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Proximate Composition, Phytochemical Profile and Antimicrobial Activity of *Andrographis paniculata* Ethanolic Leaf Extract and its Preservatives Effect in Bread

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Abstract

A study on the phytochemical profile, proximate composition, and antimicrobial activity of *Andrographis paniculata* leaf extract was carried out. The extract (300g) was obtained by extraction in ethanol at 400ml and was evaluated for antimicrobial activity for seven (7) weeks against fifteen (15) microbial species by determining the Minimum Inhibitory Concentration (MIC) based on the mean diameter zone of inhibition around the disc in millimeters. The ethanolic extract was analyzed for quantitative and qualitative composition of phytochemical profiling. The qualitative screening showed the presence of flavonoids, alkaloids, tannins, cardiac glycosides, terpenes, and glycosides while saponins and anthraquinones were not detected. The quantitative composition of the extract was; alkaloids (1.17±0.19), terpenes (11.48±0.36), tannins (4.73±0.13), flavonoids (0.73±0.00), cardiac glycosides (0.50±0.05) and glycosides (89.48±0.73). The proximate composition showed that carbohydrates and proteins were the most abundant followed by crude fiber and moisture content. The antimicrobial activity of the extract was tested against, *Pseudomonas sp, Bacillus cereus, Lactobacillus sp, Staphylococcus aureus, Micrococcus sp, Enterobacter s*p, *Aspergillus niger, Saccharomyces sp, Bacillus subtilis, Streptococcus sp, Citrobacter sp, Rhizopus sp, Mucor sp, Candida sp* and *Fusarium sp* through the measurement of zone of inhibition, the extract exhibited potent antimicrobial activity against both Gram-negative and Gram-positive bacteria under investigation with diameter of inhibition greater than 1 mm. A similar observation was recorded for the fungi species. The study has demonstrated that *Andrographis paniculata* leaf extract exhibits potent antimicrobial activity against some selected food spoilage organisms and in bread.

Keywords: *Andrographis paniculata*, Antimicrobial Activity, Phytochemical Profiling, Proximate Composition.

Introduction

Food products are prone to contamination by microorganisms during processing, handling, storage, and distribution leading to undesirable changes in food quality. Microbial food spoilage is often due to the growth and/ or metabolism of spoilage bacteria, mold, and yeast which affect all types of foods and contribute significantly to food waste and losses. The report shows that the loss of globally produced food is estimated at 40% with microbial food spoilage and quality degradation accounting for some of the major causes (Gonelimali

et al., 2018). The reported health problems associated with the consumption of food containing synthetic chemical preservatives stimulated researchers to explore the use of natural preservatives as alternatives to chemical preservatives. Plants are valuable sources of bioactive compounds with antimicrobial activities. Plant extracts that contain bioactive compounds with antimicrobial activities are among the natural preservatives that have been widely investigated as alternatives to chemical preservatives in food. Alkaloids, phenolics, terpenoids, flavonoids, tannins, and saponins are among the bioactive compounds with antimicrobial properties that are present in plants (Saleem *et al*., 2010).
Pinto *et al*. (2023) reported that plant antimicrobials represent the main group of natural preservatives. Plant-based preservatives are considered to be safer and healthier than synthetic chemical preservatives. Increasing interest in the bio-preservation of food has led to the exploration of different plant extracts for antimicrobial activities by various researchers (Beya *et al*., 2021). Vinegar (*Andrographis paniculata*) leaf extract is one of the plant extracts that have been reported to possess several bioactive compounds (Adesina *et al*., 2023) and antimicrobial activities (Idorenyin *et al*., 2020). Quantitative and qualitative analysis of *A. Paniculata* leaf extract revealed that it contains alkaloids, flavonoids, tannins, saponins, terpenoids, and phytosterol (Abiodun *et al*., 2021). The plant has gained significant attention due to its potential therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant effects. Furthermore, in the southern part of Nigeria where its locally referred as (*meje meje*) has been used traditionally by direct consumptions of the leaves pulverized form, chewing of the directly or through decoction which is orally administered for treatment of malaria (Olufunke, 2001). Several studies have reported its efficacy against common food-borne pathogens, such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp*, and *Candida albicans*. The antimicrobial activity is attributed to the presence of bioactive compounds, particularly andrographolide, which disrupt the microbial cell membrane and inhibit their growth. Bread is one of the most important and convenient types of food consumed by people around the globe including Nigeria. One of the problems associated with bread is that it is prone to microbial attack which limits its shelf-life at ambient and refrigeration storage. Bread contamination occurs after baking, during cooling, slicing, packing, and packaging which are usually done manually. Spoilage of bread and other bakery products is mainly caused by molds and yeast and occasionally by bacteria such as *Bacillus subtilis* (Saranraj and Sivasakthivelan (2015).

