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Analysis of Some Elements and Antimicrobial Activity of Evaporated Extract of Cow Dung Against Some Pathogens

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Abstract

The evaporated extract of cow dung is traditionally used in Northern Nigeria and Cameroun as food additive and in treatment of infectious diseases. In this study, the cow dung ash extract was prepared and tested for some elemental contents as well as the antimicrobial activity against *Cyanobacteria* (*C.bacteria*), *Staphylococcus aureus* (*S.aureus*), *Bacillus subtilis* (*B.subtilis*) *and Escherichia coli* (*E.coli)* using different analytical techniques. The extract was highly basic with pH of 11.7 and the elements vary in the following decreasing order of concentration; K>Na>Mg>Ca>Fe>Al>Zn. *S. aureus* was the most sensitive bacteria with minimum inhibitory concentration (MIC) value of 0.082 mg/mL while *B. subtilis* was the least sensitive with MIC value of 4.3 mg/mL. The result of this study indicate that the extract can supplement the dietary Na and K requirements for the users and supports the folkloric use of the extract in treatment of infections.

Keywords: Antimicrobial activity; Cow dung; Food additive; Infections; Physicochemical properties.

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1. Introduction

Folkloric medicine describes the traditional medicinal knowledge which developed over centuries within various countries and societies which have been used for treatment of illnesses and infections far before the era of modern medicine. Different parts of plants, stones, animal's fat, oil and wastes have been used by traditional healers in treatment of different categories of diseases with great success.

The Fulani are known in Northern Nigeria and other parts Africa for cattle rearing and they usually travel far and wide in search of grazing lands for their animals. Though they are not known for traditional healing but they use the cow dung ashes to treat wounds and infections in the course of their journey. The animals also provide them with milk, meat, cheese and fire from the dried animal dung.

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Cow dung is the waste excreted by cows which consists of undigested residues of consumed matter which has passed through the gastrointestinal system of the animal. The faecal matter ranges in color from greenish to black and it darkens on exposure to air. The dung has been used as organic fertilizer and in the production of biogas to generate electricity and heat [1]. Cow dung has also been used as foods for fungal and animal species which breaks it down and recycle it into the food chain as well as into the soil [2]. Studies have shown how dung beetles were used in recycling cattle dung back into the soil [3] while cow urine has been used as insecticides and has been reported to contain antibiotic agents [4-5]. The use of cow dung in the bioremediation of toxicants in the environment has been reported [6]. Cow dung has been identified by different names; it is referred to as cow chips or cow pit in British English while a deposit of the dung is referred to as cow pie in American English [7]. The evaporated extract of cow dung is called dalang or dalam in Northeastern Nigeria and in some parts of Northern Cameroun and has been used as soup condiment and in treatment of infections. The use of several parts of plant ashes as condiments, dietary supplements and in the treatment of various ailments have been reported [8-11]. Quite a number of antibiotics with different brands are available in the market and several antimicrobial agents of plant origin have been tested and scientifically proven to be very effective for the treatment of bacterial infections [12-14]. Furthermore, a large number of microorganisms which have biological activities and presently in use as antibiotics and antitumor agents have been reported [15]. However, the quality of herbal products is believed to depend on the active ingredients as well as method of preparation. There is also the possibility of microbes developing resistance, justifying the constant search for new, affordable, safe and effective bioactive agents of plant and animal origin. Manifestation of side effects cannot be ruled out, hence the need to explore the heavy metal contents of such preparations.

The human body requires metals and non-metals within specified limits for good health and growth. The assessment of heavy metal contents in food and related products is therefore very important in order to understand their nutritive values and their mode of action. The determination of heavy metals is also in line with the World Health Organization's recommendation which emphasized on the need to check for the presence of heavy metals in medicinal plants used as raw materials for finished products especially if they are meant for human consumption [16]. The effects of exposure to heavy metals through food or medications are usually very difficult to detect due to their gradual accumulation in the body system before manifestation.

The aim of the present study is to prepare the cow dung extract (dalang), determine the pH, the elemental composition and assess the antimicrobial potential of the extract.

2. Experimental

2.1. *Sample collection*

Cow dung samples were collected from a cow farm at Gashu'a in Yobe state Nigeria, stored in a plastic bag and taken to the laboratory for analysis.

