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Proximate Composition, Phytochemical and Antimicrobial Activities of The Fermented Ripe Fruit of Musa Paradisiaca

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Abstract

Fermentation alters the physical and chemical components of foods by enhancing their nutrients and synthesizing bioactive composition. To determine the impact of fermentation, ripe *Musa paradisiaca* (Plantain) fruit used as food and medicine across the globe was fermented and analyzed for pH, proximate composition, preliminary phytochemical constituents, *in vitro* antimicrobial activities, and active constituents by High-Performance Liquid Chromatography (HPLC). The pH of the sample dropped from 6.7 to 4.7 during fermentation while alkaloids, flavonoids, glycosides, saponins, steroids, tannins, and terpenoids were recorded. Moisture (7.84%), ash (2.10%), fat (7.28%), carbohydrate (86.4%), fiber (1.42%), and protein (2.24%) were present. Bactericidal (12.5-50.0 mg/mL against *Staphylococcus aureus* and *Escherichia coli,* respectively) and fungicidal activities (12.5-25.0 mg/mL against *Candida albicans*) were noted. At 25, 50, and 100 mg/mL sample concentrations, diameters of 7.00 mm, 10.00 mm, and 16.00 mm were recorded against *C. albicans, E. coli,* and *S. aureus.* Capsaicin, caffeic acid, beta-sitosterol, kaempferol, apigenin, syringin, quercetin, and luteolin were detected by HPLC. Consequently, the fermented ripe fruits of *M. paradisiaca* harbours active constituents and exhibit antimicrobial activities making them curative against pathogens.

1. Introduction

The bioavailability, efficacy, fewer side effects, better accessibility, and affordability [1, 2] of medicinal plants make it the most frequently used form of traditional medicine [3]. The World Health Organization (WHO) described traditional medicinal plants as those that are used without industrial processing for the management of diseases in a region [4]. Fruits with medicinal values are referred to as nutraceuticals or functional foods [5, 6]. *Musa paradisiaca* (Plantain*)* belonging to the Musaceae family, is a popular food crop growing up to 9 m long worldwide. The edible part of the crop is the fruit (finger) which is formed at maturity of the rhizome. It is known by different tribes in Nigeria as *Ogede agbagba* (Yoruba), *Abrika/okirima* (Igbo), *okamu ayaba (*Hausa).

M. paradisiaca harbors several nutrients and bioactive metabolites making different parts of the plant to be used for the management of ailments. Traditionally, unripe fruit and flowers have similar applications against ailments [7]. They are used in the control of gastrointestinal conditions including diarrhea, dysentery, and intestinal lesions in ulcerative colitis, menorrhagia, diabetes, uremia, nephritis, hypertension, and cardiac

disease [7, 8]. Aqueous extract of the fermented unripe fruits and peels possess anti-ulcerogenic, antimicrobial, and antioxidant [9]. Also, the leaves and roots have wound-healing potential [10], they are used to treat eczema, possess anthelmintic potentials, and are used to control blood disorders and venereal diseases [7].

Due to the perishable nature of some foods, fermentation techniques have become an important technology to preserve and enhance the nutritional and medicinal values of foods. However, despite the numerous applications of unripe *M. paradisiaca* and its peel in traditional medicine, there is sparse information on the fermented ripe fruit of *M. paradisiaca*, its composition, and its medicinal relevance. Being a commonly edible fruit plant that is prone to rapid spoilage, the ripe fruit of *M. paradisiaca* was fermented in this study in order to expand the available information on it and produce new fermented products with improved quality and quantity of bioactive constituents. Hence, this study was undertaken to investigate the composition and therapeutic potentials of the fermented ripe fruit of *Musa paradisiaca* with the hope of preserving it, promoting its composition, and improving its medicinal potential.