Statement of Research Problem

Microbiological spoilage by bacteria, yeast, and mould consists of visible mould growth, invisible production of mycotoxins and formation of off-flavors, which might be produced before fungi out-growth is visible. Spoiled bread hence represents a matter of concern, as it causes food waste (i.e, 5-10% world bread production losses) and economic losses both for the bakery industry and the consumers, as well as human intoxication due to contamination with fungi mycotoxins.

Despite the traditional medicinal uses of Vinegar (*Andrographis paniculata)* and its known antimicrobial properties, there is limited scientific research on its potential application as a natural preservative in food products, particularly in bread. The ingredients used in bread production promote the growth and proliferation of these microorganisms. Chemical preservatives such as calcium propionate are commonly incorporated in bread to increase its microbiological shelf life. However, this could pose health risks to consumers on prolonged exposure. The use of bio-preservatives as an alternative method of bread preservation has gained considerable interest in recent years. Thus, there's need for investigation of the effect of *Andrographis paniculata* and the spoilage microorganisms in bread.

Objectives of the Study

The aim of this research is therefore to:

- Determine the proximate composition of *Andrographis paniculata* ethanolic leaf extract
- Identify and quantify the phytochemicals in *Andrographis paniculata* ethanolic leaf extract
- Assess its antimicrobial activity on selected food spoilage organisms as well as its preservative effect on bread

Materials and Method

Source of Materials

Fresh *Andrographis paniculata* (vinegar) leaves were obtained from the Green House of the Department of Crop Science, Faculty of Agriculture, University of Uyo, Nigeria, and were taken to the Department of Food Science and Technology Laboratory for processing and analysis.

Processing of Materials

Production of dried *A. paniculata* leaf powder

Fresh *Andrographis paniculata* leaves were washed thoroughly in distilled water, drained, and shade-dried for about seven (7) days. They were protected from direct sunlight throughout the drying period. The dried leaves were ground into powder, sieved, packaged in an airtight plastic container, and kept at room temperature for further processing. (Nagajothi *et al*., 2018)

Production of dried *Andrographis paniculata* leaf extract

The *Andrographis paniculata* leaf extract was prepared according to the method described by Mishra *et al*., 2012). The fine powder (300g) was macerated in 400 ml of 70% ethanol solution for 72 hours and stirred intermittently. It was filtered using Whatman No.1 filter paper. The solution was evaporated under vacuum at 40°C to constant weight, packaged in an airtight plastic container, and kept in a refrigerator for subsequent use and analysis.

The flow chart for the preparation of *Andrographis paniculata* leaf extract is shown in figure 1.

