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CHOLINESTERASE AND BACTERIAL INHIBITORY ACTIVITIES OF *STACHYTARPHETA CAYENNENSIS*

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ABSTRACT

The cholinesterase and bacterial inhibitory activities of the leaves and stem of Stachytarphetacayennensis were evaluated due to their local applications. The results revealed that the extracts of Stachytarphetacayennensis leaves (SCL) and stem (SCS) assessed for their acetyl cholinesterase inhibitory activities showed that all the extracts had some levels of inhibitory effect depending on the solvents used. The result of bacterial inhibition showed that among all the test organisms screened, the ethanol and water extracts of leaves and stem of the plant had promising activity against Escherichia coli, Proteus mirabilis, Pseudomonasaeruginosa and klebsiella pneumonia bacteria. There were no activity shown by the ethanol and water extracts of the parts of the plants with Staphylococcus aureus. The ethanol and water extracts of the leaves had the highest zone of inhibition of 14 mm against Proteus mirabilis and Escherichia coli and klebsiella pneumonia respectively.

Keywords:anticholinesterase; antibacterial; inhibition; Stachytarphetacayennensis

INTRODUCTION

Many vegetable and fruits in Africa possess medicinal, pesticidal, antibacterial and antiseptic properties. These may include wild shrubs and weeds which are not regarded to be of any relevance both medicinally and nutritionally. Stachytarphetacayennensis is a wild shrub growing in tropical West African and has been known to possess pesticidal activity, in its local applications as a mosquito repellent. The plant has been used by different localities as a remedy to many ailments. For instance, it is used widely in Nigeria for the treatment of dysentery and as a remedy for gonorrhoea and ulcer. The juice is used to cure eye troubles and sores in children's ears. The macerated leaves and roots have been claimed to treat sore skin wounds. There are also some side effects such as vomiting, loss of appetite and unconsciousness etc (Mesia-Vela et al., 2004; Akobundu and Agyakwa, 1998 and Hatchison, 1963).

Stachytarphetacayennensis (Verbenaceae Family) is a herb commonly found in Nigeria as a weed of waste places, anthropogenic sites, roads and weeds of field crops with long growing season (Dalzipi, 1937). It is not recognized and regarded as an important plant. The plant's common names are: the blue rats tail or rough-leaved false vervian (English); Iruamure (Yoruba), WulsigaiKusu (Hausa), OkenchụanwụntaỌhịa (Igbo) (Akobundu and Agyakwa, 1998), Opapara (Abeokuta).

Stachytarphetacayennensis is an erect, shrubby perennial plant up to 1.5m high that produces seeds. The stem is four angled, woody at the base and has slender branching stems that are covered with short hairs. The leaves are opposite or sometimes alternate with short, winged stalks. The blades are ovate to elliptic in shape, about 8cm long and 4cm wide hairy on both surface and prominently net veined with rounded or pointed apex, evenly toothed margins and contracted at the base (Akobundu and Agyakwa, 1998).

The inflorescence is a long slender, hair spike, about 20cm long, occurring at the shoot terminal. The flowers are white or lilac with white centres, sessile with 4-5 mm long and five lobed petals (2 large and 3 small) about 4-5mm in diameter. The seed is a two-seeded kernel or nutlet enclosed by a persistent calyx that is embedded in a shallow groove in the inflorescence axis.

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Acetyl cholinesterase is an enzyme required for normal transmission of nerve impulses for proper nervous system function. An acetyl cholinesterase inhibitor (or anti-cholinesterase), therefore, is a chemical that inhibits the cholinesterase enzyme from breaking down acetylcholine, increasing both the level and duration of action of the neurotransmitter acetylcholine.

The plants used for this research were selected on the basis of the claims of their local use that suggest pesticidal activities, antimicrobial activities, among other local uses. This research therefore evaluated the anticholinesterase and antibacterial inhibitory activities of the plant,

MATERIALS AND METHODS

Starchytarphetacayennensis plant was harvested from uncultivated area of Michael Okpara University of Agriculture Umudike, Ikwuano Local Government Area of Abia State, Nigeria between March and April 2008. The plant was identified by Mr.IbeNdukwe of the Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State.