2. Materials and Methods

2.1 Materials

2.1.1 Collection and Preparation of the Study Material

Fresh and healthy ripe fruits of *M. paradisiaca* of Nigerian origin were purchased from Mandate market, Adewole area, Ilorin, Nigeria. They were authenticated at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Nigeria, where a voucher specimen was deposited (UILH/003/1381/2021). The ripe fruits were cleaned to remove stones, peeled using a clean sharp knife, cut into smaller sizes, and carefully rinsed with distilled water. It was then slightly mashed using a sterile mortar and pestle to increase the surface area of the fruit for microbial activities during fermentation.

2.1.2 Preparation of the Experimental Sample

The experimental sample was prepared in a sterilized Mason by mixing 620ml of distilled water and 17g of iodized salt (to promote the growth of beneficial microorganisms, inhibit the growth of spoilage organisms, and inhibit potential pectinolytic and proteolytic enzymes that may aid putrefaction from softening.) to make the brine solution. A known quantity (200g) of the mashed fruit was transferred into the jar in a ratio of 1:3 w/v (mashed sample to the brine solution). A sterilized depressing glass (to suppress the mesh and avoid contamination) was inserted and the airlock was tightly covered. This setup was kept on the desk at room temperature (28±2°C). However, the airlock was checked daily to ensure it did not dry up and the cover was turned daily and stirred twice daily to encourage natural microbial growth as well as multiplication [11]. After the 14th-day fermentation period, the sample was filtered using cheesecloth, and the filtrate was used immediately. Working concentrations were prepared by adding 2 g of the sample to a test tube containing 20 mL of distilled water, from which 5 mL was transferred into another test tube containing 5 mL of distilled water, also from which another 5 mL was transferred into the third tube with 5 mL of distilled water, to give concentrations of 100 mg/mL, 50 mg/mL and 25mg/mL, respectively.

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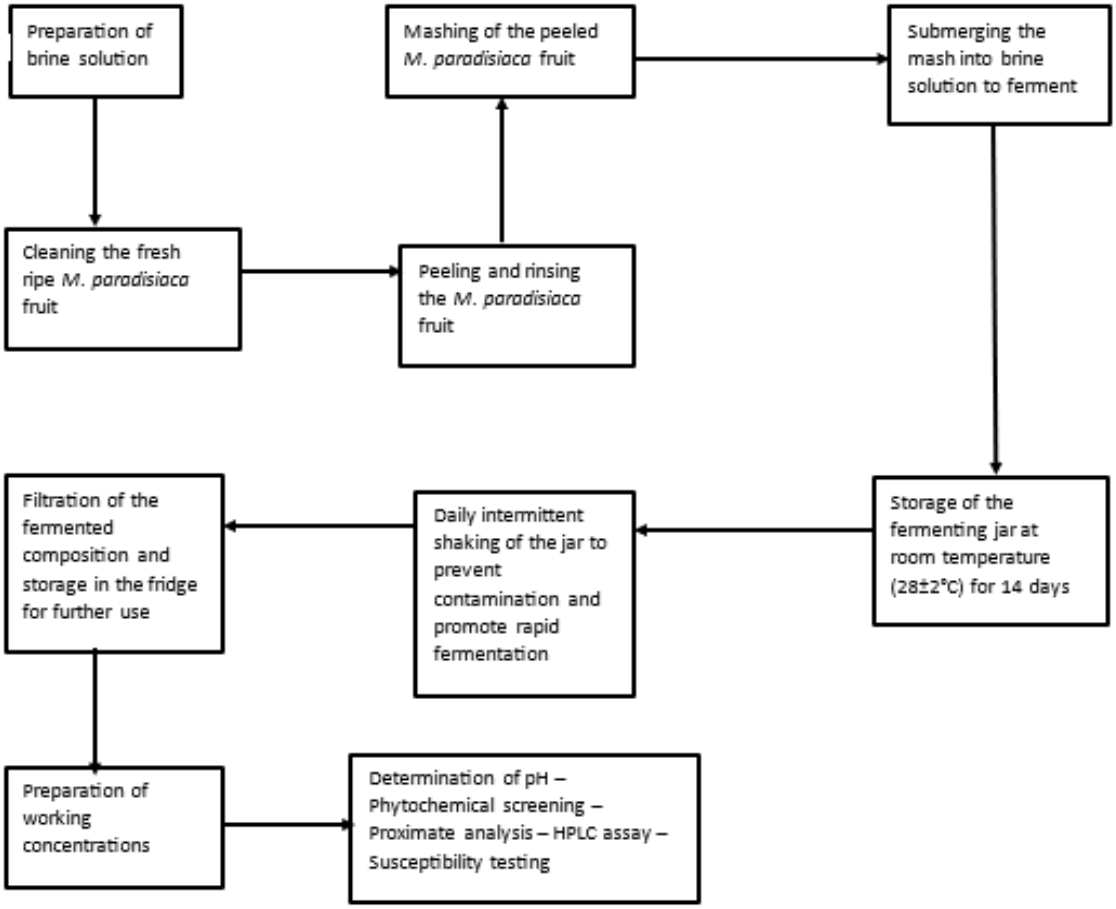