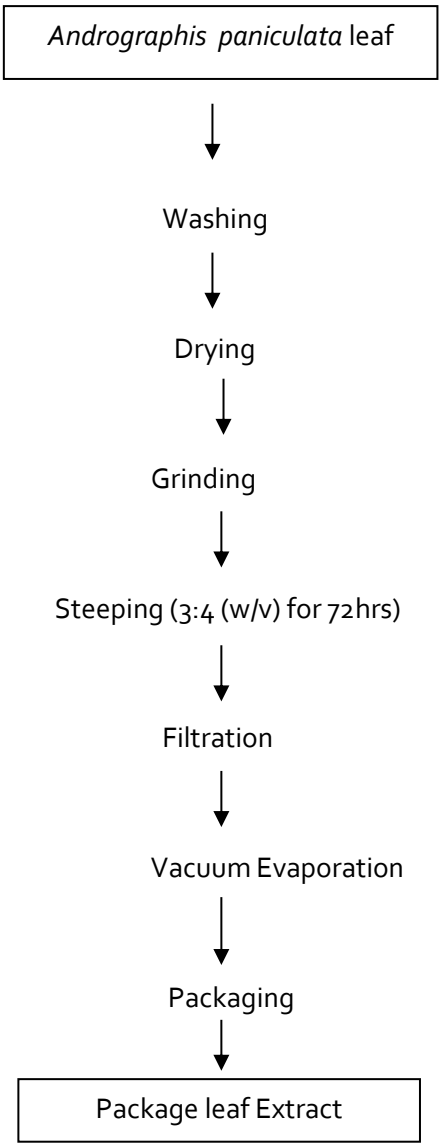

Fig 1: Flow chart for *Andrographis paniculata* leaf extract preparation (Mishra *et al*., 2012)

Ingredient formulation for bread production

The ingredient formulation used for the preparation of bread incorporated with graded doses of *Andrographis paniculata* extract is shown in Table 1

Table 1: Ingredient formulation for bread production

Ingredients	A	B	C	D	E	F
Flour (g)	100	100	100	100	100	100
yeast (g)	3.0	3.0	3.0	3.0	3.0	3.0
sugar (g)	2.5	2.5	2.5	2.5	2.5	2.5
salt(g)	1.5	1.5	1.5	1.5	1.5	1.5
water (ml)	60	60	60	60	60	60
*Andrographis Paniculata (*g)	-	0.1	0.2	0.3	0.4	0.5

Source: (Bredariol *et al*., 2019)

Bread production

The bread was produced using the formulation shown in Table 1. The leaf extract was included in the formulation at the levels of 0.00, 0.10, 0.20, 0.30, 0.40, and 0.50g. Bread produced with no *Andrographis paniculata* leaf extract inclusion served as the control sample. The straight dough method described by Bredariol *et al*. (2019) was followed in preparing the bread samples. The ingredients were mixed at low speed for 18 minutes. After this, the dough was placed to rest for 10 minutes at room temperature divided into portions, and placed to rest for another 15 minutes. After the 15 minutes, the portions were modeled using a molder (MP500, Prática, Brazil). Bread doughs were then fermented at 35 °C and 85% of relative humidity for 1 hour. The doughs were baked at 200 °C for 14 minutes, cooled, and packaged in polyethylene bags. The produced bread was stored at ambient temperature (29±2°C) for seven days and samples were drawn daily for microbial load determinations

Analytical Procedures

Determination of proximate composition of the leaf extract

The moisture, crude protein, fat, crude fiber, and ash contents in the extract were determined following the methods described by (AOAC, 2010) The carbohydrate content was determined by difference (Ihekoronye and Ngoddy,1985).

Qualitative screening of phytochemicals in the extract

The presence of phytochemicals in *Andrographis paniculata* leaf extract was qualitatively determined using standard assays as described by Shaikh and Patil (2020). Standard procedures were followed: flavonoids; Alkaline regent test was used, Saponin; Foam test was used, Tannins; Gelatine test was used, Alkaloids; Mayers test was used, Terpenoids; Salkowski test was used, Cardic glycoside; Keller-Killani test was used, Anthraquinones; Borntrager's test was used and Glycoside; Borntrager's test was used.

Quantitative determination of phytochemicals in the extract

The quantitative determinations of saponins, tannins, alkaloids, glycosides, flavonoids, terpenoids, cardiac glycosides, and anthraquinones were conducted by standard assays described by Trease and Evans (2002).

Antibacterial screening

The potential antibacterial activity of *Andrographis paniculata* extract was studied through the agar well diffusion method as described by Mazumder *et al*. (2004). The sterile petri dish was filled with 25ml of Muller Hinton agar and allowed to solidify. Before streaking the plates with bacterial culture, 5mm diameter wells were punched in the medium using a sterile cork borer. After the agar was solidified the bacterial cultures were inoculated by spreading in the Petri plates using sterile cotton swabs. Then 0.1ml, 0.2ml, 0.3ml, 0.4ml, and 0.5ml respectively of *Andrographis paniculata* extract in peptone water was directly applied to the well-made on the surface of Muller Hinton agar containing bacterial lawn. Positive control was maintained with antibiotics streptomycin (3mg) and wells containing solvent alone were maintained as negative control. The inoculated plates were incubated overnight at 37◦C following the bacterial growth, and the diameter of the zone of inhibition was measured in mm.

