxicity index. Meyer referred to plant extracts as toxic if the LC_{50} value is less than 1000 µg/mL, and non-toxic if the LC_{50} value is greater than 1000 µg/mL (MEYER *et al.*, 1982). Clarkson referred to plant extracts as non-toxic if the LC_{50} is above 1000 µg/mL, plants with LC_{50} between 500 and 1000 µg/mL possess low toxicity, plants with LC_{50} between 100–‒500 µg/mL possess moderate toxicity and plants with LC_{50} between 0-100 µg/mL possess high toxicity (CLARKSON *et al*., 2004). With reference to either Meyer's or Clarkson's toxicity indexes, both CLAE and ALAE possess high toxicity. Our study is in line with that of SANADHYA *et al.,* (2016), who revealed that the extract of *C. citratus* leaf was significantly cytotoxic at 10 mg/mL to liver cells and thus may possess an anti-tumor effect, while other studies on *A. salina* lethality assay reported moderate toxicity of the fractions from *C. citratus*, and high toxicity for the essential oil and isolated compounds. SARAVANAN *et al.* (2011) have also shown *A. indica* leaf extract to be toxic to fish during short-term exposure. NAGANO and BATALINI (2021) reported moderate toxicity of the leaves in *A. salina*. The toxicity of *C. citratus* and *A. indica* leaves is important because their use as insecticides has been shown to be effective (BRAGA *et al.*, 2021, TIBENDA *et al*, 2022) and their use in tumor suppression is also promising (HALABI *et al.*, 2014; ALZOHAIRY, 2016).

CONCLUSION

This study has shown that leaves' aqueous extract of *C. citratus* (CLAE) exhibited a higher antioxidant potential than of *A. indica* (ALAE), expressed through TAC, DPPH scavenging activity, NSA, FRAP, and TFC. The antioxidant activity of ALAE was contributed mainly by the total phenolic content. In addition, CLAE possesses lower toxicity for the *A. salina* nauplii larvae than ALAE as expressed by the LC_{50}. Therefore, CLAE may be safer and more efficient in the treatment of disease than ALAE. However, since leaves' aqueous extracts of both plants possess high toxicity, caution should be taken in their applications.

In further researches the active compounds which account for the antioxidant activities of both tested plants need to be more precisely characterized. The compounds responsible for their toxicity should be isolated, and a means of reducing their content should be derived, to adequately exploit those plants in the prevention and treatment of various health challenges.

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