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Nutritional, Phytochemical Properties and Antibacterial Potential of Lantana Camara Against Methicillin-Resistant *Staphylococcus aureus*

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major concern in clinical medicine due to the importance of β-lactams in the therapy of staphylococcal infections. MRSA strains have become a yardstick for the test of new antibiotics because of their ability to respond to newly developed antibiotics. This research was designed to investigate the antibacterial potential of *L. camara* leaves on MRSA isolated from pigs. Also, Nutritional and phytochemical constituents were analysed, so as to hypothesise whether *L. camara* can serve as feed component and simultaneously as prophylaxis against MRSA. Non-flowering *L. camara* leaves were collected, washed and the extract was obtained through aqueous extraction. Nutritional parameters, phytochemical constituents and Antibacterial potential of *L. camara* leaves were obtained using standard procedures. MRSA isolates were obtained from the anterior nares of apparently clinically healthy pigs using sterile cotton swabs. The result of proximate analysis using the AOAC method showed dry matter composition of (23.2±0.01%), crude fiber (20.9±0.03%) crude protein (20.2±0.02%), ether extract (4.3±0.03%) and ash (2.3±0.03%). Quantification of the phytochemical component showed the concentration (mg/L) of the following in the order: saponin>tannins>flavonoids>phenol>terpernoids for the oven dried-leaves and saponin>tannins>terpernoids>flavonoids>phenol for the air dried-leaves. *Lantana camara* aqueous leaf extract also had some bactericidal properties against MRSA isolates as it reduced the MRSA population size from 3.1×10^5 CFU/mL at day zero (0) to <1 CFU/mL in day 5. It can be concluded that *L. camara* leaves could be used as feed component and at the same time as prophylaxis against MRSA infections.

Key words: *Lantana camara*, nutritional, phytochemical, antibacterial, methicillin-resistant, *Staphylococcus aureus*

INTRODUCTION

The emergence and widespread of antibiotic resistance to new antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates *et al*., 2002). Confronted with a possible shortage of new antimicrobials, there is need to ensure a careful use of available drugs. This has led to calls for controlled use of antibiotics through the reduction of dosage used per regime of treatment or by regulating prescriptions in areas such as animal husbandry (Serrano, 2005). Reduced use of these antibiotics could lead to delayed antibiotic resistance. This means that the emergence of resistant strains is from an evolutionary viewpoint inevitable. It becomes imperative, therefore, that alternative approaches are explored. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids which have been found in vitro to have antimicrobial properties (Lewis and Ausubel, 2006).

One of such plants with speculated antibacterial activity is *Lantana camara*. Lantana is a genus of about 150 species of perennial flowering plants popularly used as antirheumatic, stimulant, antibacterial, biological control of insects and as ornamental plant (Ghisalberti, 2000). Phytochemical studies of Lantana species has led to the identification and isolation of terpenoids, flavonoids, phenylethanoid glycosides, furanonaphthoquinones, iridoid glycosides and steroids (Ghisalberti, 2000; Verma *et al*., 1997). The extracts of the flower, leaf, root and stem of *L. camara* showed antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus saprohiticus* (Kensa, 2011).

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One organism that has gained attention in recent years because of antibiotic resistance is Methicillin-Resistant *Staphylococcus Aureus* (MRSA). MRSA has been a threat for public health for decades, causing severe nosocomial and community infections that are generally, difficult to treat due to the multi-drug resistance of the bacterium. It is therefore, imperative to investigate the antibacterial potential of *L. camara* leaves in order to hypothesise whether *L. camara* leaves can be used as animal feed and simultaneously as prophylaxis against MRSA.

