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The Phytochemistry and Antioxidant Activity of the Ethanol Extract of *Cymbopogon citratus* (POACEAE)

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Abstract: ***Most of the synthetic corrosion inhibitors which are used to protect metals from corrosion are highly toxic to both human beings and environment and they are often expensive and non-biodegradable. There is need to produce corrosion inhibitors which are non-toxic to human beings and are environmentally safe, being green inhibitors. Therefore, the use of natural products (reducing agents or antioxidant) extracted from plants as corrosion inhibitors has become a key area of research as they are extremely rich sources of natural chemical compounds that are biodegradable, renewable, and cost-effective as well as can be extracted by simple methods. In this study, the ethanol extract of Cymbopogoncitratus (Poaceae) commonly called Lemongrass was investigated for its phytochemical constituents and antioxidant activity using Diphenylpicrylhydrazyl (DDPH). The result of the phytochemical screening showed the presence of alkaloid, cardiac glycosides, terpenes, carbohydrate, polyphenols, saponins, tannins and flavonoids. The extract also showed antioxidant activity of 0.00053mg/mL This value is higher than those of some. standard antioxidants The extract also reduced the compex Ferric ion and 2,3,5-triphenyl-1,3,4-triaza-2-azonia cyclpentane-1,4-diene chloride (TPTZ). The oily substance obtained from eluates C_{27} to C_{29}in the column chromatographywere pooled together and preserved for spectroscopic analysis. The result of the spectroscopic analysis is expected to reveal the potential corrosion inhibitor present in the extract***

Keywords: Phytochemistry, Antioxidant, Ethanol extract and *Cymbopogon citrates*

1. Introduction

Iron (III) oxide hydrate ($Fe_2O_3.H_2O$) is known as rust. Corrosion (rust) destroy food can beverages cans in industries. Rust destroys the efficiency of industrial machines. Biologically, rust promotes the growth of clostridium tetani, the bacteria that causes tetanus. Any chemical substance that can reduce Fe^{3+} to Fe^{2+} is a reducing agent or antioxidant and also a potential corrosion inhibitor on metals. This research work is designed to evaluate the antioxidant potential of the ethanol leaf extract of *cymbopogon citratus*. The result obtained if promising could expose the plant *(cymbopogon citratus)* to a better future exploitation as an anti-rust substance in chemical industries.

2. Literature Review

Cymbopogon citratus (Lemon grass) is a tropical perennial plant which yields aromatic oil. The herb originated in South Asia (Sri Lanka), Australia and South India. In the laboratory, lemon grass oil is obtained by steam distillation of the herbage using Clevenger apparatus. (Guenther 1950, Gupta 1987), Kamath *et al* 2001). Lemon grass oil has reddish-yellow to reddish-brown colour. It has a strong odour of verbena, adensity/specific gravity of 0.908. It is slightly soluble in water, soluble in ethanol (70%), chloroform and ether.

At 40^0C it has a viscosity of less than 7.0×10^{-6} m^2s^{-1} (Moyler 2010, Gumban 2013 and Merck 2015). The essential oil of C. citrates contains approximately ɑ-pinene (0.13%), ᵝ- pinene, delta -3-catrene (0.16%), myrcene (12.75%), dipentene (0.23%), ᵝ-phellandrene (0.07%), ᵝ-cymene (0.2%), methylheptanene (2.62%), Gtronellal (0.73%), ᵝ-elemene (1.33%), ᵝ-caryophyllene (0.18%), citronellyl acetate (0.96%), gevanyl acetate (3.00%), citral b (0.18%), citrala (41.82%), genaniol (1.85%), elemol (1.2%) and ᵝ-caryophyllene oxide (0.61%) (Saleem *et al* 2003 a, b). The essential oil of C. citrates showed activity against *Bacillus subtiilis, staphylococcus aureus* and *Esherichia coli*. The oil also reduced potato virus x and potato virus y by 100% (Joy *et al* 2006). Lemon grass oil repels tsetsefly, housefly and mosquitoes. Free radical reactions occur in the human body and food systems in the form of reactive oxygen and nitrogen species. Biochemically significant free radicals include: Superoxide (O_2), hydroxyl (OH^-) and peroxyl (ROO-) radicals. Others are singlet oxygen (O_2), Nitric oxide (NO), peroxy nitrite (ONOO-), hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2). H_2O_2 is not a free radical but falls in the category of reactive oxygen species (ROS). (Cheeseman and Slater (1993), Karlsson 1997, Wong *et al* 2005).

