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Evaluation of the hydro-alcohol extract of *Cola lepidota* (K Schum) (Sterculiaceae) against urease enzyme for sustainable health and agronomy

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Abstract
Natural products such as plant extracts provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. *Cola lepidota* (Sterculiaceae) commonly called Monkey kola, has been used over the years in ethno medicine practice and as food. This research was aimed at the evaluating the effect of the 70% aqueous ethanol extract from the pulp of *Cola lepidota* on the urease enzyme. The pulp of *Cola lepidota* was freeze dried and pulverized. The pulverized plant material was extracted exhaustively by cold maceration in 70% aqueous ethanol for 72 hours to afford the 70% aqueous ethanol extract. Phytochemical screening was conducted using standard methods. *In vitro* anti-urease screening was done spectrophotometrically using the modified Berthelot method. Alkaloid, phenolics, flavonoids, saponins and cardiac glycosides were present in the powdered plant. The 70% aqueous ethanol extract exhibited a significant (p<0.05) activity against urease with an IC_{50} of 3.3799 mg/ml. In conclusion, *C. lepidota* fruit has anti-urease activities and could be a source of urease inhibitors for drugs and eco-friendly agrochemicals discovery thus justifying some of its folkloric uses.

Keywords: *Cola lepidota*, sterculiaceae, urease inhibitors, eco-friendly agrochemicals

Introduction
Urease is an enzyme containing nickel that speeds up the hydrolysis of urea to the formation of ammonia and carbon dioxide [1-3]. It is a key enzyme for the global nitrogen cycle occurring in plants, fungi and bacteria and has been exhaustively investigated and proven to be strictly dependent on nickel ions (Ni^{2+}) for catalytic activity [1-3]. The functional role of urease is to provide nitrogen for organisms in the form of ammonia for their growth. However, excessive release of ammonia to the atmosphere will gradually cause imbalance in nitrogen cycle which can imply long term environmental and economic consequences [4-5].
Aside this agronomic consideration, the urease enzyme is also implicated in the pathophysiology, progression and complications of diseases associated with ureolytic organisms like: *Helicobacter pylori, Yersinia enterocolitica Proteus mirabilis S. saprophyticus* and *Mycobacterium tuberculosis* among others. Quite a good number of community- or hospital-acquired infections of the urinary tract, wound, and bloodstream have been linked to *Proteus mirabilis* [6]. Bacterial urease activity of *P. mirabilis* is a leading cause of urinary crystal formation. The *P. mirabilis* urease raises the pH of the urinary tract resulting in the crystallization, production of carbonate apatite and struvite crystals [7]. *S. saprophyticus*, a spherical bacterium of the gram-positive cocci group, is frequently the cause of urinary tract disorders [8]. Asides other virulence factors in *S. saprophyticus*, urease is a major factor contributing to the invasion of this bacterium particularly within the bladder tissues [9-12].
The human enteric pathogen *Yersinia enterocolitica* that causes yersiniosis requires urease for its action [13]. This invasive enteric pathogen enters the body through the oral route by consumption of contaminated food or water [14-15]. Clinical symptoms ranging from self-limiting gastroenteritis to mesenteric lymphadenitis, visceral abscesses, septicemia in immune compromised hosts, and reactive arthritis are associated with this disease [14]. *Y. enterocolitica* strains are quiet acid tolerant of certain meals within *in vitro* acidic conditions, can survive in their high acidity [16-17].

Helicobacter pylori is another ureolytic bacteria species associated with stomach ulcer. The high prevalence of *Helicobacter pylori* in human population indicates that such microorganism has developed mechanisms for resistance against host defenses [18]. Urease enzyme in cytoplasm and/or bound to *Helicobacter pylori* surface is its main virulence factors [19]. The ammonia formed increases the medium pH, which creates a friendly environment for *Helicobacter pylori* survival [20].

Tuberculosis (TB) is a disease caused by the acid fast *Mycobacterium tuberculosis.* TB is of appreciably high burden to the health system globally [21]. The urease enzyme has also been linked to nitrogen metabolism in *Mycobacteria* tuberculosis and is one of the crucial virulence factors associated with its pathogenesis and survival in nutrient-limited micro-environments [22].

Utilization of plants as source of remedies for the purpose of managing diseases dates back to pre-historic times and people of all continents are accustomed to this old tradition [23]. Biological materials for example plant extracts provide unlimited opportunities for novel drug discoveries owing to unprecedented availability of chemical diversity [24]. According to World Health Organization [25], a greater proportion of the world's inhabitants rely on traditional especially herbal remedies for their basic medical care needs. This is because of the effortlessness and frequency of occurrence of assessing orthodox medicine.