MATERIALS AND METHODS

Collection and preparation of aqueous extracts of *L. camara* leaves: Non-flowering *L. camara* plants were located and obtained from the Technology Village of the University of Cape Coast. Healthy leaves were selected, washed in running tap water to remove the surface contaminants and dust particles. Some of the leaves were dried at room temperature (27°C) for three days in shade and others oven-dried at 60°C for two days. After drying, the leaves were ground into powder using electric blender. Stock aqueous extract was obtained by soaking 50 g leaf powder in 500 mL of distilled water, boiled at 60°C for 30 min and was centrifuged at 5000 rpm for 10 min. Supernatant obtained after the centrifugation was filtered through a filter paper. These extracts (stock) of full strength concentration (100%) was diluted with distilled water to prepare different concentrations (20 and 50%) according to the treatments.

The rest of the powdered leaves were stored at 4°C until it was ready to be used for the phytochemical analysis.

Phytochemical screening of *L. camara* dried leaves: The *L. camara* leaves were screened for the presence of phyto-constituents (quantitatively and qualitatively) using the procedures by Sofowara (1993).

Proximate Analysis of oven-dried Leaves of *L. camara*: The dry matter, crude protein, ether extract, crude fibre and ash contents of the oven-dried *L. camara* leaf were determined as described by AOAC (2000).

Mineral determination: The determination of the levels of inorganic minerals of the pulverised *L. camara* leaf was carried out by acid digestion using nitric acid and perchloric acid mixture (HNO: HClO, 5:1 w/v). The total amounts of K, P, Ca, S, Cu, Mg, Fe and Zn in the digested samples were determined by atomic absorption spectrophotometry (Onwuka, 2005).

Isolation of methicillin-resistant *Staphylococcus aureus*: Swabs were taken from the anterior nares of pigs using sterile swabs in liquid transport medium (ESwab, Copan, Brescia, Italy) and transported to the laboratory on ice. The swabs were streaked onto selective MRSA agar plates (ORSAB, OXOID, UK). These plates were incubated for 24-48 h at 37°C and examined for discrete MRSA isolated colonies which on MRSA agar plates were distinct blue, elevated round edged colonies.

Testing the antibacterial properties of *Lantana camara* aqueous extracts: Serial broth dilution assay was used to assess the survival of MRSA in aqueous extracts of *L. camara*. A single colony of MRSA was inoculated in 9 mL peptone water which served as the stock. Initial number of MRSA in the stock solution was obtained by serial dilution, pour plate method and incubating the plates at 37°C for 24 h in a static state. About 1 mL of the stock was pippeted into 9 mL of the various concentrations of aqueous extracts (100, 50 and 20%) of oven and air-dried *L. camara* leaves. The 9 mL Peptone water plus 1 mL of the stock served as control. Everyday for the 5 day period of the experiment, colony forming units were counted using electronic colony counter. The survival of MRSA in all the treatments for the entire study period was obtained by serial dilution, pour plate method and incubating the plates at 37°C for 24 h in a static state. The experiment was duplicated to obtain a fair scientific representation of the work.

RESULTS AND DISCUSSION

Nutritional and phytochemical constituents of *L. camara*: The results of proximate analysis of the *L. camara* leaves are presented in Table 1 and 2. There was generally high percentage proximate fractions of *L. camara* leaves in terms of crude protein, crude fiber, ether extract and ash.

The phyto-constituent of aqueous extracts of the leaves of *L. camara* was qualitatively and quantitatively analysed and presented in Table 3. The extracts demonstrated some occurrence of phyto-constituents such as flavonoids, phenol, tannins, saponins and terpenoids. Alkaloids, anthraquinones, glycosides and carotenoids did not test positive in the current study. In total, five out of nine phytochemicals were present in both oven dried and air dried aqueous extracts of *Lantana camara* leaves. Statistically, it can be seen that there was a significant difference between the quantities of the various phytochemicals in the oven dried leaves and that of the air dried leaves ($p<0.01$).

Antimicrobial potential of *L. camara*: Antimicrobial activities of the plant extracts (aqueous) showed bactericidal activities against MRSA isolates with the oven dried extracts recording the highest effect. Initial MRSA number observed in the stock reduced drastically from 3.1×10^5 CFU/mL in day zero to <1 CFU/mL in day 5 when the *L. camara* extracts were introduced (Table 4). In general, it was seen that lower concentrations of *L. camara* had greater antibacterial effect than higher concentrations of *L. camara* aqueous extracts.