The two sources of free radicals are endogenous and exogenous sources. The endogenous sources include: autoxidation, enzymatic oxidation, transition metals ions and Ischemia perfusion injury. The exogenous sources include: drugs, radiation, tobacco smoking, inorganic particles ad gases. (Halliwell *et al* 1985, Halliwel et al 1992a, Evans *et al* 1994).

Benefits of free radicals include: Thyroxine synthesis, immune defence, signal transduction, cell growth and proliferation and cellular development. Takasu *et al* (1987), Baxter *et al* (2004), and Guyton (2006). The disadvantages of free radicals include oxidative stress leading to protein, lipid and DNA damages (Sies 1991, Bryan 1996, Dufeng and Arthur, 2003).

Antioxidant compounds prevent the generation of toxic oxidants, intercept any that are generated and inactivate them and thereby block the chain propagation reaction produced by these oxidants. Antioxidants terminate pro-oxidant reactions by removing free radical intermediates and inhibit other oxidation reactions. They do so by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Parker *et al* 1979, Niki *et al*, 1982 Halliwell and Gutteridge, 1985).

Antioxidants derived from natural and dietary sources like secondary products of plants which include: chlorophyll derivatives, essential oils, carotenoids, alkaloids, phytosterols, phenols, polyphenols and nitrogen containing compounds like indoles. Synthetic antioxidants are mostly used in chemistry, food industry and medicine. Exmaplesinclude butylated hydroxytoluene and butylate hydroxyanisole, probucol, nitecapone, lazaaroids, ebselen and dithioethiones. (IFR 2007, Mishra *et al* 2007).

3. Materials and Methods

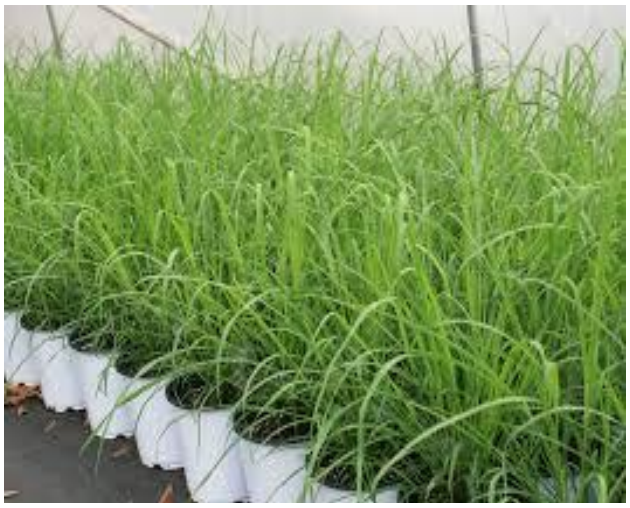

Figure 1: Photograph of Lemon grass

Collection and identification of plant

The fresh plant of Cymbopogon citraus was collected from a farmland in Uyo Local Government Area of Akwa Ibom State in July 2018. The botanical identification of the plant was done by Professor (Mrs) Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo where a voucher specimen number UU42(d) was assigned before the plant was deposited in the University of Uyo Herbarium, Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

The Lemon grass was cut into smaller pieces sun-dried, pulverized in a mixer-grinder. The coarse powder obtained was stored in anon-toxic black polythene bag for use. The pulverized plant material (400g) was weighed and extracted (using cold maceration method) with 70% ethanol (5L) at room temperature for 72hours. It was shaken inter mittently and filtered after 72hours using Whatman No. 1 filter paper. The filtrate obtained was concentrated at 40^0C and the dried extract placed in a beaker and covered with aluminum foil and stored in a refrigerator at – 4^0C until required. (WHO/AFRO, 1976).