Preparation and Preservation of Samples

The parts of the plants samples collected were separated from each other. The leaves of the plants were separated from their stem and some with flowers were left with the stem. These samples were air-dried at room temperature. The air-dried samples were pulverized into powdery form using brand new corona landers manual milling machine which were then stored in labelled polyethene bags.

Extraction of Sample for Cholinesterase Activity

The extraction method used in this study was a modification of JohnBull (2001a and b) and JohnBull and Abdu (2006). 750g to 1kg of each of the pulverized samples were soaked in different glass bottles to obtain the ethanol (crude) extract individually by percolating in about 2.5 litres of ethanol (98% analytical standard) for 72hours, followed by filteration and then concentrated under reduced pressure rotary evaporator at 34^{o}C separately (JohnBull et al, 2001a and b). These were then allowed to stand for complete evaporation of the remaining ethanol at room temperature. The crude ethanol extracts were stored in labelled covered beakers for further analyses

Partitioning of the Crude Ethanol Extract

Part of each of the crude ethanol extracts of the samples were subjected to partitioning.15 - 20 g of each of the crude ethanol extract of the sample was partitioned using 150ml/150ml v/v of chloroform and water. The sample was first dissolved using the solvents. They were continuously stirred to ensure complete dissolution and were then transferred into different separating funnels which were shaken until homogenous mixtures were obtained. They were then allowed to stand for 24hrs before separating. The chloroform extracts were allowed to evaporate completely under room temperature. These procedures were repeated for aqueous methanol/petroleum ether using equal volumes of aqueous methanol petroleum ether (40 - 60) using part of the chloroform extracts. The water fractions were stored in a refrigerator while other fractions were allowed to dry under room temperature.

Cholinesterase Assay (Enzyme Assay Technique, Eat)

The determination of an enzyme is based upon the rate of utilization of substrate or formation of product under controlled conditions. Most enzyme assays are carried out at 30^{o}C but some are perfumed at 37^{o}C because of the physiological significance of the temperature. The changes in absorbance can be used as basis of the assay.

Appropriate amounts of crude ethanol and partitioned fractions (5, 10, 20µg) were prepared in acetone. Fresh sheep liver was prepared from healthy sheep which was stored in the ice-chamber immediately after slaughter. 1% homogenate w/v of sheep was prepared in distilled water at 0^{o}C.

Cholinesterase inhibition by colorimetric method was adopted but with the following modifications; appropriate amounts of the test samples 5, 10, 20 µg were measure and 0.1ml of acetone were separately dispensed into the test tubes and the solvent acetone was allowed to evaporate. Then 0.1 ml of 1% sheep liver homogenate was preincubated with 5, 10, 20 µg of the test compounds, separately for 15 minutes at 37^{o}C in a thermostatic water bath. Standard Huperzine A was also preincubated in

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the same manner described above with 0.1 ml of 1% (w/v) to allow the inhibition of liver cholinesterase by test compounds. After pre-incubation, 0.2 ml of 0.2% green azo dye in water was added followed by 0.1 ml of 0.01M ethyl acetate substrate in acetone and the reaction mixture was again incubated for one minute for enzymatic reaction. The reaction mixture was made up to a total of 1.0 ml with distilled water prior to addition of substrate. Caution was taken to see that the enzymatic incubation time of one minute after addition of substrate did not exceed. The enzyme activity was stopped at the end of exactly one minute by adding 4 ml of glacial acetic acid. The colour developed was taken at 620 nm in a spectrophotometer.