Fig 1 *pH illustration of the experimental design*

2.1.3 Collection and Standardization of the Test Organism

The isolates used in the study (*Candida albicans*, *Escherichia coli,* and *Staphylococcus aureus*) were collected (on Potato Dextrose agar slant and Nutrient agar, respectively) from the Microbiology Laboratory of Al-Hikmah University. They were sub-cultured for viability before use. Standardization of the inocula to 0.5 McFarland standard was achieved by the aseptic method using a spectrophotometer, during which, a sterilized inoculating loop was used to introduce 2-3 colonies of the organism into a sterile test tube of normal saline, and it was mixed with a vortex mixer.

2.2 Methods

2.2.1 Chemical Analysis

The pH value was determined at each 0 h, day 5, day 10, and day 14 of the fermentation period. To do this, 50mL of the sample was transferred into a clean beaker, then the pH was then measured with a pH meter (C. A. 10001, CHAUVIN ARNOUX). Each reading was taken three times, and the average value was recorded.

2.2.2 Preliminary Phytochemical Screening

This was done by employing the method described by Trease and Evans [12] to test for the presence of tannins, steroids, saponins, glycosides, terpenoids, proteins, flavonoids, anthraquinone, alkaloids, and coumarin.

2.2.3 Proximate Analysis

Moisture, ash, protein, lipids, fat, fiber, and carbohydrate contents of the sample were determined by the method described by the Association of Official Analytical Chemists [13].

2.2.4 HPLC Method

HPLC analysis was carried out using a modular chromatographic system Shimadzu (Nexeramx) LC-10 comprising an LC-10AD pump, a CTO-10A column oven, an SPD-10A UV-DAD detector, a CBM-10A interface, and an LC-10 Workstation. A C-18 column (250 mm x 4.6 mm ID x 5 mm) from (Ubondapak C18, Bellefonte, USA) was employed at 30 ^{o}C. The sample (10 g) was extracted using mobile phase with Acetonitrile/water (70:30), and the extract stabilized with ethyl acetate was introduced into a 25 mL standard flask and made up to the mark. Separations were done in the isocratic mode using mobile phase acetonitrile: water (40:60 v/v) at a flow rate of 2 mL /min; with an injection volume ("loop") of 5 µL and the UV detection was at 254 nm [14].

2.2.5 Determination of MIC/MFC

The serial broth dilution method was employed to determine the MIC/MFC of the sample concentrations against the pathogens [15]. A quantity, 1.0 mL of 100 mg/mL of the sample solution was successively transferred into 1ml of nutrient broth (Potato dextrose broth was used for the yeast). Subsequently, 0.5 ml of an 18-hour-broth culture which had previously been adjusted to 0.5 McFarland turbidity standard (1.0 x 10 ^{8} CFU/m) was inoculated into each test tube. The tubes were vortexed to mix, and the isolates were incubated at 37^{o}C for 24 h for bacteria and at 28^{o}C for 72 h for the yeast. The MIC (99.5% killing of the original inoculum) represented the tube with no turbidity compared to the control. Two sterile test tubes were set up as control (tube one contained the sample mixed with nutrient broth without the inoculum (representing the sample control) and the other contained nutrient broth, physiological saline, and the standardized inoculum (test organism control).