Phytochemcial Screening

The extract was subjected to preliminary phytochemcial screening to determine the presence of bioactive constituents. This was done using the standard methods (Evans 2009).

Chromatographic analysis of the extract

An open glass column (gravity) was used for this chromatographic analysis. The column was packed with silica gel of 60-120 mesh. The silica gel was made into slurry with enough quantity of petroleum ether. The slurry was poured into glass column with gentle tapping. A little quantity of cotton wool was inserted to cover the gel in the column. The ethanol extract was crushed with 10g of silica gel until a powder was obtained. The powder was poured into the column and more of the solvent (petroleum ether) gradually added. The column was allowed to equilibrate. Collection of the eluates commenced the following day. The column was eluted into labelled tubes. 57 eluates were collected from different solvent systems.

A pre-coated aluminium foil TLC plates of dimensions (20cm by 20cm) was used for the analysis. The solvent system was a mixture of 2-propanol and n-butanol in a ratio of 3 to 2. The solvent mixture was mixed in a TLC tank and gently swirled and allowed to equilibrate for 10minutes. The TLC plates was then inserted into the already saturated tank for development. The plate was removed from the tank as the solvent reached the solvent front on the plate and air-dried. The spots on the TLC plates were detected using UV light (wavelength, 254nm). The visible spots were encircled faintly with pencil. The distance from the center of the spot to the origin and the distance of the solvent from the origin to the solvent front were meads measured and recorded. The retention factor (Rf) was calculated for each spot using the formula:

$$R_f = \frac{\text{Distance of spot from origin}}{\text{Distance of solvent from origin}}$$

Antioxidant analysis

0,004%w/v methanol solution of DPPH was prepared and diluted. The stock solution was serially diluted to produce 0.0004, 0.0008, 0.0020, 0.0024, 0.0028, 0.0032, 0.0036%w/v and their absorbance were obtained at 512nm, they were recorded and used in preparing a calibration curve for the DPPH reagent.

DPPH Assay with Extract

2mg of each extract was dissolved in 50ml of methanol and diluted to 0.0008mg/ml, 0.0061mg/ml and 0.09024mg/ml using methanol 5ml of each concentration was incubated with 5ml of 0.004% methanol DPPH solution for optimal analytical accuracy. After 30minutes incubation period at room temperature, the absorbance was read against a blank at 512nm. Percentage inhibition (1%) of free radical DPPH was calculated as follows:

$$1\% = [(A_{blank}-A_{sample})/A_{blank}] \times 100$$

Where A blank is the absorbance of control reaction (DPPH Solution) without the test sample and A sample is the absorbance of DPPH incubated with extract

An extract concentration which provided 50% inhibition (IC_{50}) was calculated using a graph of inhibition percentage against extract concentrations.

DPPH Assay of standard Antioxidant Drugs

The above procedure was repeated for the standard antioxidant i.e. vitamin A, C and E Tablet dosage form was used for vitamin C while for vitamin A and E, gelatin capsules were used. The weight of the standard containing 2mg of the standards were determined by proportionality and diluted. N-hexane was used to dissolve vitamin A and E, while methanol was used in dissolving vitamin C. n-hexane and methanol solutions of 0.004% were used to incubate vitamins A, E and vitamin C respectively for 30minutes.

4. Results

Phytochemical Screening

Table 1: Result of Phytochemical Screening of the Ethanol Extract of Lemon Grass

Constituents	Test	Concentration
Alkaloid	Dragendorff	+ +
Steroidal Ring	Salkowski	+ +
Terpenes	Lieberman burkchad	+ +
Cyanogenic glycoside	Sodium picrate paper	-
Carbohydrate	Molisch	+ +
Cardenolide	Legal	+
Polyphenols	Ferric chloride	+ + +
Saponins	Frothing/emulsion	+
Tannins	Ferric chloride	+
Flavonoids	Shinoda reduction	+ + +
Phlobatainins	Formaldehyde	-
Resin	Acetic Anhydride and conc. Sulphuric acid	+

