

Assessment of *Samanea saman* Whole Pod Extract as an Antimicrobial Agent and its Effect on Chicken Patties

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ABSTRACT

Purpose: *Samanea saman* whole pod (SSWP) has a potential to be used as an ingredient in broiler feed while its pod extract has antimicrobial properties. There is however, insufficient information on the use of *Samanea saman* whole pod extract (SSWPE) as antimicrobial agent in meat products. This study was aimed at assessing the antimicrobial effect of SSWPE against bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Streptococcus pyogenes*, *Salmonella typhi*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* and assessing the potential of using SSWPE on chicken patties in order to reduce the microbial contaminants.

Research Method: Agar well diffusion method and Minimum inhibition concentration (MIC) method were used to determine the antimicrobial activity in the pod extract. In order to investigate the antibacterial effect of *S. saman* extract in chicken patties, total plate counts and selective plate counts (to enumerate *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*) were carried out on refrigerated chicken patties stored for 1, 7 and 14 days. The data generated from the study were subjected to one-way analysis of variance (ANOVA).

Results: The result of Agar well diffusion method showed that the SSWPE was active against all the tested bacteria with varying mean zones of inhibition. *S. saman* ethanolic whole pod extract has shown the 25 µg/ml as the minimum inhibition concentration against both Gram positive and Gram negative bacteria used in the study when compared to the standard Ciprofloxacin at 1 µg/ml. The total microbial counts of the chicken patties with various treatments showed increase in total microbial counts with increment of the time of storage from day 1 to 14. *Salmonella* and *Pseudomonas* were absent in chicken patties at the day 1 and in other two sampling points (day 7 and 14) whereas *Escherichia coli* was present at day 1 in T4, T5 and in day 14 in T5.

Original Value: Thus the extract of *S. saman* whole pods has a potential of serving as antimicrobial agent at the minimum inhibition concentration of 25 µg/ml.

Keywords: Antibacterial, Antimicrobial, Chicken patties, Ethanolic extract, *Samanea saman* pod

INTRODUCTION

The flesh of animals called meat is very nutritious, rich in protein and other nutrients but is highly perishable and has short shelf life unless they are preserved. Microbial growth is a major challenge in areas where the ambient temperatures are high and food preservations become a problem. Poultry meat has high degree of unsaturation of phospholipids making it readily spoilt by microorganisms.

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Microbial growth is a major cause of meat quality deterioration and this determines the shelf life of meat and meat products. Thus to prevent economic loss (food poisoning and meat spoilage) and extend shelf life of the meat, certain agents with antimicrobial activities can be used to retain the products nutritional quality and flavour during storage (Yin and Cheng, 2003). It is necessary to control microbial growth in meat products because it has serious implications on human health and public health hazards (Aguirrezabal *et al.*, 2000). Komba *et al.* (2012) reported that meat being a good source of protein, fat and has adequate water activity encourages the growth of spoilage and pathogenic microorganisms causing food borne infections. Hence, the growth of microorganisms in food products result in the development of off flavor, spoilage, discolouration, slime production and deterioration which make the product unwholesome for human consumption and leads to compounds that contribute to disease such as cancer, cardiac diseases, allergy and other diseases (Mielnik *et al.*, 2008). Keeping quality of meat and maintenance is influenced by lipid oxidation and microbial growth. Thus to extend the shelf life, delay to prevent growth of pathogenic microorganisms in meat and meat products, several preservative methods such as freezing, salting, smoking, fermentation, canning and food additives have been employed in meat industries (Pokorny *et al.*, 1991).

Microorganisms present in muscle may be introduced through the vascular system during the sticking, bleeding and scalding operations. They can also be introduced through the stages of slaughter, during and after slaughtering, from animal body, slaughter house environment, equipment used, their subsequent cuts, overall handling and processing and processed meat products. Fresh meat and meat products are inhabitable to different types of microorganisms depending on the storage temperature, slaughtering process, texture, pH, environment, health of animal composition and transportation (Ercolini *et al.*, 2006; Li *et al.*, 2006). Microorganisms found on meat and meat

products come through the slaughtering process and conditions, poor husbandry practice, environment at the slaughter or abattoir, during transportation and at the sales point as muscles of healthy animals do not contain microorganisms (Ercolini *et al.*, 2006; Adzitey *et al.*, 2012). Hence, the type and extent of contamination vary with individual animals, herds or flocks and seasons of the year.

The process of spoilage of meat and meat products by the growth of microorganisms can be inhibited or diminished with the use of natural herbs, spices and medicinal plants. Since ancient time spices, flavorings and herbs have been added to foods not only as flavoring agents but also as folk medicines and food preservatives. One such medicinal plant is the *Samanea saman* of which the whole pods are known to be nutritious and contains phytochemicals useful for the prevention, controlling and treatments of various disease in human and animals. *Samanea saman* contains phytochemicals that act as strong nematocidal, hypercholesterolemic, antimicrobial agents. Parts of the tree can also be used to cure diseases and ailment like colds, diarrhea, headache, intestinal ailments, sore throat and inflammation of the gums and stomach ache (Durr, 2001; Ogunwande *et al.*, 2006; Prasad *et al.*, 2008; Ukoha *et al.*, 2011). Microbial spoilage is a major problem in the food industry and since people are becoming more conscious with their health natural antimicrobial agents need to be identified for use to inhibit the growth of these microorganisms in food. The study was conducted to assess *Samanea saman* whole pod extract as an antimicrobial agent and its effect in chicken patties.

MATERIALS AND METHODS

Samanea saman whole pods (SSWP) were collected from the trees available at the campus of Kwame Nkrumah University of Science and Technology, Ghana. The extractions and analysis of antimicrobial activity were carried out at the Department of Pharmacognosy of the

Kwame Nkrumah University of Science and Technology, Ghana.

Preparation of *Samanea saman* Whole Pods Extracts

The whole pods of *S. saman* were dried for ten days and milled with a hammer mill to produce a powder. About 100g of the powdered pod sample was used for ethanol solvent extraction and the extraction was carried out according to the method described by Fatope *et al.*, (1993). Twenty grams (20g) each of the powdered SSWP sample was percolated at room temperature (25°C) with 97% ethanol in 400 ml beakers (achieving 1:20 ratio). The beakers were covered with foil papers, shaken and left standing for two weeks with regular shaking. After two weeks, the suspensions were filtered and the filtrates—concentrated using Rotatory Evaporating Machine at 40°C. The *S. saman* whole pod extract (SSWPE) was kept refrigerated until use. The ethanolic extracts of the SSWP was measured into levels of 0.01ml, 0.05ml, 0.10ml and 0.15ml.

