QUALITATIVE AND QUANTITATIVE ANALYSIS OF PAWPAW (*Carica papaya*) LEAF EXTRACT AND ITS ANTIMICROBIAL EFFECT IN ANIMAL PRODUCTION

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Abstract
In other to improve livestock production and curb the losses from diseases occurrence in livestock animals, livestock producers resort to the use of antimicrobials as growth promoters which help to inhibit the growth of disease-causing organisms. Freshly harvested pawpaw leaves were extracted using three solvents: ethanol, methanol and n-hexane and their phytochemicals determined using standard procedure. The inhibitory activities of the extracts at low (200ppm) and high (1000ppm) concentrations against Aspergillus nigerand Escherichia coli were also determined. It was observed from the results that alkaloid, flavonoid, saponin, tannin and cardiac glycosides were pre-sent while anthraquinone was absent. The percentage yield of phenols using methanol (0.115%) and ethanol (0.214%) solvents were similar but lower than n-hexane yield (0.450%). Also the yield of flavonoid using methanol (0.700%) is significantly (P<0.05) higher than the yield using other solvents. The yield of phenols using methanol (0.480%) and ethanol (0.470%) solvents were identical but higher than n-hexane yield (0.400%). At low concentration, it was observed that the inhibitory concentrations of pawpaw leaf extract against bacteria by control, streptomycin (1.2cm) is significantly higher (P<0.05) but similar to the extract using methanol solvent (1.1cm). Methanol extract inhibition is also similar to ethanol (1.0cm) but higher than n-hexane (0.0cm). At high concentration, the inhibitory activity of the ethanol extract (1.2cm) was significantly (P<0.05) higher than the control (0.7cm) and the least observed in n-hexane (0.0cm) extract. The inhibitory concentrations of pawpaw leaf extract against fungi Aspergillus niger at low (2.2cm) and high (2.2cm) concentrations, the methanol extract was observed to be significantly (P<0.05) higher than other extracts including control.The results suggest that using methanol-extracted pawpaw leaf as alternatives to synthetic antibiotic in animal production is effective against microbial organisms. Thus the occurrence of resistance to antibiotic or its residues on animal products will be reduced.

Key words: Pawpaw, microbes, extraction solvents, phytochemicals, phytobiotics

Introduction
The presence of antimicrobial-resistant non-pathogenic commensal bacteria on farms is considered a problem, as it provides a pool of transferable resistance genes (Defrancesco *et al.*, 2004). Also there is the problem of residual effects of drugs on animal products as well as high cost of the drugs.However, available evidence indicates that indigenous health practices including medicinal plants are still being used to handle animal health problems in all livestock production systems (Maeda-Machang'u *et al.*, 1997, Minja, 1999). Several chemicals are found in different parts of pawpaw (*Carica papaya*) the leaves, the stems and fruits. Large amounts of latex are found in the leaves, stem and fruits (**Adam *et al.*, 2014**). The latex known as vegetable pepsin is rich in papain and chymopapain. It also has proteins like linamarase, protease inhibitors, and chinitasis. The leaves contain glycoside carposide, cardiac glycosides, tannins, flavonoids, saponins, alkaloids, anthraquinones, steroids, reducing sugars, cardenolides and phenolics compounds while the seeds contain myrosinase, caricin and sinigrin glycosides (**Adam *et al.*, 2014**).The fresh green extract act as anti septic and dried leaves are best as atonic and blood purifier (Nwofia *et al.*, 2012). There is need, therefore, to determining the phytochemical composition and investigate the anti microbial properties of*Carica papaya*leaves against pathogenic fungi (*Aspergillus niger*) and bacteria (*Escherichia coli*) using different solvents: ethanol, n-hexane, methanol extracts.

Materials and Methods
Plant collection and identification: Fresh samples of pawpaw leaves were collected at the back of the Department of Animal science, Faculty of Agriculture and Forestry, University of Ibadan.
Experimental area: The extractions, phytochemicals analysis and antimicrobial activities were carried out at the Department of Animal Science laboratories, University of Ibadan.
The extraction process: The leaves were cleaned with tap water and later with distilled water, then chopped on a cutting board before loading into the soxhlet apparatus. Three solvent (methanol, ethanol and n-hexane) were used for the extractions.

Phytochemical screening methods: A portion of the concentrated extract was used for the screening tests, both qualitative and quantitative analyses using standard procedure as described by Edeoga *et al.* (2005).

Preparation of bacterium and fungus seeded plates: Prepared NA and PDA that has been sterilized were poured into sterile petri- dishes at 45°C. The pathogenic organisms of interest (*Echerichia coli* and *Aspergillusniger*) were then picked and spread thinly and evenly on the prepared NA and PDA plates using a flame cork borer of 5mm diameter size, 6 holes were punched into the seeded plate (one in each hexagonal of the plate). The Agar plugs were trapped in the cork-borer which was emptied into disinfectant. The cork borer was flamed before it was used for another organism inside another petri-dish and the plugs were removed and emptied into disinfectant solution before discarding and 200 ppm (low) and 1000 ppm (high) of extracts concentrations were used in the puncture holes for inhibitions

Measurement of zone of inhibition: The diameter of the zone of inhibition or clear zone surrounding the well was measured using a ruler. The readings were taken in triplicate as suggested by Zaria *et al.* (1995). The average was taken to represent the zone of inhibition.

