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INHIBITION OF SHEEP LIVER CHOLINESTERASE ENZYME BY THE LEAF EXTRACTS OF *ANOGEISUS LEIOCARPUS*.

Abdu, K.
Department of Pure and Industrial Chemistry, Faculty of Science, Bayero University, P.M.B. 3011, Kano, Nigeria
Correspondence author: kbrabdu@yahoo.com

ABSTRACT
Anogeisus leiocarpus is known to be one of the most active ingredient responsible for the chemotherapy of tuberculosis in West Africa and Nigeria in particular. The powdered leaves were percolated with ethanol for one week and the crude extract was labeled as FE01. This was successively macerated with n-hexane, chloroform and n-butanol. Their corresponding soluble fractions were labeled as FH01, FC01 and FB01 respectively. All the crude extract fractions were subjected to anticholinesterase enzyme assay and were found to arrest the function of acetyl cholinesterase enzyme even at low concentration of 5 µg/cm^3. The efficacies of these crude extract were comparable to that of Huperzine A as a reference standard for cholinesterase inhibition. The anticholinesterase assay serves as an indicator system whose analysis always correspond to the bioluminescent Mycobacterium aurum or tuberculosis expressing firefly luciferase.

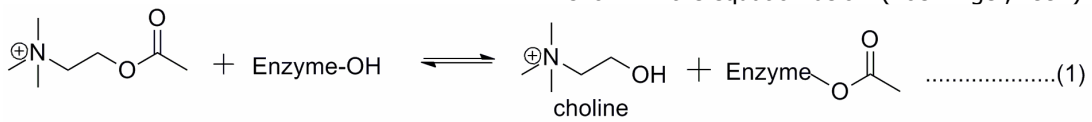
Keywords: Anogeisus leiocarpus, extraction, cholinesterase enzyme, Mycobacterium aurum.

INTRODUCTION

Literature survey shows that the plant, *Anogeisus leiocarpus* is being used in local communities in West African countries for the treatment of some diseases. The leaves of *Anogeisus leiocarpus* are widely used in West Africa as animal feeds and medication for various diseases. Hausa community in northern Nigeria use the leaves as a feed for cattle, sheep and goat suffering from influenza disease or mucus infection (Aja'afar, 1982). Decoction of the leaves with red potash is widely used for oral application as cough mixture. Similarly, decoction of the leaves with spider nut is taken orally against pulmonary tuberculosis. The flower of the plant is also known to be fried, ground and later infused in warm water as a treatment against tape worm (Aja'afar, 1982). The root and the stem have been used as chewing stick for dental hygiene (Sanni and Okor, 1983). The powdered bark is applied to wound and the aqueous extract is used for diarrhea treatment in Senegal (Burkill, 1985). A mixture of the powdered bark with *Terminalia* sp is applied to gum for tooth ache in Burkina-faso and Cote d'ivore (Burkill, 1985). The bark is also found useful in upper Guinea as a febrifuge in hot lotions and as an infusion for the treatment of leprosy in Burkina-faso. In Ghana decoction of the bark with red pepper is taken for body and chest pains. The powdered bark mixed with ordinary versiline as an ointment is applied on the skin for skin complains in Cote d'ivore (Burkill, 1985).

Tuberculosis (TB) remains the leading cause of mortality due to the bacteria, *Mycobacterium tuberculosis* (*M. tuberculosis*). It is estimated that 8.2 million new TB cases occurred worldwide in the year 2000, with approximately 1.8 million deaths in the same year, and more than 95% of these were in developing countries (Corbett, e*t al.*, 2003). In addition approximately 12% of the total death from TB was attributed to co-infection with *M. tuberculosis* and human immuno-deficiency virus (TB-HIV). Immune deficient patients with HIV are at increased risk of latent *M. tuberculosis* infection progressing to active disease and being transmitted to others (Morens *et al.*, 2004). It has been estimated that 3.2% of the world's new cases of TB were multi-drug resistant tuberculosis (MDR-TB) (Espinal, 2003; Nunn, 1997). An individual who is sick with any strain of TB will infect between 10-20 people every year (Blower and Chou, 2004), there by generating a serious problems of particular concern to public health officials.