Test microorganisms for antimicrobial activity

To evaluate the antimicrobial activity of *Andrographis paniculata* extracts, fifteen species of microorganisms were selected, namely *Staphylococcus aureus*, *Lactobacillus, Micrococcus sp, Bacillus subtilis, Enterobacter sp, Pseudomonas sp, Bacillus cereus, Streptococcus sp, Citrobacter sp, Aspergillus niger, Fusarium sp, Saccharomyces sp, Rhizopus sp, Mucor sp, Candida sp*. All these organisms' species were isolated from selected food samples sub cultured in different culture media and used for antimicrobial susceptibility tests.

Characterization of bacterial isolates

All isolates were sub-cultured to obtain a pure culture. Gram- staining technique was done thoroughly. Identification of the isolates was carried out based on the standard method used by Cheesbrough (2002)

Results and Discussion

Proximate composition of *Andrographis Paniculata* leaf extract

Proximate analysis plays an important role in assessing the nutritional significance role in foods (Pandey *et al*., 2016). The proximate composition of *Andrographis paniculata* dried leaf extract obtained in the study is presented in Table 2.

Table 2: Proximate Composition of *Andrographis paniculata* leaf Extract

Proximate Parameter	Composition (%)
Moisture	4.09±
Crude protein	18.25±
Fat	1.13±
Ash	2.55±
Crude fiber	6.45±
Carbohydrate	67.53±

The result showed that the dried leaf extract had a low moisture content (4.09%). The moisture content value obtained in the present study was lower than 5.7% and 7.1% reported by Adamu *et al*. (2017) and Tawio *et al*. (2023) for *Aspillia kotschyi* and *Adeneanthera pavonin* leaf extracts respectively. The low moisture content of the extract implies that it would be stable during storage as spoilage agents require high moisture content for their activities. The protein content in the dried leaf extract was relatively high (18.25%). The crude protein value was higher than 8.05% and 10.37% reported by Tawio *et al*. (2023) and Adamu *et al*. (2017) for *Adeneanthera pavonin* and `*Aspillia kotschyi* dried leaf respectively. The observed differences in crude protein content could be due to variations in the chemical composition of the plant leaves used for preparing the extracts. Protein is nutritionally significant in food as a source of amino acids in diets and plays a part in the organoleptic properties of food (Ogundele *et al*., 2012). It is an essential food component needed in our bodies to repair, regulate, and protect the body. In addition, essential body processes such as nutrient transport and muscle contractions require protein to function properly and the formation of enzymes and hormones as well as antibodies that fight infection (Brosnan and Brosnan,(2013). The extract had a low fat content (1.13%). The fat content was higher than values (0.85%) and (0.83%) reported by Tawio *et al*. (2023) and Adamu *et al*.(2017) for *Adeneanthera pavonin* and `*Aspillia kotschyi* dried leaf respectively. The ash content of the dried extract was 2.55%. the value obtained for ash content was lower than 4.03% and 5.09% reported by Adamu *et al*. (2017) and Tawio *et al*. (2023) for *Aspillia kotschyi* and *Adenanthera pavonia* dried leaf extract respectively. The ash content in food products is an indication of the level of mineral content in such food. The result showed that the *A.paniculata* leaf extract would be a fair source of mineral elements. The dried extract exhibited high crude fiber content (6.45%). The value obtained for the leaf extract was however lower than 9.06% and 11.09% reported by Adamu *et al. (*2017) and Tawio *et al. (*2023) for *Aspillia kotschyi* and *Adenanthera pavonin* dried leaf extracts. Crude fiber is beneficial to one's health since they have been linked to a lower incidence of various illnesses (Ajani *et al*., 2016). It is also essential for the digestion of food materials in the food canal of animals (Manalisha *et al*., (2013), aids in the absorption of trace elements in the glut (Robinson, (1978), and reduces the absorption of cholesterol (Kelsay, 1981). The result showed that extract was a good source of carbohydrates. The carbohydrate content in the extract was (67.53%) A similar observation was made by (Tawio *et al*. (2023) who reported

a carbohydrate content of 67.79% for *Adenanthera pavonin* dried leaf extract. Carbohydrates are an essential energy-given nutrient in food.

