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Effects of Aqueous Leaf Extracts of *Psidium guajava* on Bacteria Isolated from the Navel of Day-Old Chicks

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Abstract: Plants are used widely in the tropics and sub-tropical Africa and Asia for the treatment and cure of various illnesses such as malaria, diarrhoea, burns, gonorrhoea, stomach disorders and other infectious diseases; among which are livestock and poultry related diseases. Present studies on the preliminary phytochemical composition of the leaf of this plant revealed the presence of flavonoids, tannins, saponins, phenols lectins, triterpenes and carotenoids among others. Studies on the swab content from the navel of the day old chicks of both strains (broilers and layers) had revealed the presence of several gram positive and gram negative organisms such as *E. coli*, *Staphylococcus* sp., *Streptococcus* sp., *Proteus* sp., *Klebsiella* sp. and *Corynaebacterium*. The susceptibility tests on the isolated organisms by the extract under study had showed an appreciable dose dependant zone of inhibition ranging from 13-25 mm. The activity of the extract (400 mg mL^{-1}) can be favourably compared with that of the standard antibiotic-Oxytetracycline (10 mg mL^{-1}) particularly between the *E. coli* and *Streptococcus* with 25:30 mm and 20:22 mm as inhibition zone respectively where no significant difference was observed. The extract exhibited a highest MIC of 12.5 mg mL^{-1} against *Staphylococcus* sp., while concentration of 25.0 mg mL^{-1} was noted as the MIC values against both *E. coli* and *Streptococcus* sp.

Key words: Aqueous, bacteria, chicks, leaf, omphalitis, *Psidium guajava*

INTRODUCTION

Traditionally, usage of plants in curing illness has deep roots in man's history (Grabley and Thiericke, 1999; Aibinu *et al*., 2007). Plants are used in treating malaria, diarrhoea, burns, gonorrhoea, stomach disorders and other infectious diseases. Tremendous efforts of scientists have been employed in establishing plants with promising antimicrobial activity and yielding fruitful results (Adedayo *et al*., 2001; Ndukwe *et al*., 2005; Aibinu *et al*., 2007). The plants are easily available and accessible in this part of the World and cheaper than the conventional drugs. The disease condition omphalitis is technically defined as an infectious but non-contagious disease that is characterized by infected yolk sac often accompanied by unhealed navel (Jordon and Pattison, 1999). In every flock, there is usually increased mortality between 3-4 days of age due often to navel-yolk sac infection or omphalitis, commonly associated with *Escherichia coli* infections or other bacterial contamination. Because the navel is still open when the chick is hatching, or when tissue is stock in the navel after closing; it is very easy for bacteria to enter the body cavity, so infecting both the navel and the yolk sac. This is to say that navel yolk sac infection is one of the causes of high mortality during the early days of young chicks (Jordon and Pattison, 1999). Due to the significant number of birds claimed by

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omphalitis, the tragedy is called naval-yolk sac mortality. Bacterial pressure is the only determining factor through navel deformity and the chick's ability to use its natural defense mechanisms are also critical (Meigerhof, 2005). Common bacteria involved are *E. coli*, *Entrococcus* species, *Salmonella* species and *Pseudomonas* species. These bacteria can cause generalized septicemia and result in high mortality. Affected birds will show depreciation, drooping of the head and hurdling near the heat source. The navel may be inflamed and when fail to close produce a wet spot abdomen. Treatment of this condition is not specific and no specific antibiotic for the treatment. The antibiotic used is based on the prevalent bacteria type involved but of probably little value (Koteeswaran *et al*., 2004). It may not always be appropriate to be treated as if there is niggling mortality. Treatment result in infected yolk sac retention and this can lead to uneven mass in the flock (Jordon and Pattison, 1999). The escalating problems of antibacterial resistance shown by several species of bacteria to most of the antibiotics used today has made it mandatory to search for newer drugs that are effective, affordable, acceptable and available. Many research groups and organizations in many countries under take multidisciplinary research on local medicinal plants with a view of revealing that plants are potential source of drugs (Sofowora, 1993). Although, traditional medicine has been in practice as far back as 1500BC and many plants have been used to cure certain diseases, there may not be scientific data to confirm their efficacy (Sofowora, 1993). Guava is a common shade tree or shrubs in the tropics and has a long history of traditional medicinal uses, which led modern day researchers to study its extracts ((Jordon *et al*., 2003). Extracts of roots, bark and leaves are used to treat gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothaches, coughs, sore throat, inflamed gums and a number of other conditions (Morton, 1981). It has been documented to have pronounced antibacterial, antiamoebic and antispasmodic activity. Its bark and leaf extracts have been shown to have *in vitro* toxic action against numerous bacteria (Geidam *et al*., 2007).

