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Antioxidant and Antitussive Properties of *Gongronema latifolium* Leaves Used Locally for the Treatment of Fowl Cough in Nigeria

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ABSTRACT: The antioxidant and antitussive properties of *Gongronema latifolium* used locally by Nigerian poultry farmers for the treatment of fowl cough was investigated. The phytochemical results revealed the presence of saponins (0.69mg/100g), tannins (0.0416mg/100g), alkaloids (0.046mg/100g) and flavonoids (0.016mg/100g). Triterpenes and cardiac glycosides were also present in detectable concentrations. In vivo studies on the efficacy of the plant leaves in treatments against fowl cough in Hubbard broilers gave positive results. The leaf extract significantly reduced the mortality rate of the broilers by 25% within 3 weeks of treatment and by 40% in 6 weeks of administration, when the broilers were 13 weeks old broilers. The number of aerobic bacteria was reduced from 36×10^2 cfu/ml to 8×10^2 cfu/ml of viscera suspension within 3 weeks of treatment. Also the number of pleuro-pneumonia like organisms (PPLO) was reduced from to 12 x 10^2 cfu/ml to 4×10^2 cfu/ml of viscera suspension within 3 weeks of treatment. The *in vivo* antibacterial potency of the plant extract may be ascribed to the presence of antioxidative compounds like saponins, alkaloids, tannins, triterpenes and cardiac glycosides in the plant leaves. @JASEM

Gongronema latifolium (Asclepiadiaceae) is a wild climber widely distributed in the southeastern states of Nigeria. Apart from being used as bitter spice or flavouring agent in many traditional Nigerian dishes (Anaso and Onochie 1999) the plant leaves has been found very efficacious as an antidiarrhoea and antitussive (Sofowora 1982 and Iwu 1993), which confirmed the speculations made by local poultry farmers that uses it for the treatment of common respiratory diseases (CRDs) associated with broilers and laying birds in the tropics. CRD in poultry is caused mainly by a pleuro-pneumonia like organisms, Mycoplasma (Jasper 1990) although other bacteria such as the β haemolytic streptococci, Pseudomonas and Staphylococcus aureus have also been associated with tussis in animals (Thomas 1979 and Paul et al., 1982). This work was carried out to investigate the antioxidants and antitussive properties of G. latifolium.

MATERIALS AND METHODS

Collection and Preparation of Leaf Samples: Leaf samples of *G. latifolium* were collected from mature fruiting climbers in the tropical rainforest of the Niger Delta. The leaves were placed in a flat basket and sun dried for 72 hours under a mean diurnal temperature of $33.2 \pm 1^{\circ}$ C, to avoid the escape of volatile components by oven-drying. The dry leaves were then ground with a Christy-Norris hammer mill to pass through a 1mm sieve to obtained a fine powder which was stored in a clean dry airtight glass bottle at ambient temperature until analysed.

Extraction and Phytochemical Analysis of Leaf Samples: Preliminary phytochemical analysis of the petroleum ether extract of the leaves was carried out according to the methods outlined by Harbone (1973) and Trease and Evans (1989). Both petroleum ether and aqueous extracts were prepared. About 500g of the fine sample powder was successively extracted with petroleum ether at room temperature. The extract was concentrated under pressure to yield 40g of petroleum ether extract. In preparing the aqueous extract about 150g of the dry sample powder was boiled in 1.5L distilled water for 11/2 h. The resultant mixture was then decanted and filtered through a Whatman filter paper No.1. The filtrate was evaporated to dryness on a hot plate at an initial temperature of 100°C and the dry powder obtained was suspended in 10ml distilled water, stirred and refiltered. The concentration of the extract was determined in grams equivalent of the dried leaves per ml and the solutions were then stored at 4^oC until ready for use.

Screening for cardiac glycosides, phlobatannins, tannins and triterpenes were done by picric acid, hydrochloric acid, ferric chloride and sulphuric acid tests respectively. The saponnis was detected by ammonia test, the alkaloids by Keller – Williams and Dragendroffs tests, while the polyphenols was detected by the ferric chloride and potassium iodide tests. Quantitative measurement of total crude sapogenins was by the gravimetric method of Brain *et al* (1968). The Keller-Killiani test for 2-deoxy sugars (AOAC 1975) was used for the assay of the cardiac glycosides, with degoxin as standard.