Anticholinesterase agents are compounds that are said to be toxic and to a wide extent irreversibly deactivate the cholinesterase enzyme (Boehringer, 1991). In neurotransmission system the acetylcholine ester is released by a nerve impulse to trigger a muscle contraction. While the cholinesterase enzyme hydrolyses the ester to the end product. The process is a transestrification in which the acetyl group is transferred to a hydroxyl group of the enzyme as shown in the equation below (Boehringer, 1991).



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The study was set out to investigate the effect of the leaves extract of *Anogeisus leiocarpus* on the cholinesterase enzyme which serves as an indicator system whose analysis always correspond to the bioluminescence *Mycobacterium aurum* or tuberculosis expressing firefly.

The long term objective is to isolate a potential anti-tuberculosis agent(s) from the most active fraction using bioassay guided chromatography which is hope to be reported some were else.

MATERIAL AND METHODS

Plant collection

The leaves of *Anogeisus leiocarpus* (Combretacaea) were collected from Dorayi in Gwale local government area of Kano state, Nigeria. The plant was identified and authenticated at the Department of Biological Sciences Bayero University Kano, Nigeria. The sample was air-dried for two weeks, crushed, ground, weighed and kept on shelf before percolation.

Fractionation

One kilogram (1 Kg) of the air-dried powdered leaves of *Anogeisus leiocarpus* was percolated with two liters (2 L) of ethanol with vigorous shaking at regular intervals for one week. The mixture was decanted and filtered and the extract was concentrated on Rota vapor (R110) at 40^{o}C. The crude ethanol extract was labelled as FE01 and weighed 60.56 g. Fifty grams (50.00 g) of the FE01 was successively macerated with 500 cm^3 of n-hexane in parts using 50 cm^3 each. The combined n-hexane extract was evaporated to dryness on Rota vapor at 40^{o}C (Abdu and Johnbull, 2006). The residue of the n-hexane soluble fraction weighed 10.22 g and labelled as FH01. The n-hexane insoluble fraction (FH02, weighed 38.97 g) was macerated with 500 cm^3 of chloroform in parts using 50 ml each to obtain the chloroform soluble fraction. This weighed 25.17 g and labelled as FC01. The chloroform insoluble fraction (FC02, weighed 14.10 g) was also macerated with 500 cm^3 of n-butanol in parts to obtain the n-butanol soluble fraction (FB01) which weighed 5.56 g. The n-butanol in soluble fraction FB02 weighed 8.90 g (Abdu and Johnbull, 2006).

Anticholinesterase activity (Enzyme estimation assay)

Enzyme preparation

Fresh sheep liver was employed as a cholinesterase enzyme source and was procured from healthy sheep immediately after slaughter, after which 1% of the homogenate (w/v) of the sheep liver was prepared in distilled water at 0^{o}C (Ellman, 1961).

Anticholinesterase assay

The following fractions (FE01, FH01, FC01 and FB01) were subjected to *in vitro* anticholinesterase screening. The cholinesterase inhibition was carried out by colorimetric method (Ellman, 1961). Appropriate amount of extract fractions in 5, 10, 20 µg/0.1 cm^3 of acetone were separately dispensed in a test tube and the solvent was allowed to evaporate. Then 0.1 cm^3 of 1% sheep liver homogenate was pre-incubated with 5, 10, 20 µg/0.1 cm^3 of each extract fraction for 15 minutes at 37^{o}C in a thermostatic water bath. A technical grade Huperzine A (a secondary metabolite isolated from *Huperzia serrata*, is an established cholinesterase enzyme inhibitor, Ellman, 1961) was also pre-incubated in the same manner described above with 0.1 cm^3 of 1% sheep liver homogenate at 5, 10, 20 µg/0.1 cm^3 to allow the inhibition of the sheep liver cholinesterase enzyme by the fractions. After the incubation, 0.2 cm^3 of 0.2% azo dye in water was added followed by 0.1 cm^3 of 0.01 M ethylacetacetate substrate in acetone and the reaction mixture was re-incubated for 1 minute for an enzymatic reaction. The reaction mixture was made up to a total of 1 cm^3 with distilled water prior to the addition of the substrate. The enzymatic reaction was stopped at the end of exactly one minute by adding 4 cm^3 of glacial acetic acid. All the concentrations were prepared in triplicate.