Microbial cultures used

Eight (8) microorganisms of both gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus pyogenes*) and gram negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) were used. The cultures used were standard cultures with codes as *Bacillus subtilis* ATCC 10073, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes* Clinical strain, *Escherichia coli* ATCC 25922, *Salmonella typhi* Clinical strain, *Klebsiella pneumonia* Clinical strain, *Pseudomonas aeruginosa* ATCC 4853 (Department of Pharmacognosy of the Kwame Nkrumah University of Science and Technology, Ghana).

Preparation of chicken patties

Chicken fat, skin and breast muscle meat from eight weeks old, freshly slaughtered manually

deboned broiler chickens were ground using a Super Wolf (MADO CE 95, Lochschiebemit Bohrungen, Germany) and were passed through a 3mm sieve plate. Ground chicken breast muscle, chicken fats and spices were chopped using a MTK 561 meat cutter (MA^(R) Grant, Germany). The chopping temperature was maintained at 15°C for twenty minutes to obtain a meat emulsion of desirable consistency. A total of twenty four kilograms (24 kg) emulsion was prepared and used for the chicken patties preparation. The emulsion was divided into five (5) equal portions and assigned to each treatment in a completely randomized design with a total of four (4) replicate per treatment. The emulsion was portioned into one kilogram (1kg) each and thoroughly mixed with the measured levels of SSWPE.

The treatments of the emulsion were as follows;

Treatment 1 (T₁) – No *S. saman* extract added

Treatment 2 (T₂) – 0.01 ml of SSWPE

Treatment 3 (T₃) – 0.05ml of SSWPE

Treatment 4 (T₄) – 0.10 ml SSWPE

Treatment 5 (T₅) – 0.15 ml SSWPE

One hundred gram (100 g) of the freshly prepared emulsion from each treatment was formed into patties using a patty cutter and stored frozen at -18°C for a day. The experimental product formulation is shown in Table 01. The patties were cooked using dry-heat in an electric oven at 180°C until well done (internal temperature of 72°C). The oven was pre-heated for 10 minutes to ensure uniform temperature was achieved before the actual cooking process commenced. The core temperature of each patty was measured using a meat piercing thermometer. All cooked patties were conditioned at room temperature (25°C) after which they were chilled at 2°C overnight. The chilled patties was weighed and vacuum packed separately and stored at -4°C.

Table 01: Composition of the experimental chicken patties (Weights/Kg)

Ingredients %	T1	T2	T3	T4	T5
Breast Muscle	70.00	70.00	70.00	70.00	70.00
Fat	10.00	10.00	10.00	10.00	10.00
Water/Ice	15.00	14.99	14.95	14.90	14.85
Curing Salt	1.80	1.80	1.80	1.80	1.80
Phosphate	0.05	0.05	0.05	0.05	0.05
*Mixed Spices	3.15	3.15	3.15	3.15	3.15
SSWP Ethanol Extract	-	0.01	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00	100.00

*Thyme (25g), Curry powder (25g), Red pepper (25g) and Monosodium glutamate (25g). T₁ – No additive, T₂ – 0.01ml of *Samanea saman* pod extract, T₃ – 0.05ml of *Samanea saman* pod extract, T₄ – 0.10ml of *Samanea saman* pod extract, T₅ – 0.15ml of *Samanea saman* pod extract.

Detection of antimicrobial effect of *S. saman* whole pod extract

The antimicrobial activity of the extract and the standard drug ciprofloxacin were ascertained by the method of Agyare *et al.* (2012). Nutrient agar was used to determine the antibacterial activity. Different concentrations of 200 mg/ml, 150 mg/ml, 100 mg/ml and 50 mg/ml of the ethanolic pod extract and 1 mg/ml for the ciprofloxacin (standard) were used. Eight (8) microorganisms of both gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes*) and gram negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) were used to seed the nutrient agar plates. In each of the plates, four equidistant wells with diameter of 8 mm were cut out using a sterile cork borer and wells filed with different concentrations of the extracts and reference drug ciprofloxacin. This was allowed to diffuse at room temperature (27 – 30°C) for 1 hour. The zone of growth inhibition was then measured after 24 hours of incubation of the plates at 37°C for growth of the test organism. The activity was expressed in terms of zone of inhibition in mm and each test was repeated three times.

The Minimum Inhibition Concentration (MIC) of the extract against the test organisms were determined using the microdilution technique described by Berridge *et al.* (2005) and Agyare

et al. (2012). About 100 mg/ml of the pod extract solution was prepared with distilled water and serially diluted to 75, 50, 25, 1.0 and 0.4 µg/ml. The same dilution was prepared for the standard Ciprofloxacin. Then 100 µl of 10⁶cfu/ml of test bacteria, both gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes* and gram negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) were grown in nutrient broth that was added to each well in the microplates and covered. The microplates were incubated at 37°C for 24 hours and to confirm growth of the organisms, 30 µl of 3-4, 5 dimethylthiazol-2-yl-2, 5-diphenyltetrazolium bromide dissolved in distilled water was added to the well of microplates and incubated for 30 minutes at 37°C. Each experiment was repeated thrice. The MIC of the pod extract against the test microorganism was taken at the minimum concentration of the extract which did not show any microbial growth.

Enumeration of microbes in chicken patties treated with *S. saman* whole pod extract

Enumeration of total microbial contaminants were done using the standard plate count technique on the frozen chicken patties stored at 4°C for 1, 7 and 14 days as described by Andrews (1992). In order to determine the effect of *S. saman* extract on specific microbes (*Escherichia coli*, *Salmonella typhi*,

Pseudomonas aeruginosa enumerations were made on selective agar plates. The media agar used for the isolation were Bismuth sulphate agar for *Salmonella typhi*, Cetrinide agar for *Pseudomonas aeruginosa* and McConkey for *Escherichia coli*. These media agar contain chemical additives that suppress the growth of all bacteria except the group of micro-organisms that were detected and used as indicator bacteria.

Statistical Analysis

The data generated from the study was subjected to one-way analysis of variance (ANOVA) and significant differences ($P < 0.05$) between means were determined by Tukey multiple comparison test using Genstat (2010).