Determination of Antitussive Properties of G. latifolium leaves: Sixty 7 week-old cough infested Hubbard broilers weighing 1.5g were weightmatched and carefully picked from a stock of 800 birds. The birds were divided into three groups (A, B, C) of 20 broilers. Each bird was caged separately under standard poultry farm conditions and fed with standard diets and water *ad libitum*. Two sets (group A and B) of the sick birds were treated with the aqueous extract of *G. latifolium* leaves administered orally through the drinking water. The third set (group C) of sick birds which served as the control were not treated with the leave extract. The mortality rates of treated birds in group A and those in group C were determined weekly for $1\frac{1}{2}$ month. Survivors of the tussis attack were examined every week for weight loss or gain. Treatments were analysed for significant differences using Chi-square analysis for mortality and linear contrast analysis of variance for weight loss (Sokal and Rolf, 1969). The second set of treated birds (group B) served as samples for bacteriological studies.

Bacteriological Analysis of Sick Bird's Trachea: The *in vivo* effect of the extract treatment on the bacteria associated with tussis in poultry was also examined. During the bacteriological analysis the sick birds were killed and the neck cut off and dissected under total asepsis to obtain the trachea. The trachea itself was cut open to obtained the viscera. Suspensions of the viscera were prepared in sterile distilled water and diluted once to obtain 10^2 dilution levels. The bacterial load and quality of the sick birds trachea were determined by the pour and spread plate techniques (FDA, 1984) using Bacto-nutrient agar (NA) and Bacto-pleuropneummonia like organisms (PPLO) agar. The NA was used for enumeration and isolation of aerobic bacteria while PPLO agar was used for the enumeration and isolation of Mycoplasma. Inoculated agar plates were incubated at 37°C for 48 hours. Afterwards the colonies found on the agar plates were enumerated. Representative colonies of the pathogens were isolated, purified and identified by their cultural, morphological and biochemical properties as described by Cowan (1985).

RESULTS AND DISCUSSION

The results of the phytochemical analyses presented in Table 1 revealed the presence in detectable concentrations, of saponins, tannins, alkaloids, terpenes and cardiac glycosides in the petroleum ether extract of *Gongronema latifolium* leaves. Anthroquinones, polyphenols and phlobatannins were not detected while flavonoids occurred in trace amount. These are antioxidants with great antibacterial potency (Trease and Evans, 1989).

The effects of the aqueous extract of the plant leaves on the tussis infected Hubbard broilers over a 6-week period are shown in Figures 1 and 2. The antitussive potential of the plant has been established (Fig.1). The leaf extract significantly reduced the mortality rate of the broilers by 25% within 3 weeks (at 10 weeks old) of treatment and by 40% in 6 weeks, when the broilers were 13 weeks of age. These coincide with the reduction in the number of bacteria in the respiratory system of the broilers. The number of aerobic bacteria in the trachea was reduced from 36×10^2 cfu/ml of viscera suspension to 8×10^2 cfu/ml within 3 weeks of treatment. The number of pleuro-pneumonia like organism was also reduced from 12×10^2 cfu/ml to 4×10^2 cfu/ml within 2 weeks of treatment, and totally eliminated within 3 weeks of administering the extract.

The pleuro-pneumonia like organism (PPLO) particularly the Mycoplosma species and the bacteria including aerobic klebsiella and Pseudomonas species isolated from the bird's trachea have been implicated in bird's respiratory diseases (Jawetz et al., 1984). Other aerobic bacteria isolated. *Staphylococcus* aureus. Streptococcus and Corynebacterium species are known etiological agents for human tussis (Jawetz et al., 1984), which may also contribute to fowl cough (Jasper, 1990). The most affected of all the isolates in vivo were Mycoplasma, Corynebacterium, Klebsiella and Pseudomonas species (Table 2). Some of which were easily eliminated from the bird's respiratory system. This is a pointer to the fact that G. latifolium may be effective in the treatment of cough caused by the listed pathogens, although its efficacy duration may be longer than the conventional antibiotics used for the treatment of fowl cough. The remarkable antibacterial potency of the plant leaves may be ascribed to the synergistic actions of antioxidants (saponins, alkaloids, tannins, flavonoids, cardiac glycosides, and stero/triterpenes) in the plant leaf extract.