Phytochemical profiling of *Andrographis Paniculata* leaf extract

The result of qualitative screening and quantitative analysis of *Andrographis paniculata* ethanolic leaf extract is presented in Table 3.

Table 3: Qualitative and Quantitative Analysis of phytochemicals in Andrographis paniculata leaf extract

Phytochemicals	Qualitative screening	Quantity of phytochemicals (mg/g)	
Alkaloids	++	1.17±0.19	
Saponins	-	ND	
Tannins	++	4.73±0.13	
Flavonoids	+	0.73±0.00	
Cardiac Glycosides	+	0.50 ±0.05	
Terpenes	+++	11.48±0.36	
Glycoside	+++	89.48 ± 0.73	
Anthraquinones	-	ND	

ND= NOT DETECTED

The result revealed the presence of alkaloids (1.17mg/g), flavonoids (0.73mg/g), tannins (4.73mg/g), terpenes (11.48mg/g), cardiac glycosides (0.50mg/g) and Glycosides (89.48) while saponins and anthraquinones were not detected in the extracts. The quantitative analysis of the phytochemical showed that the most dominant phytochemical present in the leaf extract was glycoside (89.49mg/g) and was followed by terpenes (11.48mg/g) and tannin (4.73mg/g). Bharagavi and Kaloori (2018) similarly reported the presence of all these phytochemicals in ethanolic extract of *Andrographis paniculata* including saponin and anthraquinones that were not detected in this study. Also, Nagajothi *et al*. (2018) reported that except for glycoside that was found to be negative in the ethanolic leaf extract of *Andrographis paniculata*, alkaloids, flavonoids, tannins, saponin, cardiac glycosides, and terpenoids were detected and quantified. The differences in the phytochemicals that are not present in the leaf extract in this study and those reported by other researchers could be due to the genetic makeup, growing regions, and agro-climatic conditions. Pandey *et al*. (2019) had earlier reported that the genetic makeup, growth site, and a wide range of environmental factors interfere with the synthesis and accumulation of relevant secondary metabolites in plants.

The phytochemical compounds are plant-defense chemical compounds produced in plant tissues (Chaudhary *et al*., 2020), The presence of flavonoids is known to inhibit tumor growth and protect against gastrointestinal infection. (Rajagopal *et al*.,2003) Some of these bioactive compounds are formed as secondary metabolites along with the growth of plants

which also function as antimicrobial substances to protect plants against microbial invasion(Hsieh *et al*., 2016). Alkaloids have been used to treat diseases like malaria, and pain killers, and manage heart diseases (Oomah,2003). In addition, alkaloids and flavonoids are water-soluble antioxidants that function as anti-free radicals that prevent oxidative cell damage. Tannins at low concentrations can inhibit the growth of microorganisms and act as an anti-fungal agent at higher concentrations with a coagulation effect on the protoplasm of microorganisms as reported by Adekunle and Ikumapayi, (2006). Generally, glycosides serve as a defense mechanism against predation by many microorganisms, insects, and herbivores.

Antimicrobial activity of Andrographis Paniculata leaf extract on selected food spoilage organisms