The control enzyme reaction is the one without fraction and it was also made up to a total of 1 cm^3 with distilled water.

RESULTS AND DISCUSSION

Table 1 shows the percentage inhibition of the sheep liver cholinesterase enzyme (ChE) by the various extract fractions in comparison with the reference standard, Huperzine A. The degree of inhibition is expressed in terms of Huperzine A units (HAU) and was deduced from the Huperzine A standard calibration curve as a reference standard (Figure 1).

Table 1: Percentage inhibition of the sheep liver cholinesterase enzyme

Fractions	Concentration (µg/ml)	% ChE inhibition (Y)	% decrease or increase compared to Huperzine A % inhibition	Equivalent Huperzine A unit (HAU) deduced from the graph (X)	Remarks on the ChE inhibition
FE01	5	35.56	+17.56	28	Good inhibition at all concentrations
	10	39.63	+12.63	32	
	20	42.70	+5.70	36	
FC01	5	39.39	+21.39	32	Good inhibition at all concentrations
	10	54.07	+27.07	43	
	20	65.12	+28.14	53	
FB01	5	-2.70	-39.70	-	No inhibition at low concentrations and traces of inhibition at high concentrations
	10	21.11	-5.89	17	
	20	41.00	+23.00	31	
FH01	5	-40.00	-77.00	-	No inhibition at low concentrations and traces of inhibition at high concentrations
	10	18.00	-9.00	15	
	20	36.00	+18.00	30	

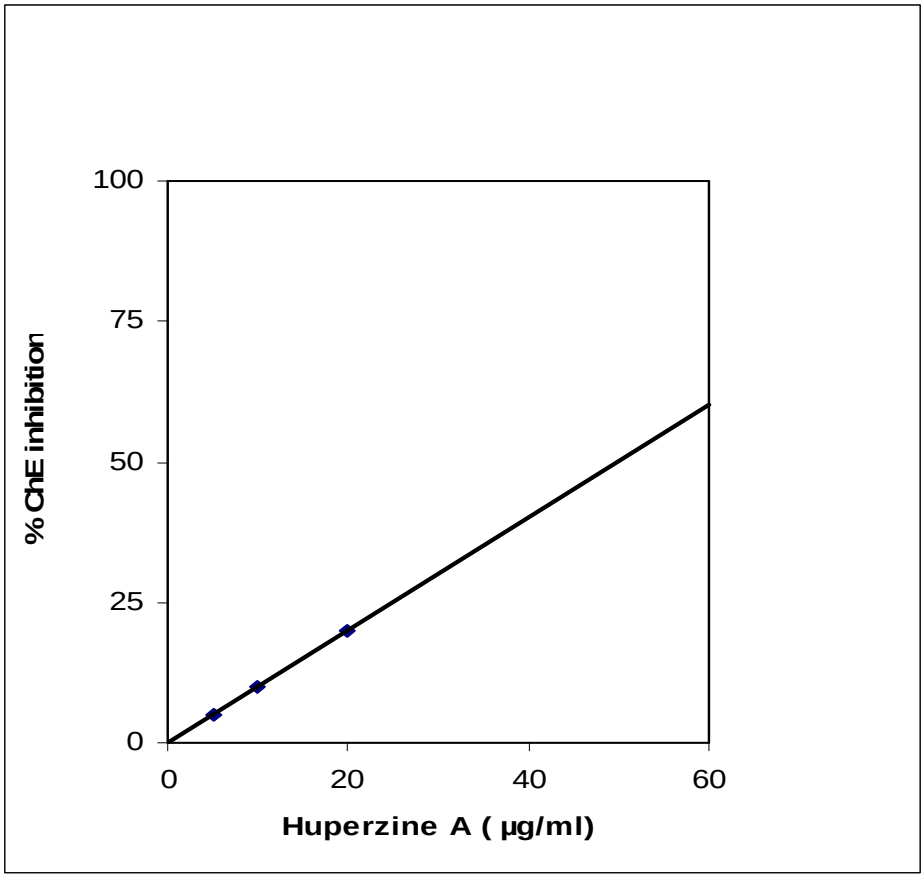

Fig. 1: Standard calibration curve with Huperzine A as a reference standard

The percent inhibition was determined using the relation;

$$\% \text{ inhibition} = \frac{C - E}{C} \times 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where C = amount of acetyl formed in control
E = amount of acetyl formed in experiment

The calibration curve (straight line) was plotted for the % ChE inhibition against Huperzine A amount (Figure 1 and Table 1) which obeys Beer lamberts law.