RESULTS

Antimicrobial Properties of the *S. saman* Whole Pod Extract

Antimicrobial activity of the *S. saman* pod extract was determined by agar well diffusion method and the MIC method.

The Agar Well Diffusion Method

The ethanolic whole pod extract was found to be active against all the test organisms (*B. subtilis*, *S. aureus*, *E. faecalis*, *E. coli*, *S. pyogenes*, *S. typhi*, *K. pneumonia*, *P. aeruginosa*) with varying

mean zones of inhibition (Table 02). There were significant differences ($P < 0.0001$) among the concentrations and between the test organisms. There was a decreasing trend in the mean zone of inhibition and antimicrobial activity as the concentration of the extract decreased. The ethanolic whole pod extract has shown the zone of inhibition ranging 10 -18 mm while the ciprofloxacin had a range of 11.50 mm to 22.50 mm considering the all tested organisms. At the concentration of 50 mg/ml the ethanolic whole pod extract, *E. coli* had 15 mm zone inhibition while the lowest activity was against *B. subtilis*, *S. aureus* and *S. typhi* with a 10.83 mm zone of inhibition. Second highest inhibition at the concentration of 50 mg/ml was against the *K. pneumoniae* and it was 13.33mm and followed by *E. faecalis* with the inhibitory zone of 12.33 mm (Table 02).

At the concentration of 200mg/ml ethanolic whole pod extract, *B. subtilis* had 15.83mm, *S. aureus* had 12.83mm, *E. faecalis* had 14.33mm, *E. coli* had 16.33mm, *S. typhi* had 16.83mm, *K. pneumonia* had 15.83mm and *P. aeruginosa* had 16.83mm as the highest zones of inhibitions respectively. While at the concentration of 150mg/ml, *S. pyogenes* had 18.83mm as the highest zone of inhibition. At the concentration of 200mg/ml, the ethanolic whole pod extract exhibited the highest antimicrobial activities against the tested microorganisms except for *S. pyogenes* which was at 150mg/ml.

Table 02: Antimicrobial activity of *Samanea saman* whole pod extracts and Ciprofloxacin (Agar well diffusion method)

Test microorganisms	Mean zone of growth inhibition (mm) and Standard error of means				Concentration of Ciprofloxacin mg/ml
	Concentration of SSWPE mg/ml				
	200	150	100	50	1
<i>Bacillus subtilis</i>	15.83 ± 0.17	15.33 ± 0.88	12.83 ± 0.17	10.83 ± 0.60	17.00 ± 0.27
<i>Staphylococcus aureus</i>	12.83 ± 0.17	11.83 ± 0.17	11.33 ± 0.33	10.83 ± 0.60	11.00 ± 0.27
<i>Enterococcus faecalis</i>	14.33 ± 0.33	13.33 ± 0.33	11.83 ± 0.17	12.33 ± 0.33	15.00 ± 0.27
<i>Escherichia coli</i>	16.33 ± 0.88	15.83 ± 0.60	15.83 ± 0.60	15.33 ± 0.88	21.75 ± 0.73
<i>Streptococcus pyogenes</i>	14.33 ± 0.33	18.83 ± 0.60	12.83 ± 0.17	11.83 ± 0.60	15.00 ± 0.27
<i>Salmonella typhi</i>	16.83 ± 0.60	15.83 ± 0.60	11.83 ± 0.60	10.83 ± 0.17	14.50 ± 0.42
<i>Klebsiella pneumonia</i>	15.83 ± 0.60	14.83 ± 0.17	13.83 ± 0.17	13.33 ± 0.88	17.25 ± 0.21
<i>Pseudomonas aeruginosa</i>	16.83 ± 0.17	16.33 ± 0.33	13.83 ± 0.17	11.83 ± 0.60	18.00 ± 0.27

The Minimum Inhibitory Concentration (MIC) of *Samanea saman* Whole Pod Extracts

The results of the MIC of the ethanolic whole pod extract against microorganisms are shown in Table 03. It indicated that the *S. saman* ethanolic whole pod extract could inhibit Gram – positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes*), Gram – negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) and at a minimum inhibition concentration of 25 µg/ml when compared to the standard Ciprofloxacin which had the minimum inhibition concentration of 1 µg/ml for the above mentioned microorganisms. Beyond the concentration of 1 µg/ml Ciprofloxacin and 25 µg/ml SSWPE, there was no noticeable growth

of the microorganism.

Microbial Load in the Chicken Patties Treated With or Without SSWPE

The study of the microbial load of chicken patties done on the Day 1 indicated that the treatment 1 (T1) and patties treated with the ethanolic pod extracts had the highest microbial counts whereas the T5 with 0.15ml chicken patties showed the lowest number of bacteria (6.079 log10 cfu/g) (Table 04). There was also the presence of *Escherichia coli* in the patties containing 0.10ml and 0.15ml pod extracts while the load was low in the treatment1 (T1). Also on the Day 14, low *Escherichia coli* counts were made in the patty that contained treatment five (T5) and few counts in the patties of T1, T2 and T4.

Table 03: Minimum Inhibitory Concentration (MIC) of *Samanea saman* Whole Pod Extract and Ciprofloxacin (Standard)

Test organisms	Concentration of Standard Ciprofloxacin (µg/ml)				Concentrations of <i>Samanea saman</i> Whole Pod Extracts (µg/ml)					
	75	50	25	1	0.4	75	50	25	1	0.4
E. coli	-	-	-	-	+	-	-	-	+	+
S. aureus	-	-	-	-	+	-	-	-	+	+
S. pyogenes	-	-	-	-	+	-	-	-	+	+
S. typhi	-	-	-	-	+	-	-	-	+	+
E. faecalis	-	-	-	-	+	-	-	-	+	+
P. aeruginosa	-	-	-	-	+	-	-	-	+	+
B. subtilis	-	-	-	-	+	-	-	-	+	+
K. pneumonia	-	-	-	-	+	-	-	-	+	+

- No growth; + growth

Table 04: Microbial Counts of Chicken Patties Stored at 4°C for 1, 7 and 14 Days

Days	Treatment log10 cfu/g	Total microbial count	E. coli	Salmonella	Pseudomonas
1	T1	6.255	ND	ND	ND
	T2	6.279	ND	ND	ND
	T3	6.209	ND	ND	ND
	T4	6.301	5.982	ND	ND
	T5	6.079	5.903	ND	ND
7	T1	6.447	ND	ND	ND
	T2	6.462	ND	ND	ND
	T3	6.322	ND	ND	ND
	T4	6.468	ND	ND	ND
	T5	6.230	ND	ND	ND
14	T1	6.279	TFTC	ND	ND
	T2	6.380	TFTC	ND	ND
	T3	6.146	ND	ND	ND
	T4	6.029	TFTC	ND	ND
	T5	6.462	5.531	ND	ND

TFTC = Too few to count cfu/g = colony forming unit/gram ND = Not detected.