The control enzyme reaction mixtures were without the test compounds and control reaction mixture was also made up to a total of 1 ml with distilled water. However, the preincubation of 15 minutes with enzyme alone and enzymatic incubation for 1 minute with addition of ethylacetate substrate and green azo dye was carried out without adding the test compounds. The control optical density was normalized to 100% and the percent inhibition was calculated as

$$\frac{C - E}{C} \times 100 = \%\text{inhibition}$$

where C = Control absorbance unit

E = Experimental absorbance unit (with test compounds)

From enzymatic activity units

$$\frac{C-E}{C} \times 100 = \%\ \text{ChE inhibition}$$

where C = Amount of ethanol formed in Control

E = Amount of ethanol formed in Experimental

Preparation of Plant Extracts for Antibacterial Activity Test

The ethanol and water extracts of the plant samples were prepared by cold percolation method. 20g of each of the pulverized samples was soaked in different 200ml of ethanol and water respectively for 48 hrs for optimum extraction with intermittent shaking to get a concentration of 10%, after which they were filtered with Whatman paper No. 1 into vials. The filtrate of each extract was dried until a constant dry weight of each extract was obtained. The extracts were stored at 4^{o}C for further use.

Preparation of Culture Media

Nutrient agar was used as medium in the antibacterial assay. The milky white powder has the formulation 5.0 g/l peptone, 12.0 g/l agar, 8.0 g/l sodium chloride and 3.0 g/l beef extract. This was prepared by dissolving 28g of nutrient agar powder in 800ml of distilled water in a calibrated, covered glass bottle and shaken properly. The solution was allowed to stand for 10 minutes, swirled to mix in order to achieve total dissolution of the powder. The dissolved solution was then placed in an autoclave for sterilization at 121^{o}C for 15 minutes after which it was allowed to cool to 47^{o}C and mixed well before pouring into plates.

Preparation of Inoculum

Stock Cultures were maintained at 4^{o}C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient agar broth (NAB) and incubated without agitation for 24 hours at 37^{o}C. The cultures were diluted with fresh nutrient agar to achieve optical density corresponding to 2.0 x 10^{6} colony forming units (CFU/ml) for bacteria.

Antibacterial Activity Screening

The ethanol and water extracts of the samples were screened against a total of five bacterial strains. The test bacteria organisms were Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa and Escherichia coli which were all obtained from the stock Culture of the Microbiology Lab of Federal Medical Centre, Umuahia. Abia State Nigeria.

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The antimicrobial activity screening was performed by filter paper disc method (Valsaraji, et al, 1997). In vitro anti-bacterial activity was screened by using nutrient agar. The plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the innoculum was allowed to dry for 5 minutes. About 5 mg of the extract was loaded on 4mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation for 24 hours at 37°C. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. The experiment was repeated two more times for reaffirmation.

Antibacterial Activity Assay

The plates were prepared and inoculated as in the screening test 0.1g of dried ethanol and water extract were respectively dissolved in 1 ml of ethanol and 1 ml of water to get concentrations of 0.1 g/ml. Standard solution containing 0.1 g/ml ciprofuxacin was also prepared. About 0.2 ml plant extract, ethanol and water, standard ciprofuxacin were loaded on separate 4 mm sterile disc with ethanol and water respectively served as controls for ethanol and water extracts.

Standard ciprofuxacin disc was used to compare the efficacies of the antibacterial activities of the extracts. A loaded disc was placed on the surface of inoculated medium and the substance allowed to diffuse for 5 minutes and plates were kept for incubation for 24 hours at 37°C. At the end of incubation, inhibition zones formed around the discs were measured with transparent ruler in millimeter. The experiment was performed in triplicate and the mean value was recorded.

The percentage inhibition relative to standard anti-microbial agent was calculated as follows:

$$\% \text{ inhibition} = \frac{Xe - Xc}{X_s} \times \frac{100}{1}$$

where Xs = inhibition of standard

Xc = inhibition of control and

Xe = inhibition of extract.

RESULTS AND DISCUSSION

Cholinesterase Inhibition

The results of the extracts of Stachytarphetacayennensis leaves (SCL) and stem (SCS) assessed for their acetyl cholinesterase inhibitory activities showed that all the extracts showed some levels of inhibitory effects depending on the solvents used.