2.2.6 Susceptibility Testing of the Test Pathogens

The disc diffusion method was employed to check the susceptibility of the pathogen to the sample [16]. The pour plate method was used to prepare the inoculated plates by which 1 ml of the standardized inoculum was aseptically introduced into a sterilized petri dish. It was overlaid with 15- 20 mL Mueller-Hinton agar (MHA; Becton-Dickson, USA) for bacteria and Potato dextrose agar (PDA) for the yeast, respectively. The inoculated plates were swirled for proper distribution of the inoculum within the medium. The prepared discs containing the sample were distributed using sterile forceps on the inoculated agar surface (ensuring the discs were 22 mm from each other and 14 mm from the edge of the plate). The discs were gently pressed for uniform contact with the surface of the medium, then the plates were allowed to stand for 30 minutes for the diffusion of the sample from the disc into the inoculated medium. The clear zones around the discs were measured using a Vernier caliper (mm). The average of each triplicate that was done for each organism, concentration, was determined.

2.2.7 Data Analysis

The results of the proximate composition were mean values of the triplicate determinations reported with their standard deviations. Hence, values were presented as mean ± standard deviation.

3. Results and Discussion

The pH values of the sample dropped during the period of fermentation from 6.7 on day 1 to 4.7 on day 14. (Fig 2). Fruits do not only serve as nutritional but also as therapeutic agents for ages [17] as the birth of herbal medicines coincides with the evolution of isolation, purification, and discovery of plant metabolites [18]. In the present study, a spontaneous fermentation technique was employed in the preparation of the ripe fruits of *M. paradisiaca* for further studies on its compositional and medicinal values. Fermentation, a process that breaks down complex organic molecules through the controlled action of microorganisms into simpler ones [19], is no longer considered just a method of food preservation, rather, it is seen as a method that promotes the growth of beneficial microorganisms which enhance the bio-preservative effects of food and inhibit the growth of pathogens [20,21]. Thus, the decline in pH observed in this study depicts the conversion of soluble carbohydrates in the sample to organic acids which accumulate due to fermentative organisms, and this may be responsible for an increase in the shelf life of fermented foods. A similar trend of reduced pH of fermented banana peels was reported by Duponte *et al*. [22].

The proximate composition of the sample showed the presence of varying levels of moisture content, ash, fat, carbohydrate, fiber, and protein (Table 1). Fermentation biochemically modifies food to become functional agents or nutraceuticals with valued health-promoting potential [23]. Plant metabolites are useful natural sources of therapeutic agents and serve as better sources of nutrition for human well-being [24]. The considerable levels of nutritional components recorded in this study may be a function of the fermentation process which significantly degraded the toxic and anti-nutritive components of the sample to bioavailable nutrients that improve the nutritional components and enhance the organoleptic properties such as taste and texture [25, 26].