At the 7th day of sampling, there was an increment in the microbial load as well as the load of fungus compared to the Day 1. On Day 14, the microbial load counts of the patties with the highest inclusion level 0.15ml (T5) was the highest with a value of 6.462 log₁₀cfu/g. The growth of microorganisms increased from Day 1 to 14. The microbial load counts of the patties indicated the absence of *Salmonella typhi* and *Pseudomonas aeruginosa* from Day 1 to 14. The study also indicated that the total microbial load count of the patties was due to the presence of other microorganisms other than the ones tested.

DISCUSSION

Antimicrobial Properties of the S. saman Whole Pod Extract

The agar well diffusion method relies on the ability of the test antimicrobial agent to diffuse through the culture media while the minimum inhibition concentration uses broth dilutions which is reproducible and does not rely on the antimicrobial agent diffusion (Cos *et al.*, 2006). The results of the agar well diffusion method revealed that the ethanolic pod was able to inhibit the growth of tested microorganisms. The decreasing trend of the antimicrobial activity of *S. saman* pod extract as the concentration decreased because the potency of the activity kept reducing as a result of the dilution.

The significant differences among the concentrations are probably due to the extent of the dilution from one concentration to the other. There was also a significant difference between the tested microorganisms which could be attributed to the organisms having different genetic make-up. It was also realized that *S. saman* ethanolic pod extract was more effective on the Gram-negative organisms than the Gram-positive organisms which is good, since the Gram-negative organisms tend to be more dangerous to human health than the Gram-positive although both groups are harmful. This observation is contrary to the findings of

Thippeswamy *et al.* (2011) and Gonzales and Paombong (1990) who indicated that acacia or rain tree leaves extract has strong potency against Gram-positive organisms (*S. aureus*, *S. lutea* and *B. subtilis*) but weak activity against Gram-negative organisms especially *E. coli*. However, the findings of Prasad *et al.* (2008) support the antimicrobial result of this study because they established that *S. saman* leaves extract has mild to medium inhibition against Gram-negative organisms. Therefore, the antimicrobial property exhibited by the pod extract could be attributed to the presence of phytochemicals in the extract especially the alkaloids and tannins which was directly related to the size of the zone (diameter) of growth inhibition around the disc that contains the extract. Hence a wider zone of growth inhibition indicates more susceptibility of the organism to the alkaloids or tannins in the pod extract.

Obasi *et al.* (2010) attested to the fact that antimicrobial activities in plants is due to the presence of phytochemicals thus the higher content of different phytochemicals especially the flavonoids and tannins present in the methanolic *S. saman* pod extracts might have contributed to the higher inhibitory activity against the *B. subtilis* and *S. aureus*.

Ferdous *et al.* (2010) also used agar or disc diffusion method on a methanolic extract of *S. saman* bark to test for antibacterial and antifungal properties against 13 Gram-positive and Gram-negative bacteria and 3 fungi. Of the 3 fractions of the methanol bark extract namely hexane soluble fraction, carbon tetrachloride soluble fraction and chloroform soluble fraction obtained, only the carbon tetrachloride showed highest antibacterial and antifungal activities. The carbon tetrachloride extract showed the highest and moderate inhibition against *B. cereus*, *B. subtilis*, *S. lutea*, *P. aeruginosa*, *S. dysenteriae* having a diameter zone of inhibition ranging from 10 mm to 12mm and low inhibition for *B. megaterium*, *S. paratyphi*, *E. coli*, *S. typhi*, *S. aureus* at a range of 7mm – 9 mm, while for the fungus, *C. albicans* (7mm), *A. niger* (9mm) and *S. cereyaciae* (9mm) respectively. Also, an

investigation conducted through agar diffusion method by Prasad *et al.* (2008), indicated that the leaves extract at 5mg/ml inhibited *E. coli* growth whilst the minimum inhibition of *S. aureus* and *C. albicans* is at 10mg/ml. Through the agar well diffusion method, it was established from this study that at the minimum concentration of 50 mg/ml, the growth of *E. coli* was highly inhibited by the ethanolic whole pod extract than the other microorganisms and *B. subtilis*, *S. aureus* and *S. typhi* having the lowest inhibition. However, the ethanolic whole pod extracts with concentration of 200 mg/ml exhibited highest antimicrobial activity against the test organisms (*B. subtilis*, *S. aureus*, *E. faecalis*, *E. coli*, *S. pyogenes*, *S. typhi*, *K. pneumonia* and *P. aeruginosa*) growth whilst the minimum inhibition was at 50 mg/ml.

The result of this study had the highest inhibition of the pod extract at a concentration of 200 mg/ml indicated that either the solvent used or the part of plant used (pod) was not able to extract enough of the phytochemicals from the plant part to cause efficient inhibition against the test pathogens compared to the ciprofloxacin. This result is in contrast to what was observed by Obasi *et al.* (2010) that out of the solvents used on the *S. saman* pods, only the methanolic and ethyl acetate extracts exhibited measurable inhibition against *B. subtilis* and *S. aureus* at concentration of 20 and 10 mg/ml and *S. aureus* at concentration of 20 mg/ml. Ukoha *et al.* (2011) conducted similar test using different solvents and realized that the ethanolic extract of the pod of the *S. saman* was not as effective on the tested organism than when n-hexane solvent extract was used. Also the part of plant used, extraction method employed and concentrations used are also factors. The ethanolic extract of the *S. saman* pod does not have enough secondary metabolites in it compared to the methanolic extract as well as not having a lot of the flavonoids which work as an antimicrobial agent as was evident in this study. The presence of phytochemicals especially when there are large numbers of different phytochemicals indicates an antimicrobial potential (Obasi *et al.*, 2010).