The dose dependent inhibitory effects of the different plants extracts using different solvents are shown in Figures 1 - 5. For the water extract (Fig. 1), SCL and SCS shared maximum inhibitory effect at the concentration of 0.5mg/l with percentage inhibitory effects of 56.31 and 55.13% respectively.

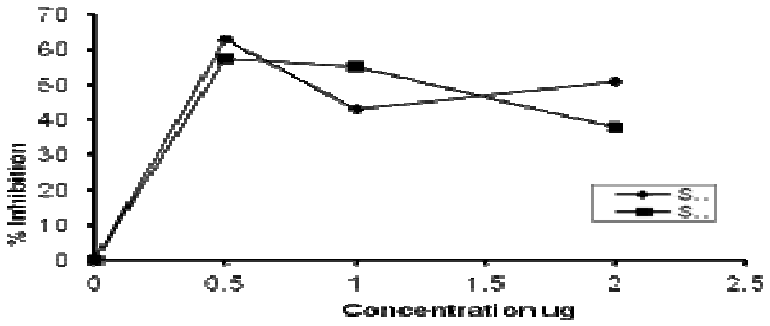

Figure 1.Dose dependent inhibition of Acetylcholinesterase (ACHE) activity by the water extracts of Starchytarphetacayennensis leaves and stem

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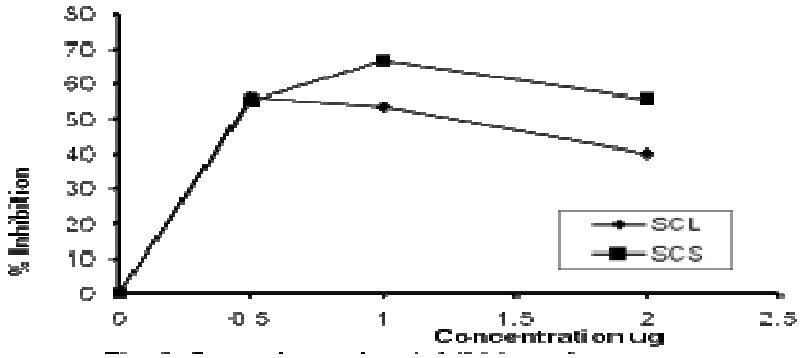

Figure 2.Dose dependent inhibition of Acetylcholinesterase (ACHE) activity by the crude ethanol extracts of Starchytarphetacayennensisleaves and stem

Figure 2 showed dose dependent inhibitory effect of the ethanol extracts (crude extracts) of the all the parts of the plants on the acetyl cholinesterase; SCS had maximum inhibitory effect at 1.0mg/l with percentage inhibitory effect of 66.71%. Furthermore, all the parts of the plant studied (Fig. 3) showed maximum inhibitory effect at 0.5mg/l for the chloroform extract. For the Petroleum ether extracts (Fig.4), maximum inhibitory effect was observed for SCS (67.50%) at concentration of 1.0mg/l while SCL (47.63%) had maximum inhibitory effects at 0.5mg/l concentration. For the Aqueous methanol extract (Fig. 5), SCL had maximum inhibitory effects at 1.0mg/l while the maximum inhibitory effect of SCS was at 0.5mg/l.

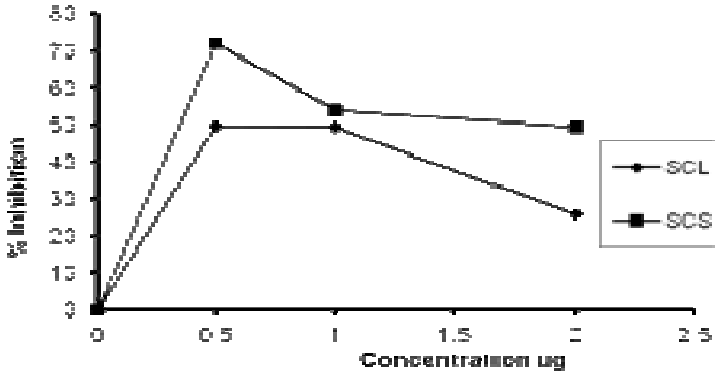

Figure 3.Dose dependent inhibition of Acetylcholinesterase (ACHE) activity by thr chloroform extracts of Starchytarphetacayennensisleaves and stem