Biological activity in many plant tissues is attributed partly to the presence of proteinaceous substances in their tissues (Ghongade, 2013). This study showed an optimum level of protein in the leaves of *L. camara* and it could be utilised as a supplementary source of protein in animal diets. The level of protein in the leaves of *L. camara* compared favourably well with a few proteinaceous plants including cowpeas 24.7% (Igbal *et al*., 2006), pigeon pea 20.4% (Nwokolo, 1987), lima bean and bambara groundnut 23-26% (Olaofe *et al*., 1993). Crude fiber content also helps to maintain the motility of food through the gut and may be broken down by some bacteria in the gut to provide energy. The ash content is generally recognised as a measure of quality for the assessment of the functional properties of foods (Hofman *et al*., 2002). The ash content is also a reflection of the mineral contents preserved in the leaf. The ether extract will help provide energy while the moisture content will reflect shelf-life of the plant. The significance of the presence of these elements suggest that the leaves of *L. camara* could also be a good source of the aforementioned minerals

The existence of the anti-nutritional factor, tannins which are known to have various deleterious effects, ranging from reduction in feed intake, reduction in bioavailability of minerals to causing death of animals (Osagie and Eka, 1998) is an indication of the limitation of the use of the leaf as protein source in animal diets. Hence, for *L. camara* to be used as a source of protein and minerals in animal diets, it will be necessary that the plant be subjected to processing techniques or a regulation of its percentage in a feed formulation. This will either reduce or eliminate its anti-nutritional factors.

This study confirmed the fact that *L. camara* contains some major phytochemicals that have some bactericidal and bacteriostatic activity. The amounts of phytochemicals obtained in this study are lower compared to previous studies by Ambasta (1986) and Kiruba *et al*. (2011) who reported better extraction rates for several bioactive compounds using ethanol as the solvent. The rational for aqueous extraction was to find what an ordinary farmer can use at his or her level as opposed to total extraction which is a laboratory procedure. In this study, though some of the bioactive compounds were found in abundance, others were in trace amount. High

Table 1: Proximate composition of the leaves of oven-dried *Lantana camara*

Proximate composition	Percentage	SEM
Dry matter	23.2	0.01
Crude protein	20.2	0.02
Crude fibre	20.9	0.03
Ash	2.4	0.03
Ether extract	4.3	0.03

Table 2: Mineral Constituent of oven-dried *Lantana camara* Leaves

Minerals	Composition (µg/g)	SEM
Phosphorus (P)	2243.60	1.73
Potassium (K)	16847.00	1.16
Sodium (Na)	6672.80	1.16
Iron (Fe)	1233.70	1.45
Copper (Cu)	554.90	1.73
Zinc (Zn)	70.70	0.58
Calcium (Ca)	2.10	0.09
Magnesium (Mg)	0.03	0.01

Table 3: Qualitative and quantitative phytochemical screening of *Lantana camara* leaves

	Lantana camara			
Phytochemicals	Oven dry(mg/L)	Air dry(mg/L)	SED	Sig.
Terpenoids	1.45^a	2.91^b	0.01	***
Flavonoids	4.12^b	1.23^a	0.01	***
Tannins	45.78^b	43.86^a	0.01	***
Saponins	56.76^a	59.90^b	0.06	**
Phenol	1.46^b	0.74^a	0.01	***
Alkaloids	----	----	----	----
Anthraquinone	----	----	----	----
Glycoside	----	----	----	----
Carotenoids	----	----	----	----

*** = p<0.01; ** = p<0.05; Means in a row with different letter superscripts are significantly different (p<0.05); ---- = Not detected qualitatively

Table 4: Quantitative Effect of Aqueous leaves extracts of *L. camara* on MRSA isolates