Cola lepidota is a member of the family Sterculiaceae. Together with its closely related species like *C. parchycarpa and C. laterita*, they are commonly called monkey or cockroach cola [26-27]. In Cameron it is called Duala mbwid. While in Southeastern part of Nigeria, it is known as Oji ochicha or achicha as reported by [28]. *C. lepidota* possess a yellow pulp whereas *C. parehycarpa and C. laterita* possess white and red pulp respectively [29]. *C. lepidota* is a plant that grows up to 18m high with a twisted trunk and calciferous lump. Asides it nutritional uses [30-31], it is used in Nigeria as alternative medicine in curing pulmonary disorders, fever, and cancer with previous scientific reports justifying these claims. [32-35]. It is also employed in prevention of dysentery, headache and sleep [36-38]. This research is geared towards evaluating the pulp extract of *Cola lepidota* as a potential source of urease inhibitors for sustainable health and agronomy

Materials and Method

Materials

Reagents and solvents used in this study were of analytical grade and are products of JHD and Sigma Aldrich Chemicals. Thiourea as reference standard and Urease test kits (Agape diagnostics Switzerland GmbH).

Sample collection and extraction

Cola lepidota fruits were purchased from Olobolo market in Ogu-bolo Local Government Area of Rivers State. It was authenticated at the herbarium of Plant Science and Biotechnology, University of Port Harcourt, Choba, Rivers State, Nigeria. The exocarp of the fruits was carefully removed, same as the seeds. The pulp was neatly washed and shredded. The shredded pulp was freeze dried, pulverized and preserved in an air tight container until use. A 50g of dried *Cola lepidota* pulp was macerated in 500ml of 70% aqueous ethanol with intermittent agitation for 72 hours. Fresh replacement of solvent was done after every 24 hours. The combined aqueous ethanol extract was filtered using Whatman No. 1 filter paper.

Phytochemical Screening

Phytochemical screening was done using the dried powdered plant material obtained according to [39-40]. The analysis was conducted to determine the phyto-constituents like alkaloids, flavonoids, anthraquinone, saponins, phenolics, carbohydrate, triterpenoids/steroids and glycosides from the sample.

Approval

University of Port Harcourt Office of Research Management and Development, Research Ethic Committee (UPH/CEREMAD/REC/MM76/032) approved the work.

Urease Inhibitory Assay

The modified Berthelot method [41] was used in carrying out this assay and thiourea was used as the reference drug. Briefly, 100mg of thiourea was dissolved in 10 ml of ethanol to afford a 10 mg/ml thiourea stock solution. A 1ml aliquot of this thiourea stock solution was further diluted to 10ml giving the 1mg/ml thiourea reference standard test solution. The test 70% aqueous ethanol solution (30, 15, 7.5, 3.75, and 1.875 mg/ml) were prepared following a two-fold serial dilution approach from the stock 30 mg/ml solution. The Urease enzyme was reconstituted with 100ml of distilled water. A 10ul of each of these test solutions were separately mixed with 1ml of enzyme, 1ml of substrate (urea) and allowed to incubate for 10mins. This was followed by addition of 1ml of colour developer and 10mins incubation. The absorbance was taken thereafter using a spectrophotometer at 630nm using ethanol as blank and a solution containing 1ml of enzyme, 10ul of ethanol and 10ul of the substrate as negative control. The percentage inhibition was calculated using the formular below:

$$\% \text{ Urease Inhibition} = \frac{100[A \text{ (negative control)} - A \text{ (sample)}]}{A \text{ (negative control)}}$$

Where

A (negative control) = Absorbance of the negative control solution (containing all the reagents except the test fractions)

A (sample)] = Absorbance of the test fraction.

The IC_{50} was obtained by regression analysis from a plot of % inhibition against concentration using the AAT Bio quest software [42].

Results

Table 1: Result showing % Inhibition of Urease Activity of 70% Aqueous Ethanol Extract of *Cola lepidota* pulp

Test Samples	Concentrations mg/ml	% Mean Inhibition ± SEM	IC_{50} (mg/ml)
70% Aqueous Ethanol Extract of *C. lepidota* Fruit pulp	1.875	14.9 ± 0.001	3.3799
	3.75	56.5 ± 0.002	
	7.5	84.1 ± 0.001	
	15	*96.4 ± 0.000	
	30	*96.9 ± 0.000	
Standard (Thiourea)	1.0	92.4 ± 0.000	

Key: *represent the values significantly different from the standard at $p<0.05$, Number of determination = 3