Therefore this study aims to isolate the common bacterial agents from the navel of day-old chicks from different hatcheries, determine the *in vitro* anti bacterial properties of *Psidium guajava* aqueous extract against the bacteria isolated from the navel of day-old-chicks and then establish the minimum inhibitory concentration of *Psidium guajava* aqueous leaf extract against the isolated bacterial agents.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh samples of the leaves were collected in May 2006 from a matured guava tree within University of Maiduguri campus. The plant was identified and authenticated by a plant taxonomist, Dr. S.S. Sanusi of the Department of Biological Sciences and voucher specimen number (Chem: 242B) was deposited at Research Laboratory, Chemistry Department University of Maiduguri, Maiduguri-Nigeria.

Preparation of Extract

The fresh leaves of *Psidium guajava* collected were then air-dried in the laboratory under room temperature. The dried leaves were ground into powder using mortar and pestle. Two hundred and fifty grams of the dried powdered leaves of *Psidium guajava* were exhaustively extracted with water using the reflux method for 3 h. The extract was then filtered and concentrated *in vacuo* to yield a dark-green coloured mass.

Experimental Birds

Sixty day-old-chicks (30 broilers and 30 layers) were acquired from the 3 main sources of day-old-chicks in Maiduguri all of whom are agents of different hatcheries in Nigeria.

Collection of Samples

Swaps were taken using sterile swap sticks from the naval of the 60 day-old chicks. These were inoculated into the plates containing MacConkey and blood agars. Using the half and quarter plate streaking method, respectively.

Culturing and Identification of the Organisms

The inoculated plates were incubated immediately for 24 h at 37°C, the growth were then identified using colonial appearance, Gram stain, examination of the organisms under microscopes and the use of sub-culture using different media for confirmation of the organisms earlier identified. All media used were prepared according to manufacturer's instructions.

Determination of Anti-Bacterial Properties

Bacterial Isolates

The bacterial isolates from the naval of day-old-chicks were used for determining the anti bacterial properties of *Psidium guajava* leaf extract. The isolates were propagated and stored on nutrient agar plates. All the isolates were maintained on nutrient agar plate at 4°C and sub-cultured in nutrient broth at 37°C for 8 h prior to antimicrobial testing. One milliliter of the broth culture was then used to flood the agar plates.

Concentration of Extracts

Stock solutions of the extract were prepared by dissolving known weight of the extract in known volume of distilled water 0.01, 0.02 and 0.04 g of the extracts were dissolve in 1 mL of distilled water to afford 100, 200 and 400 mg mL^{-1} of the extract, respectively. Standard antibacterial agent oxytetracycline (Pfizer, Inc., USA) at a concentration of 10 mg mL^{-1} was also used on all the bacteria and the zones of inhibition compared with those of the plant extract.

Antibacterial Sensitivity Testing

Bauer-Kirby disc diffusion method as described by Bauer *et al*. (1966) was used to determine the antibacterial activity. Discs containing different concentrations of dissolved extract were prepared. Sterilized filter papers (Whatman No. 1, 6 mm in diameter) soaked in beakers containing different concentrations (100, 200 and 400 mg mL^{-1}) of the extract.

Over night cultures of each bacterial isolate was spread on the surface of dried nutrient agar plates. The plates were incubated at 37°C for 30 min before the discs were applied aseptically. The treated plates were incubated at 37°C for 48 h. The same procedure was carried out with the oxytetracycline (10 mg mL^{-1}) as standard antibiotic. Plates without the antibiotic or extract discs were set up as control experiment. The zones of inhibition above 6 mm diameter of each isolate were used as measure of susceptibility to the extracts and were compared to that of the standard antibiotic.

Determination of Minimum Inhibitory Concentration (MIC) of the Extracts

The MIC was determined using the method described by Greenwood (1989). For each extract six sterile test tubes were arranges in a test tube rack in a row for each organisms and 0.5 mL of sterile nutrient broth was pipetted into each tube. Half a millimeter of the crude extract containing 100 mg mL^{-1} was pipetted into tube one to obtain a concentration of 50 mg mL^{-1}. There after there was a serial dilution of the extract to obtain concentrations of 25, 12.5, 6.25 and 3.13 mg mL^{-1}, respectively. 0.5 mL of the test organism was pipetted into each test tube and incubated at 37°C for 24 h. The MIC was recorded as the least concentration of plant extract that completely inhibit the growth of the test organism.

RESULTS AND DISCUSSION

Two hundred and fifty grams of the dried powder leaves of *Psidium guajava* was exhaustively extracted with 1.5 L of distilled water in a reflux apparatus and then concentrated to yield 70.8 g of the crude extracted that is 28.3% w/w with respect to the dried powdered extract.

Six different bacterial organisms were isolated from the 60 swabs taken from the navel of broilers and layers day-old-chicks. The isolated organisms include; *Staphylococcus* sp., *E. coli*, *Proteus* sp., *Klebsiella* sp. and *Corynaebacteria* sp. All these six organisms were isolated from layer day-old-chicks while only four of the organisms (*Staphylococcus* sp., *Streptococcus* sp., *E. coli* and *Proteus* sp.) were isolated from the broiler day-old-chicks (Table 1).