+ + + = present in abundance,
+ + = moderately present, + = present, - = Absent

Table 2: Calibration of Methanol Solution of DPPH

Absorbance at 512nm	Concentration (% w/v)
0.065	0.0004
0.131	0.0008
0.191	0.0012
0.227	0.0016
0.264	0.0020
0.332	0.0024
0.373	0.0028
0.446	0.0032
0.518	0.0036
0.553	0.0040

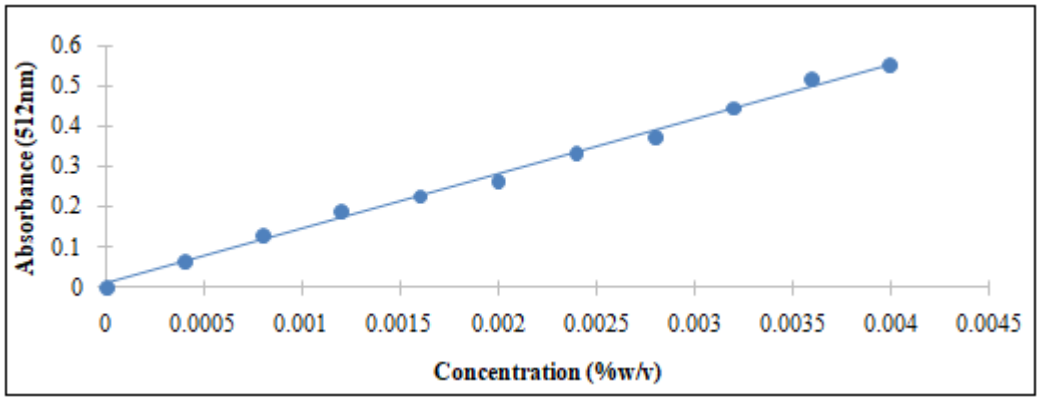

Figure 1: Graphical representation of UV Spectrophotometric curve for calibration of Methanolic solution of DDPH

Antioxidant analysis

The results of the antioxidant analysis of the extracts and standards using DDPH Assay are presented in Tables 3 and 4 and figure 2

Table 3: The Absorbance (512nm) of test samples and standards at different concentrations

Three concentrations of samples (mg/ml)	Absorbance of samples			
	LG	Vit A	Vit C	Vit E
0.0008	0.1140	0.2920	0.1020	0.1740
0.0016	0.1020	0.2572	0.0920	0.1731
0.0024	0.0930	0.2422	0.0731	0.1601

KEY:
Vit = vitamins A, C and E
LG = Lemon Grass

Percentage Inhibition of DDPH (%)

Table 4: Percentage inhibition of DDPH at different concentrations and their 1C50

Concentration (mg/ml)	% Inhibition			
	LG	Vit A	Vit C	$VitE_{blank}$
0.0008	79.42	47.30	81.59	68.60
0.0016	81.59	53.57	83.40	69.75
0.0024	83.21	56.20	86.81	71.10
IC_{50}	0.00053	0.00119	0.00057	-

Blank Absorbance (512nm) of 0.004% methanolic DDPH = 0.554

$$\text{Percentage Inhibition} = \frac{[(A_b - A_s) \times 100]}{A_b}$$

A_b = Absorbance of blank
A_s = Absorbance of sample

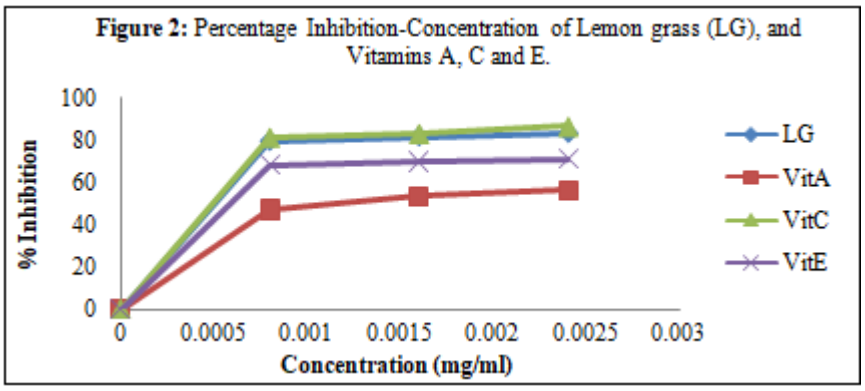

Figure 2: Percentage Inhibition-Concentration of Lemon grass (LG), and Vitamins A, C and E

5. Discussion

The collected plant parts were dried under shade. This helped to reduce possible enzymatic and microbial activities which could lead to rapid deterioration of the plant (Evans, 2009).