			Oven dried			Air dried		
Days	A	Con.	100% (CFU/mL)	50% (CFU/mL)	20% (CFU/mL)	100% (CFU/mL)	50% (CFU/mL)	20% (CFU/mL)
0	3.1×10^5	3.1×10^5	3.1×10^5	3.1×10^5	3.1×10^5	$3.1\times10^5\times10^5$	3.1×10^5	3.1
1		6.0×10^7	1×10^5	1.8×10^5	2.5×10^5	1.6×10^5	7×10^4	5×10^5
2		7.2×10^7	7×10^4	9×10^4	1.2×10^5	9×10^4	5.5×10^4	3×10^4
3		8×10^7	3×10^3	2×10^4	3×10^3	1.5×10^5	2×10^4	3×10^3
4		8.2×10^7	2.5×10^3	5×10^3	1×10^3	6×10^4	7×10^3	1.5×10^3
5		5.1×10^5	<1	<1	<1	<1	<1	<1

A = Initial colony number; CFU = Colony Forming Unit, Con. = Control

amount of these phytochemicals were found in the oven dried leaves of *L. camara*. The higher phytochemical content of the oven dried samples can be partly attributed to the rapid inactivation of enzymes (Lim and Murtijaya, 2007). Oven drying may be a good method for drying and preserving phytochemicals in plants in that it can be completed in a shorter time and under more closely monitored conditions than the other drying methods. The drying of herbs inhibits microbial growth on them and forestalls biochemical changes but at the same time, it can give rise to other changes that affect the herb quality. In addition, the drying of herbs is often accompanied with the loss of bioactive compounds, although some of the phytochemicals are more thermo stable (Bravo, 1998).

In this study, it was seen that MRSA isolates were sensitive to *L. camara* aqueous leaves extracts. This complements the study by Kensa (2011) who predicted antibacterial potential of *L. camara* leaves against *Staphylococcus aureus*. According to Udayprakash *et al*., *L. camara* recorded better antibacterial activities than other herbaceous plants of which this study reiterates this fact. The phytochemicals present in *L. camara* may make it more successful than any pure compound against most MRSA isolates. It has been widely reported that the activities of secondary metabolites like alkaloids, saponins, tannins and cardiac glycosides might be responsible for the microbial activities against microbial infections (Okigbo *et al*., 2009). Flavonoid present in the leaves have been found in-vitro to be effective antimicrobial substances against wide array of microorganisms. They do this by breaking the bacterial cell walls (Rauha *et al*., 2000). Savoia (2012) reported that phenols are widely distributed in plants and they are toxic to micro-organisms. This may have accounted partly for the bactericidal activity of the leaves of *L. camara* in this study.

Although, the bactericidal activity of the extracts varied between the oven dried extracts and that of the air dried extracts, it was observed that, in both instances, as the concentrations decreased, the antimicrobial potential of the extracts increased. The difference in the activity of the oven and air dried extracts may be partly attributed to the differences in the amount of phytochemicals. Annan-Prah *et al*. (2012) also reported lower concentrations of aqueous extracts of goat weed (*Ageratum conyzoides*) as more effective though the plant was used as antiprotozoan agent. The reason for this is still not understood but is suggested to be attributed to the high ionization of the extract as one dilutes the absolute (100%). We should, however not lose sight of the fact that the effectiveness of a bactericidal agent also depends on the initial population size. In Table 4, despite the same inoculum size, the decrease in population of MRSA varied with days. The antibacterial activity of *L. camara* leaf extract against MRSA isolates implies that the plant contains bioactive compounds that can be developed as alternative to synthetic drugs. This work is purely academic research as it lacks toxicity studies with animals.

CONCLUSION

It can be concluded that *L. camara* leaf is rich in nutrients such as protein and minerals which can be used partly as a source of nutrients in animal diets. Although, tannins may serve as an anti-nutritional factor, the presence of phenols, terpenoids and flavonoids affirms the plant as medicinal plant and a potential source of antimicrobials against MRSA population. It is concluded that *L. camara* could be guardedly used simultaneously as animal feed and as a prophylaxis against MRSA.

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