The antimicrobial activity of *A.paniculata* leaf extract is presented in Table 4. The results showed that the extract of *Andrographis paniculata* had a concentration-dependent antibacterial activity with more sensitivity for gram-negative bacteria than gram-positive bacteria used in the study. The extract showed considerable antibacterial activity at all five (5) concentrations (0.1, 0.2, 0.3, 0.4, and, 0.5g respectively). The study clearly showed that the ethanolic extract of *Andrographis paniculata* acted as a significant growth inhibitor against a broad spectrum of pathogens and as a potent antimicrobial activator. Also, the extract exhibited varying degrees of inhibitory activity against the growth of all the bacteria tested. The minimum antimicrobial activity of the extract was exhibited against zone inhibition ranging from 12mm at 0.4 mg/ml to 13mm at 0.5g respectively. The maximum antimicrobial activity of the extract of *Andrographis paniculata* was exhibited against Pseudomonas sp, with zone inhibition ranging from 12mm at 0.2g concentration of the extract to 18mm at 0.5mg/ml. The effect of *Andrographis paniculata* leaf extract on the test organisms may be due to the presence of the above phytochemical components. The various phytochemical constituents found are known to be beneficial in medicinal science. According to the results of Humnabadkar and Kareppa (2012), chloroform extract of *Andrographis paniculata* inhibited the growth of *Staphylococcus aureus* with zone of inhibition ranging from (10 – 16mm) and showed no inhibitory growth on *Pseudomonas aeruginosa* also reported that acetone and alcohol extract of *Andrographis paniculata* exhibited higher inhibitory activity against *Bacillus substilis* and *Staphylococcus aureus*. Furthermore, research was conducted on other plants. Turker *et al*. (2009) examined the aqueous and alcoholic extracts of *Nupharlutea*, *Nymphaea*, *Alba*, *Vinca minor*, and *Fragaria* herbs of Turkey and reported antibacterial activity against *Enterococcus*, *Streptococcus agalactiae*, and *Yersinia ruckeri* bacteria isolated from fish.

The results showed that seven (7) bacteria species) out of 15 bacteria species that were isolated from the food products, had minimum growth which shows that the extract exhibited potency against the bacteria species, the extract also had no antibacterial potency for *Citrobacter sp*, *Aspergillus niger*, *Fusarium sp*, *Saccharomyces sp, Rhizopus sp*, *Mucor sp*, *Candida sp*, and *enterobacter sp*. the *A.paniculata* ethanolic extract possess antimicrobial activity as they could inhibit the growth of tested food pathogens and

spoilage organisms. The different percentages of microbial growth inhibition can be attributed to different chemical compositions and modes of action of the plant extract (Urzua *et al*.,2006). Results obtained from this study indicated that the extracts showed the highest antimicrobial activity against bacteria than fungal isolates. It has been reported that gram-negative bacteria are usually more resistant to plant-originated antimicrobials and even show no effect compared to gram-positive bacteria (Stefanello *et al*., 2008). Generally, gram-positive bacteria are more sensitive to plant extract because of the presence of the mesh-like peptidoglycan layer which is more accessible to permeation by the extracts (Tajkarimi *et al*., 2010). The resistance of the gram-negative bacteria could be attributed to its cell wall structure. Gram-negative bacteria have a powerful permeability barrier, composed of a thin lipopolysaccharide exterior membrane which could restrict the penetration of the extruding plant extract. The finding indicates that the plant extract tested in this study could be used as a natural preservative agent in food to eliminate or control the growth of spoilage and pathogenic organism.

Table 4: Preliminary screening of antimicrobial activity of ranging concentrations of ethanolic extracts of *Andrographis paniculata*

Organism	Concentration (mg/ml) 0.1mg/ml Inhibition zone diameter (mm)	Concentration (mg/ml) 0.2mg/ml I inhibition zone diameter (mm)	Concentration (mg/ml) 0.3mg/ml Inhibition zone diameter (mm)	Concentration (mg/ml) 0.4 mg/ml Inhibition zone diameter (mm)	Concentration (mg/ml) 0.5mg/ml Inhibition zone diameter (mm)
Aspergillus niger	-	-	-	1.2	1.3
Micrococcus	-	-	1.1	1.2	1.7
Enterobacters	-		1.1	1.1	1.2
Saccharomyces sp	-	-	-	-	-
Staph aureus	-	1.1	1.1	1.1	1.1
Bacillus cereus	-	1.3	1.5	1.7	1.8
Lactobacillus	-	-	1.2	1.4	1.7
pseudomonas sp	-	1.2	1.5	1.5	1.8
Fusarium sp	-	-	-	-	-
Citrobacter sp	-	-	-	-	-
Rhizopus sp	-	-	-	-	-
Mucor sp	-	-	-	-	-
Streptococcus sp	-	-	-	-	-
Candida sp	-	-	-	-	-

Effect of *A. paniculata* leaf extracts inclusion on the microbial load of Bread during storage