On the other hand when the minimum inhibition concentration (MIC) of the pod extract was conducted, the ethanol *S. saman* pod extract was able to inhibit *E. coli*, *S. aureus*, *S. typhi*, *E. faecalis*, *P. aeruginosa*, *B. subtilis* and *K. pneumonia* at a minimum inhibition concentration of 25 µg/ml as shown in Table 3. The MIC is the minimum concentration of an extract that did not show any microbial growth. The antimicrobial activity may be attributed probably due to the presence of the phytochemical content in the extracts (Nweze *et al.*, 2004; Edeoga *et al.*, 2005). Therefore, the higher the numbers of different phytochemicals present in the extract, the higher the exhibition of strong and effective antimicrobial potential. Taguri *et al.* (2004) also confirmed that the antimicrobial activity of *Pistacia terebinthus* L. extract is due to the presence of phenolic compounds present in that extract. The leaf extract of *S. saman* contains alkaloids and this exhibits strong antimicrobial activity against varied bacteria because its potency is equivalent to that of gentamycin with a minimum inhibition concentration (MIC) value of 7 – 20µg/ml (Prasad *et al.*, 2008). The leaf extract of the *S. saman* at a concentration of 25 mg/ml was more resistant to bacteria than Streptomycin and has the same potency as that of Chloramphenicol at 50 mg/ml but more lethal than penicillin at 50 mg/ml (Prasad *et al.*, 2008).

The results of the antimicrobial activity obtained in this study corroborates the findings of Thippeswamy *et al.* (2011) where antimicrobial efficacy of the ethanolic extract of the *S. saman* leaves showed a broad spectrum activity ranging from 12.0 ± 0.7 , 11.0 ± 0.4 , 8.5 ± 0.2 , 10.0 ± 0.5 , 9.7 ± 1.7 , 13.5 ± 0.5 , 26.5 ± 0.2 , 11.0 ± 0.4 mm at 1mg/ml concentration for *E. coli*, *K. pneumonia*, *P. vulgaris*, *P. aeruginosa*, *S. typhi*, *S. aureus*, *S. faecalis* and *X. campestris*, respectively whilst that of the methanolic extract is in the range of 11.0 ± 1.1 to 30.5 ± 0.5 mm. The methanol extract MIC (minimum inhibition concentration) value for the tested bacteria ranged from 15µg/ml to 500µg/ml with the most susceptible organism being *Streptococcus faecalis* with a minimum

inhibition concentration (MIC) value of 15µg/ml followed by *Staphylococcus aureus* with 62µg/ml and the most resistant was *Proteus vulgaris* with 500µg/ml MIC. Also the methanol and ethanol extract exhibited strong antifungal potential against the tested fungi with inhibition ranging from 20.4% to 81.6% and 10.4 to 66.9% at concentrations of 1mg/ml and had IC₅₀ value of 0.3mg/ml for *Fusarium moniliforme* being highly susceptible and the least sensitive was *Aspergillus tamari* with IC₅₀ 5mg/ml. According to Thippeswamy *et al.* (2011) the antifungal and antibacterial activities of methanol and ethanol extract were almost equivalent to the synthetic fungicides (Blitox and Dithane M-4) and antibiotic (Erythromycin and Penicillin-G).

The high MIC value of 25 µg /ml or the low antimicrobial activity of the pod extract may be due to low content of the active ingredients in the extract or attributable to the extraction procedure used. The potency of an antimicrobial and antifungal agent is affected by certain factors such as temperature, pH, concentration, lethal effects and microbial population. Chung *et al.* (1998) and Min *et al.* (2008) indicated that tannins antimicrobial activity is affected by the source and concentration of the microbial agents. Ukoha *et al.* (2011) observed that the antimicrobial effect of the *S. saman* pods in the tannin ethyl acetate extract had the highest inhibition zone and this was followed by tannin methanol extract, tannin acetate extract and lastly the tannin ethanolic extract showed no zone of inhibition against *S. aureus* and *K. pneumonia* but only tannin ethyl acetate inhibited against the fungus, *C. albicans*. They suggested that the ethyl acetate extract of tannins from the *S. saman* pod can serve as a natural source of antimicrobial since it was effective against the selected pathogens because of its strong concentration and lethality. Based on the MIC result of 25 µg /ml (2.5 mg/ml) obtained in this study, one can comment that the *S. saman* pod extract may be a remarkable antimicrobial compound because Fabry *et al.* (1998) reported that plant extract with MIC range of 2.5 and 8 mg/ml is a potential source

of effective antimicrobial agent. However, Adu *et al.* (2009) observed that plant extract with low antimicrobial property may possess some phytoconstituents that may modify the antimicrobial activities of known antimicrobials against resistant bacteria. Although a higher minimum inhibitory concentration (MIC) value was needed to inhibit *Candida albicans* and *Aspergillus niger* (125 and 500 µg/mL, respectively), the inhibitory effect of the pod extract was comparable to the commercial drugs such as nystatin and griseofulvin (Viput and Anjana, 2011). Also, a potent antibacterial property, including inhibition against *Candida albicans*, have been exhibited by the ethyl acetate fraction of the *Samanea saman* pods at 10,000 ppm (Ukoha *et al.*, 2011). The result of this study shows that a higher minimum inhibitory concentration (MIC) value above 25ug/ml was needed to inhibit all tested bacteria and that the potency of the extract was equivalent to that of gentamycin with a minimum inhibition concentration (MIC) value of 7 – 20ug/ml (Prasad *et al.*, 2008).

Microbial Load in the Chicken Patties with Ethanolic S. saman Pod Extracts

The control and prevention of spoilage by microorganisms could be done by the use of antimicrobials agents. The result obtained implies that the ethanolic pod extract had low antimicrobial activity in the patties since it did not show reduced microbial load counts compared to the control (Treatment 1, Table 4). The result obtained may be influenced by many factors, eg; mixing of the extract in the patties as well as the concentration of the extract in the patties. This result is contrary to what Omojola and Adediran (2014) observed because the extract of garlic, ginger and roselle contained antimicrobial activity, and they reduced the microbial load of the patties across the treatments in each of the storage period as compared with the control.