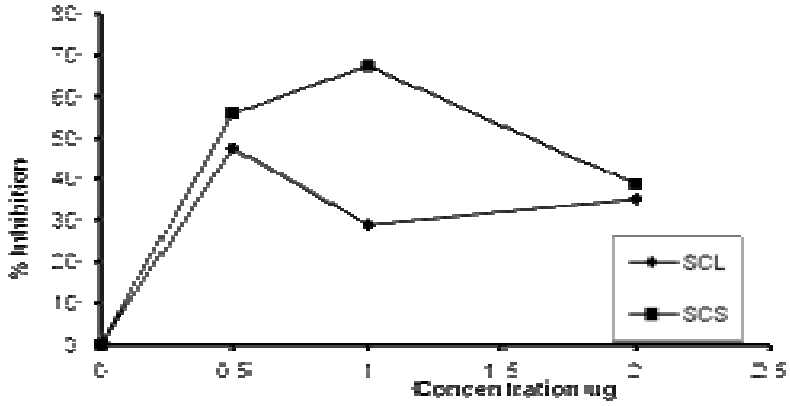

Figure 4.Dose dependent inhibition of Acetylcholinesterase (ACHE) activity by the Pet. ether extracts of Starchytarphetacayennensis leaves and stem

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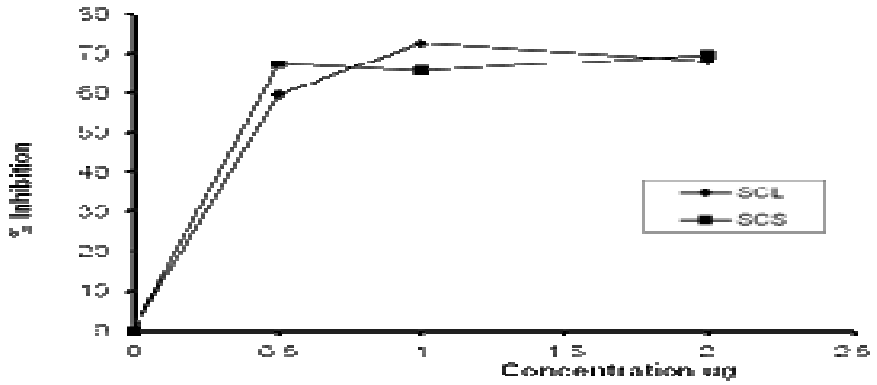

Figure 5.Dose dependent inhibition of Acetylcholinesterase (ACHE) activity by the Aqeuous Methanol extracts of Starchytarphetacayennensisleaves and stem

Moreover, the dose dependent inhibitory effects of the acetyl cholinesterase activities of the parts of the plants extracts studied were evaluated based on the different solvents used which are shown in Figures 6 and 7. The methanol extracts had better inhibitory effects with maximum inhibition observed at 1.0mg/l with a value of 72.50% for Stachyterphetacayennensis leaves (Fig.6). Maximum inhibitory effects were observed at 1.0mg/l concentration with pet. ether and ethanol having a value of 67.50% and 66.71% respectively while chloroform and water extracts had maximum inhibitory effects at 0.5mg/l concentration for Stachyterphetacayennensis stem (Fig. 7). Koelle (1975) reported that for significant effect to occur in-vivo, an anti-cholinesterase agent must generally inhibit from 50 – 90%. Also, several studies have reported the anti-cholinesterase activities of plant extracts (Raffeeq et al., 2006; Viegas et al, 2005; Ortega et al., 2004 and Orhan et al., 2004). Furthermore, Raffeeq et al., (2006) and Ahmad et al., (2003) in their studies of some plants extracts reported the presence of steroidal galactoside contents as may have a role in its enzyme inhibitory activity.