Bread is a rich source of nutrients that promote the growth and proliferation of spoilage and pathogenic microorganisms during storage. This contributes to the short shelf-life of bread at ambient temperature. The incorporation of natural or chemical preservatives as one of the ingredients during bread making is aimed at extending the period that it would remain safe for human consumption. The effects of incorporating graded levels of *A. paniculata* dry leaf extract on the total plate counts of heterotrophic bacteria, fungi, and coliform in bread during storage (in days) are presented in Tables 5,6 and 7 respectively. The result showed that the number of heterotrophic bacterial counts in the bread was affected by the storage period and the level of extract in the bread (Table 5). The freshly prepared bread for all the samples (day 0) recorded no growth of heterotrophic bacteria count. This might be because during baking, the high temperature of the oven killed most of the bacteria in the dough thereby rendering the bread free of microorganisms when it came out of the oven. Similar observations had been reported by other authors for wheat flour bread (Ijah *et al*., 2014). However, bread contamination can occur at various post-baking steps, including cooling, slicing, packaging, transportation, and storage which are carried out manually. Bread handlers could also contribute to the contamination of bread. During storage of the bread samples, the heterotrophic bacteria counts increased for all the samples with storage time. However, it was observed that the control sample recorded higher counts than all the samples with *A. paniculata* dry leaf incorporation. A similar observation was reported by Al-shammari, (2024). Although all the samples that contained leaf extract recorded an increase in the number of heterotrophic bacteria with storage time, there was a reduction in their number with an increase in extract level. While the counts for the sample that contained 1g extract per 100g of flour increased from 2.0×10^3cfu/g on day 1, to 1.5×10^4cfu/g on the last day of storage, the sample with 5g of extract per 100g of flour increased from 1.9×10^3cfu/g on day 1 to 1.0×10^4cfu/g on the last day of storage. This shows that higher levels of extract were able to control bacterial growth more than the lower levels. According to international microbiological standards, Total Heterotrophic bacteria (THBC) of dry ready-to-eat (RTE) foods should be below 10^3cfu/g. An aerobic bacterial count not exceeding 100cfu/g is recommended by the Standard Organization of Nigeria for bread. The addition of plant extracts to bakery products is seen as a future solution to improve their nutritional and functional properties due to the bioactivity derived from the phytochemicals of the plant ingredients (Gao *et al*., 2022). The result showed that the inclusion of the extract in bread formulation would lead to an increased shelf life of bread compared to the control sample.

Fungi are one of the major organisms usually associated with the spoilage of bread during storage. The result obtained from this study (Table 6) showed that no growth of fungi was detected for all the freshly prepared bread samples (day 0). This could be attributed to the destruction of microorganisms that might be present in the dough by the oven temperature during baking.

This observation is in agreement with the report by Ijah *et al*. (2014) for freshly prepared bread samples from wheat flour. During storage at ambient conditions, the fungi counts increased exponentially for all the bread samples with the control sample having the highest counts each day the bread samples were checked for fungi counts. The fungi counts for the control sample increased from 1.3×10^3cfu/g on day 1 to 2.7×10^4cfu/g on the 7th day of storage. It was found that fungi count for the bread samples that contained *A. paniculata* dry leaf extract as preservatives depended on the level of extract in the bread. Although the fungi count generally increased with storage time, it was observed that each day enumeration, bread samples with lower doses of *A. paniculata* leaf extract recorded higher fungi counts than those with higher levels of extract inclusion. A similar observation was reported by Al-Shammari (2024). The fungi count for bread samples with 1% extract inclusion increased from 1.2×10^3cfu/g on the first day of storage to 2.5×10^4cfu/g on the 7th day of storage while the fungi count of bread samples with 5% extract inclusion increased from 1.0×10^3cfu/g on the 5th day storage to 1.4×10^3cfu/g on the 7th day of storage. It was observed that the bread sample that contained 1% of the leaf extract was shelf stable for 3 days; and the samples with 2 to 4% extract were shelf stable for 4 days while the sample with 5% extract level was shelf stable for 6 days as their fungi counts were within the safe limit of 103cfu/g recommended by the standard organization of Nigeria (Khanom *et al*., 2016). The preservative effect of the leaf extract could be attributed to the antifungal effect of the constituents in the extract.

As expected, no coliform growth was detected in all the bread samples. A similar observation was reported by Alpers *et al*. (2021).