Works done by Obasi *et al.* (2010) on different solvent extract of *S. saman* pod indicated the presence of antimicrobial activities of the pod

extract depending on the extraction method. According to Obasi *et al.* (2010) methanolic extract of the *S. saman* pod inhibited *Staphylococcus aureus* and *Bacillus subtilis* at concentrations of 20 and 10 mg/ml while ethyl acetate inhibited only *Staphylococcus aureus* at 20 mg/ml. The ethanolic extract of the *S. saman* pod does not have enough secondary metabolites in it compared to the methanolic extract as well as not having a lot of the flavonoids which work as an antimicrobial (Rattanachaikunsopon *et al.*, 2007; Redko *et al.*, 2007; Abou-Donia *et al.*, 2008). This corroborates the result of this study; in that the various levels of the ethanolic pod extract show no effects on the microorganism and thus there are no reductions in the load count as the storage days lengthen. Also, probably the concentrations used in this study were not strong enough to cause any antimicrobial effect. Nonetheless, the type of meat product chicken patties on which the pod extract was applied to may have been the cause. For instance, Mitsumoto *et al.* (2005) reported of a reduced lipid oxidation by adding tea catechins to beef patties but this was not effective when used on chicken patties.

The result observed in this study might be due to the concentrations used in the patties were low and not strong enough to cause an antimicrobial effect as was reported by Obasi *et al.* (2010). The above result shows that the ethanolic extract of the *S. saman* pod cannot be used as an antimicrobial agent, but if it should be used, then the concentrations should be increased above what was used in the present study and that of Obasi *et al.* (2010). The result obtained also might be due to the extraction method used as a study by Obasi *et al.* (2010) that revealed that antimicrobial activities of the *S. saman* pod extract is influenced by the extraction method. According to Obasi *et al.* (2010) the methanolic extract of the *S. saman* pod inhibited *Staphylococcus aureus* and *Bacillus subtilis* at concentrations of 20 and 10 mg/ml whilst ethyl acetate inhibited only *Staphylococcus aureus* at 20 mg/ml. However, Gonzales and Paombong, (1990) in using the leaves and bark extract of acacia indicated that it exhibited complete

activity against gram-positive organisms (*Staphylococcus aureus*, *Sarcina lutea* and *Bacillus subtilis*) and partial activity against gram-negative organism (*E.coli*). The presence of phytochemicals especially when there are large numbers of different phytochemicals indicates an antimicrobial potential (Obasi *et al.*, 2010). According to Sodipo *et al.* (1991) tannins, an antimicrobial agent inhibit microbial growth by precipitating the microbial protein for growth and development by the microorganism. Tannins were detected in the methanolic extract but not in the other extracts and this was expected to have given the inhibitory characteristic to the extract.

According to Obasi *et al.* (2010) *S. saman* pods' therapeutic potential hinges on the solvent used for the extraction, and recommended methanolic extracts. However, all hope is not lost as higher concentrations of the ethanolic extract of the *S. saman* pods could be tried to ascertain the antimicrobial potency of the pod. Nevertheless, the chicken patties is a consumable product so methanolic extract cannot be used as suggested by Obasi *et al.* (2010), because methanol is toxic, in the same way there is a limit to the concentration of the ethanolic pod extract that can be used since the *S. saman* pod is known to contain some toxic substance such as alkaloids, tannins, saponins (Ihekoronye and Ngoddy, 1985; Enwere, 1998). Ukoha *et al.* (2011) reported 7.9% of tannins in the *S. saman* pod and recommended it for industries but for human consumption excess of the tannins in the body is toxic as it leads to anaemia and cancer. However, the tannin concentration in the pod is not so high to induce toxicity so can be used in beverages and herbal teas. El-Waziry *et al.* (2005) also related esophageal cancer to consumption of herb with high tannin content.

The Enterobacteriaceae are the harmful food poisoning bacteria such as *E.coli* and *Salmonella* and are mostly used as indicator bacteria thus their number should not exceed 100 per cm. El-Khateib *et al.* (1988) also indicated that the total bacterial count of chicken products as sausage, burger, luncheon and frankfurter was 10⁷,

10^7 , 10^6 and 10^6 cfu/g respectively. Microbial spoilage occurs as a result of the growth and metabolic activities of spoiling bacteria and the criterion of microbiological acceptability (total viable counts reaching 7 log CFU/g) has been used to define spoilage (Höll, *et al.*, 2016; Zhang, *et al.*, 2012). These patties from the various treatments can be consumed since they have not reached the recommended threshold of 10^7 cfu/g and also they contain no harmful bacteria such as *Salmonella*, *Pseudomonas* but few *E. coli* counts which may cause food poisoning and or food borne infections. This observation is contrary to the findings of Thippeswamy *et al.* (2011) and Gonzales and Paombong (1990) who indicated that acacia or rain tree leaves and bark extracts have strong potency against Gram-positive organisms (*S. aureus*, *S. lutea* and *B. subtilis*) but weak activity against Gram-negative organisms especially *E. coli*.

CONCLUSION AND RECOMMENDATIONS

This study had two objectives, the first objective was to assess the antimicrobial effect of *Samanea saman* pod extract against some bacteria and the second objective was assessing the potential of using *S. saman* whole pod extract in chicken patties in order to reduce the microbial contaminations.

With regards to objective one it can be concluded that *S. saman* whole pod extract has the potential to be used as antimicrobial agent. *Samanea saman* whole pod extract has a therapeutic potential as it proved to be a strong antimicrobial agent using the ethanol solvent as it inhibited against the tested microorganisms both Gram negative and Gram positive bacteria. Based on the MIC result of 25 µg /ml (2.5 mg/ml) obtained in this study, one can comment that the *S. saman* pod extract may be a good antimicrobial compound because plant extract with MIC range of 2.5 and 8 mg/ml is a potential source of effective antimicrobial agent.

With regards to the second objectives, the effect of *S. saman* whole pod extract in

reducing microbial counts in chicken patties is inconclusive which can be attributed to several factors such as mixing of extract with the chicken patties and concentration level of the extract. It was established that at the present level of inclusion in the chicken patties the *S. saman* pod extracts has minimal effect on the pathogenic microorganisms of the chicken patties. However, the therapeutic potential of *S. saman* pods was dependent on the ethanolic solvent used as the antimicrobial activities are conferred by the phytochemicals present in the plant. Therefore, the extraction, characterization, isolation, quantification and purification of the detected phytochemicals present in *S. saman* whole pods might result in the clarification of its active beneficial compound as a potential cure for diseases and a reduction in food deterioration caused by microorganisms.