From Table 1 above the cholinesterase inhibition of the leaves extract shows certain level of inhibition. Generally, good inhibition at both higher and lower concentrations was observed in both chloroform and ethanolic fractions. While only traces of inhibition was observed in both n-butanol and n-hexane fractions at higher concentrations. The order of inhibition caused from the highest to the least is: FC > FE > FB > FH. Chloroform fraction (FC01) was observed as the most active while n-hexane fraction (FH 01) was the least active. Due to the significant level of sheep liver cholinesterase enzyme inhibition observed from both the chloroform and ethanol fractions, it is expected that these two fractions may inhibit the growth of the cells of *Mycobacterium aurum* or tuberculosis expressing firefly luciferase. This is because a positive cholinesterase enzyme inhibition test serves as a good indicator system for a positive bioluminescent *Mycobacterium aurum* or tuberculosis expressing firefly luciferase test.

The results of this work tend to rationalize the fact that the plant, *Anageisus leiocarpus* is ethnomedically used in the treatment of tuberculosis. This is because both the FC01 and FE01 were found to significantly inhibit further growth of cholinesterase enzyme of sheep liver. The cholinesterase enzyme inhibition assay serves as an indicator system whose analysis always correspond to the bioluminescent *Mycobacterium aurum* or tuberculosis expressing firefly luciferase (one of the standard antituberculosis bioassay). Therefore, it is suggested that an *in vitro* ChE inhibition model should be used for screening of the pure compounds isolated from the most active fraction before proceeding to other toxicological protocols.

REFERENCES

Abdu, K. and Johnbull, E.O. (2006): Phytochemical and antimicrobial screening of the ethanolic extract of the leaves of *Anogeisus leiocarpus*, *Bio. Environ. Sci. J. Trop.* **3** (6), 162-164.

Aja'afar, B.I. (1982): Northern Nigeria medicinal plants as sources of drugs, *B. Sc Thesis,* Department of Chemistry, Bayero University, Kano Nigeria.

Blower, S.M. and Chou, T. (2004): Modeling the emergence of the 'hot zones': tuberculosis and the amplification dynamics of drug resistance. *Nat. Med*. **10**, 1111-1116.

Boehringer, M. (1991): Cholinesterase technical bulletin 124117, Indianapolis, USA.

Burkill H.M. (1985): *The useful plants of tropical Africa,* 2nd edition, vol. 1 Royal botanical garden, kew, pp. 389-391.

Corbett, E.L.; Watt, C.J.; Walker, N.; Maher, D.; Williams, B.G.; Raviglione, M.C. and Dye, C. (2003): The growing burden of tuberculosis: global trends and interaction with HIV epidemic. *Arch. Intern. Med*. **163**, 1009-1021.

Ellman. G.L. (1961): Cholinesterase enzyme inhibition by organophosphorus compounds: *Biochem. Pharmacol*. **88**, 126-129.

Espinal, M.A. (2003): The global situation of MDR-TB, *Tuberculosis*, **83**, 44-51.

Nunn, P. (1997): WHO global TB programme, tuberculosis research and surveillance unit of WHO global TB programme. Press release WHO/74; (http://www.scielo.br/www.who.int/gtb/press/who74.htm).

Morens, D.M.; Folkers, G.K. and Fausi, A.S. (2004): The challenges of emerging and re-emerging infectious diseases, *Nature,* **430**, 242-248.

Sanni, S.B. and Okor D.I. (1983): Fluoride content of Nigerian chewing sticks, *IRCS, LBR Compend.* **117**, 604.