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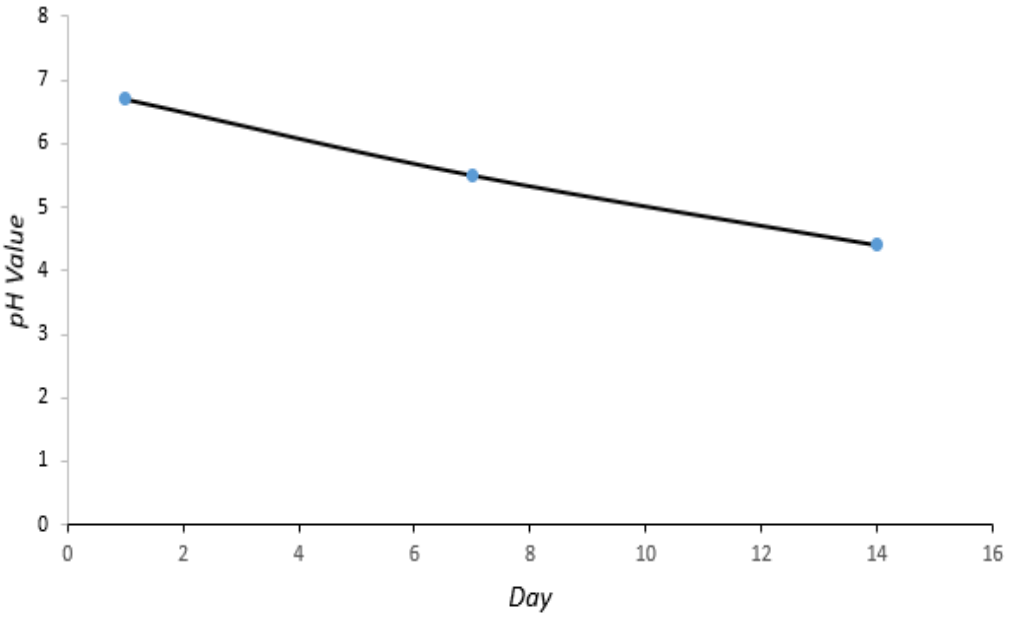

Fig. 2 *pH values of the ripe fruit of M. paradisiaca during the period of fermentation*

Moisture is a marker for measuring the shelf-life of foods, while high moisture enhances the nutritional content of foods, low moisture promotes their shelf-lives [27]. The perishable nature of *M. paradisiaca* was supported by the high moisture content recorded in this study, this corresponds to the level of moisture of the leaf and fruit peel of *M. paradisiaca* reported by Oyeyinka and Afolayan [28]. In concurrence with this study, Oko *et al*. [29] reported a low level of ash content, an essential component that aids digestion and promotes gastrointestinal health, in *M. paradisiaca*.

Lower fat content in fermented foods, as observed in this study may be due to the activities of lipolytic enzymes in fermenting foods which hydrolyze the fat content in food into fatty acid and glycerol and it may mean a better shelf life. Fatty acids were used as sources of energy by some microorganisms such as molds [30]. Consumption of a high level of dietary fat is detrimental to health [31], hence, a low level of fat in this study may signify the health-promoting potentials and an increased storage potential of the food. Carbohydrates, being the prime source of energy [32] seen in this study are in tandem with various findings on the high level of carbohydrate content in different species of Musa, respectively [33, 34].

Varying levels of protein were reported in fermented foods. This study observed a low level of protein observed. This agrees with the report that in some fermenting foods, the organisms involved are capable of using the protein monomers (amino acids) which could reduce the quality and protein level of such food [35, 23]. Fermenting microorganisms also use amino acids thereby lowering the protein content and quality of fermented food. However, the increase in the protein level of some fermented foods may be attributed to microbial degradation of proteins to peptides and amino acids [23]. Similarly, Ozabor *et al*. [36] reported a high moisture, low fat, high carbohydrate, and low protein content of fermented peels in *M. paradisiaca.*

Table 1 *Proximate composition of the fermented ripe fruits of M. paradisiaca*

Proximate parameter (%)	**Proximate value**
Moisture content	7.84 ±0.41
Ash	2.10 ± 0.31
Fat	7.28 ± 0.12
Carbohydrate	86.4 ± 0.24
Fiber	1.42 ± 0.48
Protein	2.24 ± 0.32

Values mean ± standard deviation

Phytochemical screening of the sample showed the presence of tannin, alkaloids, flavonoids, glycoside, saponin, steroids, and terpenoids (Table 2). Phytochemicals play important roles in the bioactivity of medicinal plants [37]. This study reported similar phytochemical constituents, such as alkaloids, saponin, steroids, and tannins as previously mentioned in a study on the peel of *M. paradisiaca* [38]. The presence of flavonoids, tannins, and alkaloids has been reported in the fruits of bioactive metabolites present in the fruits of *M. paradisiaca* [39, 40, 41]. Alkaloids promote hemoglobin formation and function in cell activity [42]. Ghani [7] reported the presence of tannins in the pulp of *M. paradisiaca.* Agama-Acevedo *et al.* [43] reported the presence of flavonoids in *M. paradisiaca* peel flour. However, factors such as microbial activities can reduce phytochemicals.