Table 5: Effect of storage on Total heterotrophic bacterial count (THBC) in the bread (cfu/g)

Storage time(days)	A	B	C	D	E	F
0	NG	NG	NG	NG	NG	NG
1	1.7×10^3	1.9×10^3	2.1×10^3	2.2×10^3	2.0×10^3	1.9×10^3
2	2.3×10^3	1.7×10^3	1.9×10^3	1.8×10^3	1.9×10^3	1.7×10^3
3	2.4×10^3	1.7×10^3	1.8×10^3	1.6×10^3	1.7×10^3	1.5×10^3
4	2.7×10^4	1.7×10^3	1.8×10^3	1.5×10^3	1.5×10^3	1.3×10^3
5	2.9×10^4	1.6×10^3	1.7×10^3	1.5×10^3	1.4×10^3	1.2×10^3
6	3.0×10^4	1.6×10^3	1.6×10^3	1.5×10^3	1.2×10^3	1.1×10^3
7	3.2×10^4	1.5×10^3	1.5×10^3	1.4×10^3	1.1×10^3	1.0×10^3

Keys: A= control sample, B = Sample with 0.1g/100gflour, C= sample with 0.2g/100gflour, D=sample with 0.3g/100gflour, E = sample with 0.4g/100gflour, F =sample with 0.5g/100gflour, NG = No growth

Table 6: Effect of storage on Total fungal count (TFC) in the bread (cfu/g)

Storage time(days)	A	B	C	D	E	F
0	NG	NG	NG	NG	NG	NG
1	NG	NG	NG	NG	NG	NG
2	1.3×10^3	1.2×10^3	NG	NG	NG	NG
3	1.8×10^3	1.5×10^3	1.1×10^3	NG	NG	NG
4	1.8×10^4	1.9×10^3	1.3×10^3	1.6×10^3	1.1×10^3	NG
5	2.1×10^4	2.1×10^3	1.5×10^3	1.5×10^3	1.1×10^3	1.2×10^2
6	2.3×10^4	2.5×10^3	2.2×10^3	1.5×10^3	1.3×10^3	1.1×10^2
7	2.7×10^4	2.7×10^3	2.5×10^3	1.4×10^3	1.5×10^3	1.4×10^2

Keys: A= control sample, B = Sample with 0.1g/100gflour, C= sample with 0.2g/100gflour, D=sample with 0.3g/100gflour, E = sample with 0.4g/100gflour, F =sample with 0.5g/100gflour, NG = No growth

Table 7: Effect of storage on Total coliform count (TCC) in the bread (cfu/g)

Storage time(days)	A	B	C	D	E	F
0	NG	NG	NG	NG	NG	NG
1	NG	NG	NG	NG	NG	NG
2	NG	NG	NG	NG	NG	NG
3	NG	NG	NG	NG	NG	NG
4	NG	NG	NG	NG	NG	NG
5	NG	NG	NG	NG	NG	NG
6	NG	NG	NG	NG	NG	NG
7	NG	NG	NG	NG	NG	NG

Keys: A= control sample, B = Sample with 0.1g/100gflour, C= sample with 0.2g/100gflour, D=sample with 0.3g/100gflour, E = sample with 0.4g/100gflour, F =sample with 0.5g/100gflour, NG = No growth

Conclusion

The extract of *Andrographis paniculata* showed the presence of a wide array of phytochemicals with terpenes, glycosides, and tannins as the most abundant. Also, the plant is a rich source of carbohydrates, protein, and crude fibers. Furthermore, the extract of *A. Paniculata* exhibited potent antimicrobial activity against selected food spoilage microorganisms such as *Micrococcus sp*, *B. Cereus*, *Lactobacillus sp*, and *Pseudomonas sp*. In addition, the extract showed potent preservative potential when incorporated into bread at concentrations of 0.3, 0.4, and 0.5 g/100gflour exhibited its potency thereby extending the shelf life of the bread.

Recommendations

From the above result;

- It is recommended that *A. paniculata* extract could be used as a preservative by home and commercial bakers at 0.3 – 0.5 g/100g flour concentrations.
- Also, further research should be carried out to assess the sensory characteristics of the bread at production and after storage.

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