On the basis of the findings of the study, it can be recommended that further study is required to assess the MIC level of the *S. saman* whole pod ethanolic extract applicable to bacteria. Also the mixing of the extract with the meat product should be done properly since it has the potential of affecting the result. The inclusion of the ethanolic whole pod extract at higher concentrations should be considered to eliminate pathogenic microorganism in meat products. Finally, the financial benefit of including higher levels of the ethanolic whole pod extract in the meat product should be explored.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

- Abou-Donia, A. H., Toaima, S. M., Hammada, H. M., Shawky, E., Kinoshita, E. and Takayama, H. (2008). Phytochemical and biological investigation of *Hymenocallis littoralis* SALISB. *Chemistry and Biodiversity*. 5(2): 332-340. DOI: <https://doi.org/10.1002/cbdv.200890031>
- Adu, O. B., Falade, A. M., Nwalutu, E. J., Elemo, B. O. and Magbagbeola, O. A. (2009). Nutritional status of under graduates in a Nigerian university in south-west Nigeria. *International Journal of Medicine and Medical Sciences*. 1(8): 318-324. <https://academicjournals.org/journal/IJMMS/article-abstract/47C0E9D434>
- Adzitey, F., Rusul, G. and Huda, N. (2012). Prevalence and antibiotic resistance of *Salmonella* serovars in ducks, duck rearing and processing environments in Penang, Malaysia. *Food Research International*. 45: 947–952. DOI: <https://doi.org/10.1016/j.foodres.2011.02.051>
- Aguirrezabal, M. M., Mateo, J., Dominguez, M. C. and Zumalac-arregui, J. M. (2000). The effect of paprika, garlic and salt on rancidity in dry sausages. *Meat Science*. 54: 77–81. DOI: [https://doi.org/10.1016/s0309-1740\(99\)00074-1](https://doi.org/10.1016/s0309-1740(99)00074-1)
- Agyare, C., Koffor, G. A., Boamah, V. E., Adu, F., Mensah, K. B. and Adu-Amoah, L. (2012). Antimicrobial and anti-inflammatory activities of *Pterygotamacrocarpa* and *Cola gigantea* (Sterculiaceae). *Evidence Based Complementary and Alternative Medicine*. 2012: 1-9. DOI: <https://doi.org/10.1155/2012/902394>
- Andrews, W. (1992). Manual of food quality control. 4.1. Microbiological Analysis. In: Food and Agriculture Organization of the United Nations, Rome.
- Berridge, M. V., Herst, P. M. and Tan, A. S. (2005). Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. *Biotechnology Annual Review*. 11: 127–152. DOI: [https://doi.org/10.1016/s1387-2656\(05\)11004-7](https://doi.org/10.1016/s1387-2656(05)11004-7)
- Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W. and Lin, Y. (1998). Tannins and human health: a review. *Critical Review Food Science Nutrition*. 38(6): 421-464. DOI: <https://doi.org/10.1080/10408699891274273>
- Cos, P., Vlietinck, A., Vanden, J., Berghe, D. and Maes, L. (2006). Anti-infective potential of natural products: how to develop a stronger in vitro ‘proof-of concept’. *Journal of Ethnopharmacology*. 106: 290–302. DOI: <https://doi.org/10.1016/j.jep.2006.04.003>
- Durr, P. A. (2001). The biological, ecology and agroforestry potential of the rain tree, *Samanea saman* (Jacq.) Mer. *Agroforestry system*. 51: 223-237. DOI: <https://doi.org/10.1023/A%3A1010765022497>
- Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*. 4(7): 685–688. DOI: <https://doi.org/10.5897/ajb2005.000-3127>
- EL-Khateib, T., Abd El-Rahman, H., Hamdy, M. and Lotfi, A. (1988). Poultry meat products in Egypt Proximal chemical composition and microbiological quality. *Fleischwirtsch*. 68(6): 756–757.

- El-Waziry, A. M., Nasser, M. E. A. and Sallam, S. M. A. (2005). Processing methods of soybean meal. Effect of roasting and tannic acid treated soybean meal on gas production and rumen fermentation *in vitro*. *Journal of Applied Science*. 1: 313-320. https://www.researchgate.net/publication/303494630_El-Waziry_AM_MEA_Nasser_and_SMA_Sallam_2005_Processing_methods_of_soybean_meal_1_Effect_of_roasting_and_tannic_acid_treated-soybean_meal_on_gas_production_and_rumen_fermentation_in_vitro
- Enwere, N. J. (1998). In: Foods of Plant Origin. 1st Edition. Afro-Obis Publ. Ltd., Nsukka, Nigeria. 301pp.
- Ercolini, D., Russo, F., Torrieri, E., Masi, P. and Villani, F. (2006). Changes in the spoilage-related microbiota of beef during refrigerated storage under different packaging conditions. *Applied Environmental Microbiology*. 72(7): 4663-4671. DOI: <https://doi.org/10.1128/aem.00468-06>
- Fabry, W., Okemo, P. O. and Ansorg, R. (1998). Antibacterial activity of East African medicinal plants. *Journal of Ethnopharmacology*. 60(1): 79–84. DOI: [https://doi.org/10.1016/s0378-8741\(97\)00128-1](https://doi.org/10.1016/s0378-8741(97)00128-1)
- Fatope, A. O., Ibrahim, H. and Taked, Y. (1993). Screening of higher plants reputed as pesticides using brine shrimp lethality bioassay. *International Journal of Pharmacognosy*. 31: 250-256. DOI: <https://doi.org/10.3109/13880209309082949>
- Ferdous, A., Mohammad, Z. I. and Tajnin, A. (2010). Antioxidant, Antimicrobial and Cytotoxic Activities of *Samaneasaman* (Jacq.) Merr. *Stamford Journal of Pharmaceutical Sciences*, 3(1): 11-17. DOI: <https://doi.org/10.3329/sjps.v3i1.6792>
- Gonzales, M. V. M. and Paombong, A. A. (1990). The antiseptic activity of the extract from *Samanea saman* (Acacia) leaves. Unpublished Master's Thesis, Batangas City.
- Höll, L., Behr, J. and Vogel, R. F. (2016). Identification and growth dynamics of meat spoilage microorganisms in modified atmosphere packaged poultry meat by MALDI-TOF MS. *Food Microbiology*. 60: 84–91. DOI: [10.1016/j.fm.2016.07.003](https://doi.org/10.1016/j.fm.2016.07.003).
- Ihekoronye, A. I. & Ngoddy, P. O. (1985). In: Integrated Food Science and Technology for the Tropics, Macmillan Publishers Limited, London. UK. 293–298.
- Komba, E. V. G., Komba, E. V., Mkupasi, E. M., Mbyuzi, A. O., Mshamu, S., Luwumbra, D., Busagwe, Z. and Mzula, A. (2012). Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: implications for public health. *Tanzania Journal of Health Resources*. 14(2): 6. DOI: <https://doi.org/10.4314/thrb.v14i2.6>
- Li, M. Y., Zhou, G. H., Xu, X. L., Li, C. B. and Zhu, W. Y. (2006). Changes of bacterial diversity and main flora in chilled pork during storage using PCRDGGE. *Food Microbiology*. 23(7): 607-611. DOI: <https://doi.org/10.1016/j.fm.2006.01.004>
- Mielnik, M. B., Signe, S., Bjorg, E. and Grete, S. (2008). By-products from herbs essential oil production as ingredient in marinade for turkey thighs. *Food Science and Technology*. 41: 93–100. DOI: <https://doi.org/10.1016/j.lwt.2007.01.014>