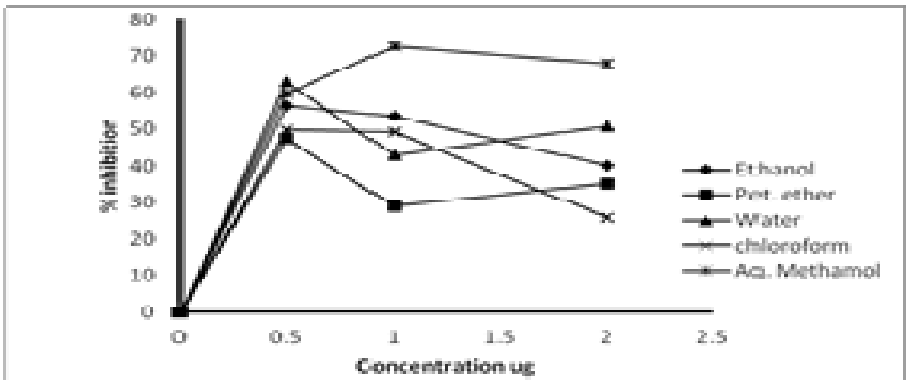

Figure 6.Dose dependent inhibitory effects of the acetyl cholinesterase activities of the leave extract on the different solvents

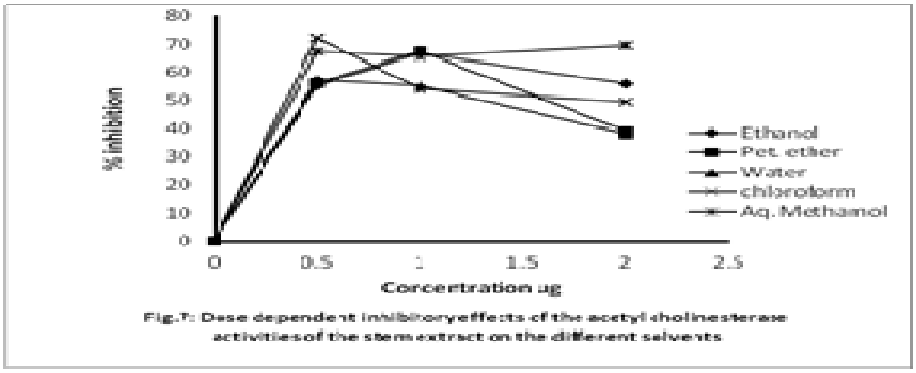

Figure7.Dose dependent inhibitory effects of the acetyl cholinesterase activities of the stem extract on the different solvents

An increase in pest toxicity is usually accompanied by an increase in the inhibitory effect of acetyl cholinesterase activity. Cholinesterase is an enzyme that catalyses the hydrolysis of the neurotransmitter acetyl choline (Lester, 1977) into choline and acetic acid. The reaction allows cholinergic neuron to return to its resting state after activation. Anticholinesterase substances (cholinesterase inhibitors) suppress this action of the enzyme. Substances that interfere with the action of cholinesterase are potent neurotoxins causing excessive salivation and eye-watering, low doses followed by muscles spasms (sudden uncontrollable strong tightening of the muscles) and ultimately death. Anti-cholinesterase agents as a group have received more extensive application as toxic agents in the form of agricultural insecticides and potential chemical warfare.

The acetyl cholinesterase inhibitory effects of Stachytarphetacayennensis could be attributed to its known pesticidal activity in its local applications as a mosquito repellants (Akobundu and Agyakwa, 1998; Nwaiwu and Aka, 1996).

Anti-cholinesterase substances are used as biochemical warfare in addition to its use as anesthesia or in the treatment of myasthenia gravis, glaucoma (eye disease causing gradual lost of sight) and Alzheimer's disease (a serious disease of the brain that prevents its normal function and causes loss of memory, loss of ability to speak clearly) etc.