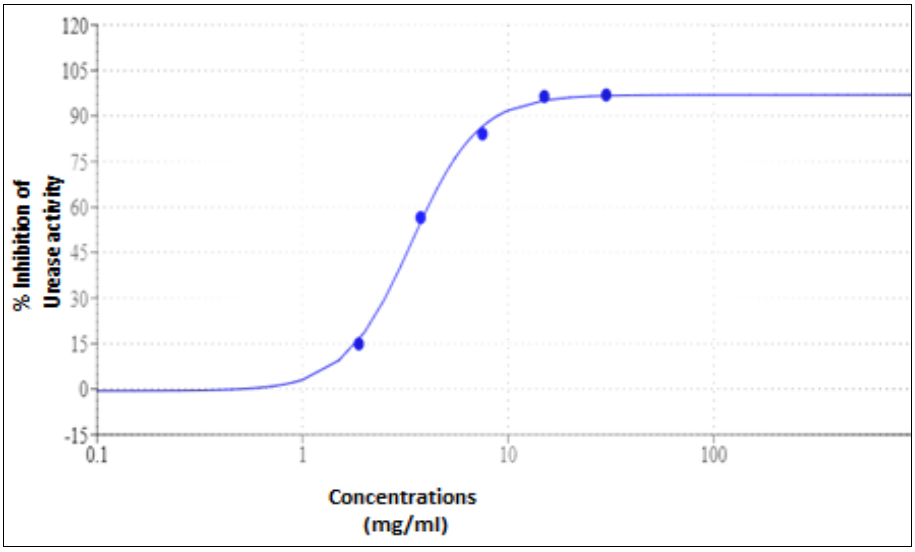

Fig 1: Concentration-response curve for the urease inhibitory activity due to the 70% aqueous ethanol extract of *Cola lepidota* fruit pulp.

Table 2: Result of phytochemical screening of the *Cola lepidota* pulp

Screened phytochemical test	Results
Alkaloid	
Dragendorff test	+
Hager's test	+
Anthraquinone	
Bontrager test	-
Free anthraquinones	-
Combined anthraquinones	-
Saponins	
Frothing test	+
Emulsion test	-
Test for triterpenoids	
Salkowski test	+
Liebermann Burchard test	+
Phenolics	
$FeCl_3$ test	+
Phlobantannin test	-
$AlCl_3$ test(Flavonoids)	+
Carbohydrate	
Molisch test	+
Reducing Sugars (Fehlings test)	+
Deoxy-Sugars(Keller-Killiani test)	+

Key: + means present – means absent

Discussion

In the anti-urease screening result (see Table 1 and Figure 1), the aqueous ethanol extract had concentration dependent inhibition activity against urease. The extract displayed a peaked percentage inhibition at concentration ≥15 mg/ml which were significantly (p<0.05) higher compared with the reference standard thiourea at 1 mg/ml. It has an IC_{50} of 3.3799 mg/ml. The phytochemical screening results (Table 2) showed the presence of alkaloids, phenolics and flavonoids, triterpenoids, saponins, deoxy and reducing sugars with anthraquinones absent in the pulp of *Cola lepidota*. Alkaloids have an extensive range of pharmacological activities including anti-malaria, anti-asthma, anti-cancer, anti-bacterial, analgesic and anti-hyperglycemic activities [43-45]. Flavonoids and several other phenolic compounds are seen in plant parts like seed, bark, leaves, stem bark and flower. They are reported scientifically to have antioxidant, anti-inflammatory, anti-allergic, anti-tussive, anti- diarrhea, anti-microbial, anti-cancer and cholesterol lowering [46-47]. Plant derived phenolic aldehydes have been reported to have urease inhibition activity [48]. *Cola lepidota* pulp also contained triterpenoids several of which have been reported to exhibit varying pharmacological activities including: anti-inflammatory, antioxidants, anti-bacterial, anti-viral, gastro protective in addition to hepatoprotective [49-50]. The presence of carbohydrate in the pulp of *Cola lepidota* may be allied to its sugary taste. Carbohydrates are recognized to supply vigor to the body. Saponins were found in *Cola lepidota* pulp. They are acknowledged to be surface active in water. This could impose *Cola lepidota* as an ingredient in cosmetics production. Deoxy sugars are a pointer to the presence of naturally occurring cardiac glycosides which are steroidal triterpenoidal glycoside with at least one deoxy sugar as part of the aglycone, are known for their pharmacological activities principally in the heart [51-52] and are as used as potent ingredient in managing weak heart and correlated heart malfunction disorder. This is a pointer to justify the report on the use of *C. lepidota in* ethnomedicine practice to treat heart failure [33-34].

Conclusion

Cola lepidota pulp in this research has confirmed scientifically as a potential source of urease inhibitors that could be utilized as scaffold for the development of drugs and eco-friendly agrochemicals for sustainable health and agronomy. It also justify it's used in ethno medicine for ailments having their pathogenesis and pathophysiology linked to urease activity.

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Disclosure of conflict of interest

The authors declare a no conflict of interest in this work.

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