The effect of the three different concentrations of the extract on the bacteria isolated is presented in Table 2. The extract showed concentration dependent antibacterial activity against *E. coli*, *Streptococcus* sp., *Staphylococcus* sp. and *Proteus* sp. However, *Klebsiella* and *Corynaebacteria* appeared to be resistant to the extract. The result of the MIC of the extract on the three susceptible organisms is presented in Table 3.

Table 1: Bacterial organisms isolated from the navels of day-old-chicks

	Chick type	
Bacterial isolate	Broilers	Layers
E. coli	+	+
Staph	+	+
Strep	+	+
Proteus	+	+
Klebsiella	-	+
Corynaebacterium	-	+

+: Presence; -: Absence

Table 2: Antibacterial Activity of *Psidium guajava* aqueous leaf on organisms isolated from the navel of day-old chicks

Bacterial isolates	Concentration of extract/Drug (mg mL^{-1})	Zones of inhibition (mm)
E. coli	400	25
	200	18
	100	16
	Oxytetracycline 20	30
Streptococcus sp.	400	20
	200	16
	100	13
	Oxytetracycline 20	22
Proteus sp.	400	25
	200	20
	100	18
	Oxytetracycline 20	40
Klebsiella sp.	400	R
	200	R
	100	R
	Oxytetracycline 20	20
Corynaebacterium sp.	400	R
	200	R
	100	R
	Oxytetracycline 20	20

R = Resistant

Table 3: The minimum inhibition concentration of *Psidium guajava* aqueous leaf extract against some of the isolated bacteria

	Concentration of extract (mg mL^{-1})				
Organisms	50.0	25.0	12.5	6.25	3.13
Staphylococcus sp.	-ve	-ve	-ve*	+ve	+ve
Streptococcus sp.	-ve	-ve*	+ve	+ve	+ve
Escherichia coli	-ve	-ve*	+ve	+ve	+ve

+: ve = With bacterial growth; -: ve = Without bacterial growth; *: = MIC value

A study of the antibacterial effect of *Psidium guajava* aqueous leaf extract on bacterial organisms isolated from the navel of day old chicks was carried out. The result of the study showed that *Psidium guajava* leaf extract have concentration dependant inhibitory effect on the growth of *E. coli*, *Staphylococcus* sp., *Streptococcus* sp. and *Proteus* sp. isolated from the navel of day-old chicks. These results agree with those obtained by Jarlar and Wongkrajang (1999), who observed growth inhibition of *Staphylococcus aureus*. Similar results on growth inhibition were obtained by Gnan and Demello (1999), when testing the effect of the extract on *Staphylococcus aureus* by using guava leaf water extract. Iwu (1993) reported antibacterial effect *Psidium guajava* leaf extract against *E. coli*, *Staphylococcus aureus*, *Streptococcus* and *Proteus mirabilis*. All the bacteria inhibited by the leaf extract have been incriminated in omphalitis as shown by Whittam and Wilson (1988) and Jordon and Pattison (1999).

The susceptibility test of the extract (400 mg mL^{-1}) against most of the organisms screened indicated that *E. coli* exhibited the highest inhibition zone of 25 mm which could be compared favourably with 30 mm of Oxytetracycline (20 mg mL^{-1}). The activity of the extract against *E. coli* is important since many avian pathogenic *E. coli* strains have been reported to be resistant to common antibacterial agents used in poultry production (Ewers *et al*., 2003). It has been reported that *E. coli* can be frequently isolated from a clear infection of Yolk sac (Whittam and Wilson, 1988). The minimum inhibitory concentration against the susceptible organisms indicated that *E. coli* and *Proteus* sp. had the lowest, suggesting that the extract can be a potential antibacterial agent if the active compound responsible is isolated.

Phytochemical evaluation of the leaf has shown the presence of flavonoids, tannins, saponins, Phenols lectins, triterpenes and carotenoids (Geidam *et al*., 2007). These compounds are known to be biologically active. The antimicrobial activity of the leaf extracts demonstrated can be attributed to the presence of flavonoids (Ali and Shamsuzzaman, 1996a). Similarly, Berdy *et al*. (1981) demonstrated that the antibacterial effect could also be due to guajaverine and psydiolic acid, which are also present in the leaf. Flavonoids derivatives have been found to inhibit the growth of *Staphylococcus aureus* at the dilution of 1: 10,000 (Ali *et al*., 1996b). This is medically important in the treatment of inflamed tissues (Mota *et al*., 1985). And lectins in guava were shown to bind to *E. coli* preventing its adhesion to the intestinal wall and thus preventing infection (Berdy *et al*., 1981). Therefore, the activity of the extract against the isolated organisms in this study could be linked to the aforementioned reports. These effects can explain the long history of guava use in traditional medicine as a cure for many bacterial diseases. In conclusion, this study has provided a basis for the use of *Psidium guajava* in the treatment of yolk sac infection caused by *E. coli*, *Staphylococcus* sp. *Streptococcus* and *Proteus* either primarily or in combination. However, it is necessary to further investigate the *in vivo* antibacterial activities of the extracts in chicks.

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