Pounding the dried plant material help to increase the surface area of the plant that come in contact with the solvent of the extraction. This also help to increase the yield of the extract.

During maceration the extraction tank was agitated regularly. This helped to disperse local concentration gradient and also improve extraction yield (Carter, 2009).

Exhaustive extraction was done using n-hexane, chloroform and ethanol. The ethanol extract recorded the highest yield. That is the reason this extract was used for the research work.

The phytochemical test of the extract indicated that the plant contain alkaloid, cardiac glycoside, terpene, carbohydrate cardenolide, polyphenols, saponnins, tannins and flavonoids. Cyanogenic glycoside, resin and phlobatanins were absent. DPPH obeys Beer's law at concentrations within 50 – 100 µm.

The concentration and Absorbance of the reagent are linearly proportional to each other.

It gives a linear regression line through the origin of its calibration curve provided the instrument is zeroed. This is in agreement with work of Blois, 1958

The Absorbance of the DPPH solution increase with increase in concentration. It can be suitably used to correctly extrapolate subsequent concentrations of the residual DPPH free radicals during the antioxidant test.

The standard drugs used for this work were vitamins A, C and E.

They were not pure samples but formulated products. Estimation of the weight of the formulated product containing the required weight was done. From the estimation, 1.6 mg, 6.01 mg and 2.42 mg of vitamins A, C and E respectively were used for the test.

The molecule of 2, 2-diphenyl-1-1-picrylhydrazyl is a stable free radical because its spare electron undergoes delocalization over the molecule as a whole so that the molecule does not dimerize when a solution of DPPH is mixed with a substance that can donate a hydrogen ion. This gives rise to the reduced form, 2,2-diphenyl-1-picryl hydrazine. As the free radicals are being reduced, the concentration of the residual free radical reduces. This concentration is determined from the absorbance of the incubated solution at 512nm because DPPH complies with Beer's law.

Methanol solution of the sample was used in the analysis. This is in accordance with the recommendation by Blios 1958. Vitamins A and E were dissolved in n-hexane (this is non-polar solvent) .The purple colour of DPPH was bleached to yellow when the extract and vitamin C were incubated with methanolic DPPH. This indicated a reasonable level of antioxidant activity. The change in colour from purple to yellow revealed that the extract of lemon grass and vitamin C have sufficiently scavenged the free electrons such that the DPPH molecule loses its purple colour to yellow.

Normally, the lower the IC_{50}, the higher the antioxidant activity. The extract show antioxidant activity of 0.00053 mg/ml which is higher than thoe of vitamin A (0.00119 mg/ml) and E (0.00071 mg/ml).The order of antioxidant activity is as shown below:

Vitamin C >Extract > Vitamin E >Vitamin A

This high antioxidant activity of the extract is believed to be caused by phenolic compounds such as polyphenols and flavonoids observed during phytochemical screening.

The ethanol extract of the lemon grass demonstrated antioxidant properties higher than some standard antioxidants. The extract also reduced the complex ferric ion and 2, 3, 5-triphenyl-1,3,4-triaza-2-azonia cyclo pentane-1, 4-diene chloride (TPTZ) to the ferrous form at low pH.

6. Conclusion

The ethanol extract of lemon grass demonstrated antioxidant activity of 0.00053mg/mL. This value is higher than those of some. standard antioxidants The extract also reduced the complex Ferric ion and 2,3,5-triphenyl-1,3,4-triaza-2-azonia cyclpentane-1,4-diene chloride (TPTZ). The oily substance

obtained from eluates C_{27} to C_{29} in the column chromatographywere pooled together and preserved for spectroscopic analysis. The result of the spectroscopic analysis is expected to reveal the potential corrosion inhibitor present in the extract

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