Apart from the antibacterial activities of the plants studied, their cholinesterase properties could further be used to explore their potential in drug discovering for the treatment of the above mentioned ailments. However, a good number of plant extracts and pure components of natural origin have been reported to have anticholinesterase property (JohnBull, and Muluh, 2009; Rafeeq et al., 2006; Ortega et al., 2004; Orhan et al., 2004; Ahmed et al., 2003 and Sung et al., 2002). Furthermore, the acetyl cholinesterase inhibitory effect of Stachytarphetacayennensis could however, also be justified by its traditional use of the juice to cure eye troubles such as cataract (Akobundu and Agyakwa, 1998) and in the treatment of other bacterial causes diseases due to their inhibition against some bacterial pathogens.

Bacterial Inhibition

The result showed that among all the test organisms screened, the ethanol and water extracts of leaves and stem of Starchytarphetacayennensis plants had promising activity against Escherichia coli, Proteus mirabilis, Pseudomonasaeruginosa and klebsiella pneumonia bacteria. There were no activity shown by the ethanol and water extracts of the parts of the plants with Staphylococcus aureus.

Table 1.The results of the zone of inhibition (mm) of antibacterial activity of the leaves and stem of Starchytarphetacayennensis and that of the standards

Microbes	*Standard ciprofuxacin Inhibition*	*Leaves*		*Stem*	
		Ethanol	*Water*	*Ethanol*	*Water*
Escherichia coli	20.0	12.0	14.0	8.8	12.0
Staphylococcus aureus	21.0	-	-	-	-
Proteus mirabilis	19.8	14.0	10.0	8.0	12.0
Pseudomonas aeruginosa	17.0	7.0	-	-	-
klebsiella pneumonia	21.0	10.5	14.0	9.0	7.0

Values are means of three replicates. Standards ciprofuxacin solutions was used

Table 1 shows the results of the zone of inhibition (mm) of antibacterial activity of the leaves and stem of Starchytarphetacayennensis plant and efficacies of the extracts relative to the standards. The water extracts of the leaves and stem of Starchytarphetacayennensis against Escherichia coli were more active than the ethanol extracts. The zones of inhibition for the water and ethanol extracts were 14.0mm and 12.0mm for the leave and 12.0mm and 8.8mm for the stem respectively, while no activity was observed against bacterial strains neither with the ethanol nor water extract of the Starchytarphetacayennensis. The least inhibitory activities were observed for the ethanol extract of the leave against Pseudomonas aeruginosa and water extract of the stem against klebsiella pneumonia with zone of inhibition of 7.0mm

Furthermore, the observed in-vivo bacterial inhibitory effects by the plants parts against some the selected pathogenic microorganisms studied justified the traditional applications in disease management. Stachyterphetacayennensis is used as a remedy for syphilis, gonorrhea, catarrh condition, skin wounds and sores in children (Hatchison et al., 1963). Both the water and ethanol extracts exhibited some level of inhibitory effects against some of the studied pathogens which have been implicated in one bacterial infection to the other in human and plant.

Escherichia.coli and Proteus mirabilis cause urinary tract infections; Klebsiella pneumonia causes pneumonia. In plant Pseudominasspp causes bacterial blight in guinea corn. The antimicrobial activities of plant extracts are usually due to the activities of plant to be toxic to microorganism due to enzyme inhibition. Many plant extracts have been reported to exhibit antibacterial activities which proved effective in the management and control of the microorganisms. Plants have these potential to control pests because they contain toxic compounds and these could be used to formulate natural control products as alternative to synthetic chemical (Cutler and Hill, 1994).

CONCLUSION

Starchytarphetacayennensis plants showed cholinesterase and bacterial inhibitory activities. Its bacterial inhibitory activity against some of the selected pathogens showed that it could use in the management of disease caused by such microorganisms. The findings from this research justified some of the reasons for the local use of the plant in pest control and in the management disease caused by bacterial pathogens.

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