The antimicrobial properties of flavonoids, saponins and alkaloids have been established (Trease and Evans, 1989). However, the apparent slow action of the plant extract may be attributed to presence of constituents (proximate the components) other than antioxidants which might have retarded the potency of the extract. Staphyloccocus aureus was noticeably the most stubborn and prevalent of the bacteria species isolated from the birds trachea. The susceptibility of Klebsiella, Corvnebacterium and Mycoplasma species may be ascribed partly to the weak competitive parasitism of the bacteria and to the age-dependent increase in the resistance of the broilers. All the survivors (broilers) exhibited variable loss in weight (Fig.2). The untreated birds recorded a significant loss in weight while birds treated with the plant extract regained loss weight within 5 weeks of treatment. The gain in weight may be as result of a reduction in the physiological effect of the disease on affected birds and increase in the metabolic activities of the birds. The ability of Gongronema latifolium leaves extract to check the proliferation of pathogenic bacteria and the elimination of Mycoplasma species in the trachea of the sick birds have justify its use by local

farmers in controlling fowl cough. This has also confirmed its antitussive and antidiarrhoeal efficacy in humans. However, the present knowledge of the chemical properties of the leaf extracts makes it difficult to identify with certainty

the actual antitussive component of the plant leaves. Further research is therefore necessary to isolate and identify the active components and their antibacterial specificity for higher efficacy and safety in rural poultry management.

Table 1: Phytochemisty of Gongronema latifolium leaves

Property	Observation	Remark	Concentration			
	(precipitate, ppt)		(mg/100g) dry seeds			
Saponins	Persistent foam	++	0.69mg/100g			
	Brown ppt.	++	0 0			
Tannins	Green ppt	++	0.0435mg/100g			
Poly phenols	No ppt	-				
Alkaloids	Green ppt	++	0.046mg/100g			
	Red ppt	++				
Phlobatannins	No ppt	-				
Flavonoids	Faint-yellow ppt	+				
	Faint-yellow ppt	+	0.016mg/100g			
Anthroquinones	Yellow ppt	-				
Sterol/triterpenes	Pink ppt	++				
Cardiac glycosides	Reddish brown ppt	++				

Present in trace amount +++

Absent

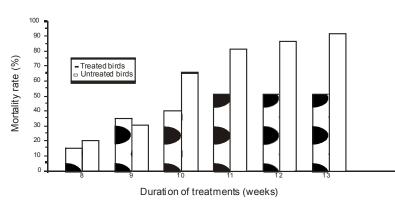


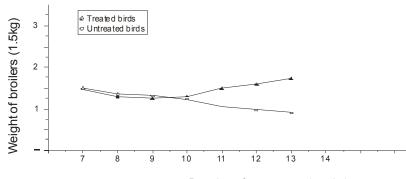
Fig. 1: Mortality rate of tussis infected broilers treated with G. latifolium leaf extract.

Table 2: Bacteriological properties of the sick Hubbard broiler's trachea

Properties		Ag	e of bii	rds (we	eks) Ui	ntreated	birds							
-		-		(cc	ntrol)					Т	reated	oirds		
	7	8	9	10	11	12	13	7	8	9	10	11	12	13
Aerobic count (cfu 10 ² /ml)	36	36	39	40	38	42	52	36	24	15	8	11	10	11
Count of PPLO (cfu x 10 ² /ml)	12	12	15	17	16	19	18	12	11	4	0	0	0	0
Isolates														
Corynebacterium sp	+	+	+	+	+	-	-	+	+	+	+	-	-	-
Klebsiella sp	+	+	+	+	+	-	-	+	-	-	-	-	-	-
Mycoplasma sp	+	+	+	+	+	+	+	+	+	+	-	-	-	-
Pseudomonas sp	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Streptpcoccus sp	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Present; - = Absent or present in an undetectable number

Remarkably present



Duration of treatments (weeks)

Fig. 2: Changes in weight of sick broilers treated G. latifolium leaf extract

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