2.2. *Preparation of extract (Dalang)*

About 200 g of the cow dung was dried to a constant weight in an oven at about 80°C and later burnt to ashes in a furnace at 600°C. The ash obtained was dissolved in 500 mL of distilled water and filtered. The filtrate was heated until crystals start to form and about 20 mL of the filtrate remains. The products were evaporated to dryness and the crystal formed is the evaporated extract of the cow dung (dalang).

2.3. *Preparation of stock solution of extract for analysis*

1 g of the extract sample was dissolved in 20 mL of distilled water and made up to 100 mL in a volumetric flask. The solution was allowed to stay overnight and filtered.

2.4. *Determination of pH and elemental analysis*

The pH of the solution was measured using a digital pH meter (Jenway model). Different portions of the solution were used for the analysis of potassium and sodium using flame photometer (model 405, UK) while all other elements were determined by Atomic Absorption Spectrophotometer (Shimadzu AA-6880). The procedures employed in these determinations were as reported in the manufacturer's manual for the equipment.

2.5. *Test organisms*

The following bacterial cultures were collected from the Department of Veterinary Microbiology, University of Maiduguri, Nigeria; *Cyanobacteria (C. bacteria.), Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis (B. subtilis) and Escherichia coli* (*E. coli*). The cultures were maintained on nutrient agar at 37ºC for 18 h and used for the antimicrobial activity test.

2.6. *Antimicrobial activity test and determination of minimum inhibitory concentration*

The antimicrobial activity was tested by the disc diffusion technique [17]. Different concentrations of the extract were prepared by reconstituting the stock with distilled water. The test microorganisms were seeded into nutrient agar medium by spread plate method. The paper was allowed to dry and the filter paper discs, now covered with the extract were placed on the test microorganism seeded plates. The plates were incubated at 37ºC for 24 h and the zones of inhibition were measured. Minimum inhibitory concentration (MIC) was determined for each concentration which shows antimicrobial activity against test bacteria by the broth micro dilution method [18].

3. Results and Discussions

3.1. *pH and Elemental analysis*

The pH of the extract was 11.7 indicating that the extract is highly alkaline. This alkaline property means that the dung has provided suitable environment for the growth of

microbes. The results of the elemental analysis of the cow dung extract are presented in Figs. 1 and 2. The levels of the elements in decreasing order of concentration were; K>Na>Mg>Ca>Fe>Al>Zn. The elements; K, Na and Mg exhibited highest concentrations as presented in Fig. 1 when compared with the other elements (Fig. 2). Elements are essential in the assimilation and utilization of nutrients in the human body and the daily intake of elements depend on both the amount and concentration of food consumed. As the human body consumes these elements through daily functioning, it has to replace them in order to stay very healthy. The greatest source of these elements is through the right foods or dietary supplements to ensure the proper maintenance of the chemical balances in our bodies. The role of metals in biochemical processes by acting as cofactors for various enzymes has been studied [19-21]. The elemental content of the extract indicates that all the elements are within the recommended dietary allowances, except for K which exceeds the recommended value [22-23]. Though the concentration of K is high but the K: Na ratio of 3:1 means that the cow dung extract (Dalang) used as food additive can provide supplementary body need of both minerals. Similar K: Na ratios of 1:1, 2:1 and 4:1 in food additives have been reported [24]. However, high levels of Na and K are not favorable for hypertensive and obese patients as high intake can lead to cardiac failure [25]. Therefore such patients should avoid 'dalang' or take it with caution to safeguard their lives. The high levels of these elements in the extract may also be responsible for the activity against the tested bacteria. Similar studies have shown that Fe, Mg, Cu and Zn inhibit the growth of some bacteria [26-27].