Statistical analysis: Data obtained were subjected to analysis of variance using SAS (2008). Means were separated using Duncan's Multiple Range Test (DMRT) of the above software package

Results and Discussion

The result of qualitative analysis of phytochemical constituents of pawpaw leaf extract is shown in Table 1. The pawpaw leave extracts using methanol, ethanol and n-hexane solvents have tannin, saponin, phenol, cardiac glycoside, flavonoids and alkaloids. The methanol extract has shown to contain more saponin (++) than the ethanol and n-hexane extract. Meanwhile none of the solvent extracts has anthraquinone. The presence of tannin, saponin, flavonoids, alkaloids, phenols in the ethanol, methanol and n-hexane extract of pawpaw leaves is in agreement with research work by Doughari (2006) while the absence of anthraquinone in the present study contradicts the findings of Doughari (2006) but in accordance with the research work carried out by Ajani *et al.* (2013). Isela *et al.* (2014) reported that the presence or absence of metabolites may be due to differences in polarity of the solvents used for the extraction.

The quantitative analysis of phytochemicals constituents of pawpaw leaves is reported on Table 2. The percentage yield of phenols using methanol (0.115%) and ethanol (0.214%) solvents were similar ($P>0.05$) but lower than n-hexane yield (0.450%). Also the yield of flavonoid using ethanol (0.500%) is similar to n-hexane (0.480%) but significantly ($P<0.05$) lower than the yield using methanol (0.700%). The percentage yield of phenols using methanol (0.480%) and ethanol (0.470%) solvents were similar ($P>0.05$) but higher than n-hexane yield (0.400%). Brun *et al.* (1993) concluded that the quantity of chemical substances varies in the fruit, latex, leaves, and roots and varies with the extraction method, age of the plant part, and the cultivar and sex of the tree.

The inhibitory concentrations of pawpaw leaf extract against bacteria are shown on Table 3. At low concentration, the zone inhibited by control, streptomycin (1.2 cm) is significantly higher ($P>0.05$) but similar to the extract using methanol solvent (1.1cm). Methanol extract inhibition is also similar to Ethanol (1.0 cm) but higher than n-hexane (0.0 cm).

At high concentration, the inhibitory activity of the ethanol extract (1.2 cm) was significantly ($P<0.05$) higher than the control (0.7 cm) and the least observed in n-hexane (0.0 cm) extract.

The results from Table 4 showed the inhibitory concentrations of pawpaw leaf extract against fungi *Aspergillus niger*. At low (2.2 cm) and high (2.2 cm) concentrations, the methanol extract was observed to be significantly ($P<0.05$) higher than other extracts including control. Thus, the methanol extract has the highest inhibitory activity against *Aspergillus niger*.

From these results, the methanol extracted pawpaw leaf, at a low concentration was observed to have better inhibitory activity against the test bacteria and fungus. The result of the methanolic extract is in compliance with the findings of Anibijuwon and Udeze (2009) that the methanolic extract of pawpaw leaf has a high antimicrobial property at low concentration.

The n-hexane extract did not inhibit any antibacterial activity at low and high concentration which means that n-hexane extraction of pawpaw leaf is not effective against the test bacterium and fungus. However this result contradicts the findings of Chandra *et al.* (2011) who reported that the n-hexane extract of pawpaw leaves possess antibacterial activities. This may be because of the hot extraction used in this study as against the cold extraction used in the study by

Chandra *et al.* (2011).

Conclusion

The results suggest that using methanol-extracted pawpaw leaf as alternatives to synthetic antibiotic in animal production is effective against microbial organisms at low (200 ppm) concentration. Thus the occurrence of resistance to antibiotic or its residues on animal products will be reduced.

Demonstration of antibacterial activity against the test isolates is an indication that there is possibility of sourcing alternative antibiotic substances and scientific bases for the local usage of pawpaw plant extracts for the development of newer antibacterial agents.

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Table 1: The results of qualitative analysis of phytochemical constituents of pawpaw leaf extract

Phytochemical	Methanol extract	Ethanol extract	n-Hexane extract
Tannin	+	+	+
Saponin	++	+	+
Phenols	+	+	+
Anthraquinones	-	-	-
Cardiac glycosides	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+

+ means the presence of the phytochemicals; - means the phytochemical is absent

Table 2: Results showing quantitative analysis of pawpaw leaves of pawpaw leaf extract

	Percentage yield (%)		
Phytochemicals	Methanol extract	Ethanol extract	n-Hexane extract
Phenols	0.115 ± 0^{b}	0.214 ± 0.001^{b}	0.450 ± 0.01^{a}
Flavonoids	0.700 ± 0.05^{a}	0.500 ± 0.005^{b}	0.480 ± 0.005^{b}
Alkaloids	0.480 ± 0.01^{a}	0.470 ± 0.01^{a}	0.400 ± 0^{b}

^{abc} mean with different superscript are significantly different ($P<0.05$)

Table 3: Inhibitory concentrations of pawpaw leaf extract against bacteria

	Zone of inhibition (cm)	
Extract	low concentration	high concentration
Methanol	1.1 ± 0.01^{ab}	0.4 ± 0^{c}
Ethanol	1 ± 0.1^{b}	1.2 ± 0^{a}
n-Hexane	0 ± 0^{c}	0 ± 0^{d}
Control	1.2 ± 0.1^{a}	0.7 ± 0.1^{b}

**Control is streptomysin*

Table 4: Inhibitory concentrations of pawpaw leaf extract against fungi

Extract	low (cm)	high (cm)
Ethanol	1.6 ± 0.05^{c}	1.8 ± 0.2^{c}
Methanol	2.2 ± 0^{a}	2.2 ± 0^{a}
n-Hexane	0 ± 0^{d}	0 ± 0^{d}
Control	1.6 ± 0^{b}	1.8 ± 0^{b}

*Control is Copper sulphate