- Min, B. R., Pinchak, W. E., Merkel, R., Walker, S., Tomita, G. and Anderson, R. C. (2008). Comparative antimicrobial activity of tannin extracts from perennial plants on mastitis pathogens. *Scientific Research and Essay*. 3(2): 066-073. http://www.academicjournals.org/app/webroot/article/article1380355448_Min%20et%20al.pdf
- Mitsumoto, M., O'Grady, M. N., Kerry, J. P. and Buckley, D. J. (2005). Addition of tea catechins and vitamin C on sensory evaluation, colour and lipid stability during chilled storage in cooked or raw beef and chicken patties. *Meat Science*. 69: 773–779. DOI: <https://doi.org/10.1016/j.meatsci.2004.11.010>
- Nweze, E. I., Okafor, J. I. and Njoku, O. (2004). Antimicrobial activities of methanolic extracts of *Tremaguineensis* (Schumm and Thorn) and *Morinda Lucida* Benth used in Nigerian Herbal Medicinal Practice. *Journal of Biological Research and Biotechnology*. 2(1): 39-46. DOI: <https://doi.org/10.4314/br.v2i1.28540>
- Obasi, N. L., Egbonu, A. C. C., Ukoha, P. O. and Ejikeme, P. M. (2010). Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* (fabaceae or mimosaceae) pods. *African Journal of Pure and Applied Chemistry*. 4(9): 206-212. http://www.academicjournals.org/app/webroot/article/article1379501137_Obasi%20et%20al.pdf
- Ogunwande, I. A., Walker, T. M., Setzer, W. N. and Essien, E. (2006). Volatile constituents from *Samanea saman* (Jacq.) Merr. Fabaceae. *African Journal of Biotechnology*. 5(20): 1890-1893.
- Omojola, A. B. and Adediran, O. A. (2014). Enhancing Oxidative Stability and Quality of chicken meat using natural products. Meat Science, Animal Science Department, University of Ibadan, Ibadan, Nigeria.
- Pokorny, J. (1991). Natural antioxidants for food use. *Trends in Food Science and Technology*. 2: 223–227. DOI: [https://doi.org/10.1016/0924-2244\(91\)90695-f](https://doi.org/10.1016/0924-2244(91)90695-f)
- Prasad, R. N., Viswanathan, S., Renuka, D. J., Nayak, V., Swetha, V. C., Archana, B. R., Parathasarathy, N. and Rajkumar, J. (2008). Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*. *Journal of Medicinal Plants Research*. 2(10): 268-270. http://www.academicjournals.org/app/webroot/article/article1380526442_Naveen%20et%20al.pdf
- Rattanachaikunsopon, P. and Phumkhachorn, P. (2007). Bacteriostatic effect of flavonoids isolated from leaves of *Psidium guajava* on fish pathogens. *Fitoterapia*. 78(6): 434–436 DOI: <https://doi.org/10.1016/j.fitote.2007.03.015>
- Redko, F., Clavin, M. L., Weber, D., Ranea, F., Anke, T. and Martino, V. (2007). Antimicrobial isoflavonoids from *Erythrina crista galli* infected with *Phomopsis* sp. *Z Naturforsch*. 62(3-4): 164-168. DOI: <https://doi.org/10.1515/znc-2007-3-402>
- Sodipo, O. A., Akanji, M. A., Kolawole, F. B. and Odutuga, A. A. (1991). Saponin is the active antifungal principle in *Garcinia kola*, heckle seed. *Bioscience Resource Community*. 3: 1- 71
- Taguri, T., Tanaka, T. and Kouno, I. (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease, *Biological and Pharmaceutical Bulletin*. 27(12): 1965–1969. DOI: <https://doi.org/10.1248/bpb.27.1965>
-

- Thippeswamy, S., Praveen, P., Mohana, D. C. and Manjunath, K. (2011) .Antimicrobial evaluation and phytochemical analysis of a known medicinal plant *Samanea saman* (jacq.) merr. against some human and plant pathogenic bacteria and fungi. *International journal of pharma and biological sciences*. 2(2): 443-452.
- Ukoha, P. O., Egbuonu, A., Cemaluk, C. Obasi, L. N. and Ejikeme, P. M. (2011). Tannins and other phytochemical of the *Samanaeasaman* pods and their antimicrobial activities. *African Journal of Pure and Applied Chemistry*. 5(8): 237-244. <https://academicjournals.org/journal/AJPAC/article-abstract/D96D4481286>
- Viput, S. D. and Anjana, K.V. (2011). Antifungal activity of crude extracts of *Cassia occidentalis*. *International Journal Research Phytochemical Pharmacology*. 1: 36–38.
- Yin, M. and Cheng, W. (2003). Antioxidant and antimicrobial effects of four garlic-derived organosulfur compounds in ground beef. *Meat Science*. 63: 23-28. DOI: [https://doi.org/10.1016/s0309-1740\(02\)00047-5](https://doi.org/10.1016/s0309-1740(02)00047-5)
- Zhang, Q. Q., Han, Y. Q., Cao, J. X., Xu, X. L., Zhou, G. H. and Zhang, W. Y. (2012). The spoilage of air-packaged broiler meat during storage at normal and fluctuating storage temperatures. *Poultry Science*. 91: 208–214. DOI: 10.3382/ps.2011-01519. DOI: <https://doi.org/10.3382/ps.2011-01519>