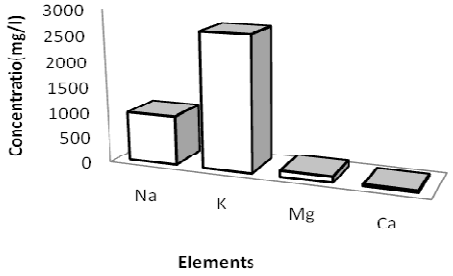

Fig. 1. The elemental composition of the cow dung extract showing predominant elements

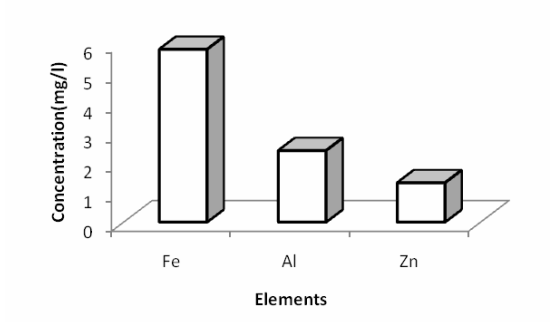

Fig. 2. The elemental composition of the cow dung extract showing other elements analyzed

3.2. *Antimicrobial activity*

The antimicrobial activities at different concentrations of the cow dung extract against Cyanobacteria, *Staphylococcus aureus, Bacillus subtilis and Escherichia coli* in terms of inhibition zone exhibited by the bacteria are shown in Table 1. Results obtained revealed that the cow dung extract posses potential antibacterial activity against some of the tested bacteria.

Table 1. Antimicrobial activity of the cow dung extract against the tested microorganisms.

[Extract] (mg/mL)	Test microorganisms			
	B. subtilis IZ	*C. bacteria* IZ	*E. coli* IZ	*S. aureus* IZ
0.20	-	-	-	-
0.35	-	7.0±0.32	-	10±0.36
0.70	-	8.0±0.22	-	11±1.54
2.50	0.5±0.01	8.5±0.43	-	13±2.25

Values are expressed as mean±SD, *n*=6, IZ= Inhibition zone , - = No activity.

Highest antibacterial activity was noted at 2.5 mg/mL concentration when compared with the other concentrations of 0.2 mg/mL, 0.35 mg/mL and 0.7 mg/mL. Maximum antibacterial activities were observed in *Staphylococcus aureus* (10 mm, 11mm, and 13 mm in diameter) and *Cyanobacteria* (7 mm, 8 mm and 8.5 mm in diameter) when compared to the other tested bacteria (Table 1). This indicates that *Staphylococcus aureus* and *Cyanobacteria* are the most susceptible organisms in the investigation as they showed significant inhibition zones at three out of the four tested concentrations, but all the tested bacteria showed no activity at 0.2 mg/mL concentration. The results are similar to earlier investigations where the extracts of *sorghum bicolor* were inhibitory against *Streptococcus faecalis* (*S. faecalis*) and *Staphylococcus aureus* (*S. aureus*) [24]. The antimicrobial activities of cow urine have been studied and the distillate showed different activities against different microorganisms similar to the test microorganisms in the present study [4]. *E.coli* showed no activity at all concentrations while *Bacillus subtilis* was observed to be the most resistant microbe because it only showed activity at high concentrations of the extract (2.50 mg/mL).

The MIC results showed that *S. aureus* was the most sensitive bacteria with MIC value of 0.082 mg/mL while *B. subtilis* was the least sensitive bacteria with MIC value of 4.3 mg/mL (Table 2). The results of the investigation clearly indicate that the cow dung extract has antibacterial activity and the low MIC values will also be helpful in establishing the antibiotic property of the extract.

Table 2. Minimum Inhibitory Concentration (MIC) of the cow dung extract against the tested microorganisms.

Extract (mg/mL)	Test microorganisms			
	B. subtilis	*C. bacteria*	*E. coli*	*S. aureus*
0.20	-	-	-	-
0.35	-	0.412±0.01	-	0.210±0.15
0.70	-	0.320±0.02	-	0.170±0.10
2.50	4.3±0.10	0.250±0.10	-	0.082±0.00

Values are expressed as mean (MIC) ±SD (standard deviation), n=6, - = No inhibition.

4. Conclusion

The elemental content of the extract indicates that all the elements are within the recommended dietary allowances and the evidence from the antimicrobial activities tentatively suggests possible benefits from the cow dung extract, supporting the folkloric use of the extract. Further research will be conducted to identify the active ingredients, compositional analysis, antifungal activity and possibly clinical trials to provide more conclusive proof of the efficacy of the cow dung extract.

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