Table 2 *Phytochemical constituents of the fermented ripe fruits of M. paradisiaca*

Proximate parameter (%)	Proximate value
Alkaloids	+
Anthraquinones	-
Flavonoids	+
Glycosides	+
Phlobatannins	-
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+

Keys: + = present
- = absent

The MIC of the sample showed inhibitions between 12.5 mg/mL – 25 mg/mL concentrations against all the bacterial pathogens and MFC between 12.5 mg/mL – 50 mg/mL concentrations against the yeast sample (Table 3). In addition to the nutritional and phytochemical constituents of *M. paradisiaca*, corresponding antimicrobial potentials were also detected in this study. This may accentuate the antimicrobial activities by MIC and diameters of zones of inhibition recorded.

Table 3 *MIC/MFC of the fermented ripe fruits of M. paradisiaca*

Organism	Concentration (mg/mL)			
	100	**50**	**25**	**12.5**
S. aureus	-	-	+	+
E. coli	-	+	+	+
C. albicans	-	-	+	+

Keys: + = present
- = absent

The Diameter of zones of inhibition (mm) of the different concentrations of the sample showed varying degrees of concentration-dependent activities against the test pathogens (Fig 3). A higher zone of inhibition (16 mm) was obtained at 100 mg/mL concentrations against *S. aureus*, while the least zone of inhibition (7 mm) was obtained at 25mg/mL against *C. albicans.* In traditional medicine, different parts of *M. paradisiaca* are employed in the management of numerous ailments and conditions such as diarrhea, burns, diabetes, hypertension, and ulcers [9]. In concurrence with this study, Musa spp. has been reported to possess antibacterial and antifungal activities [44, 45].

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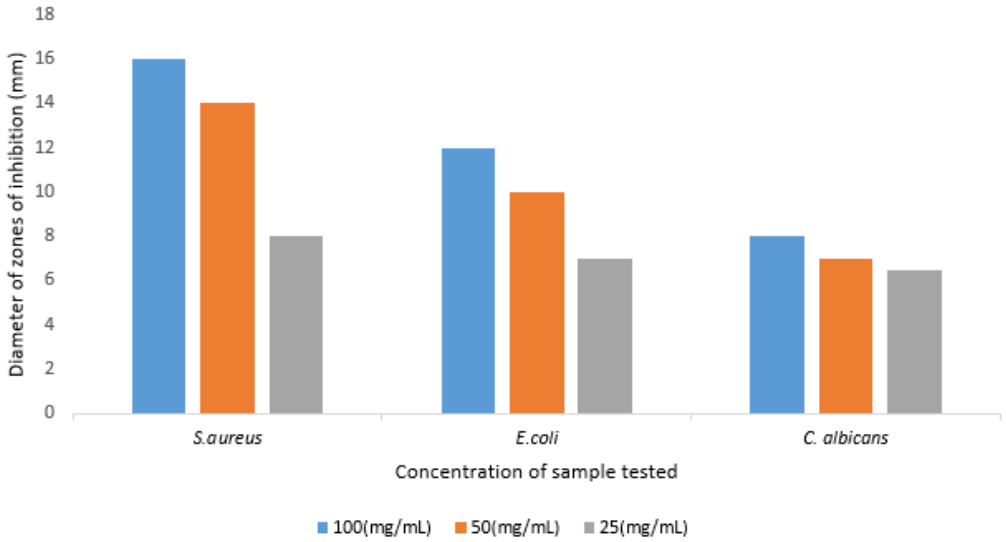
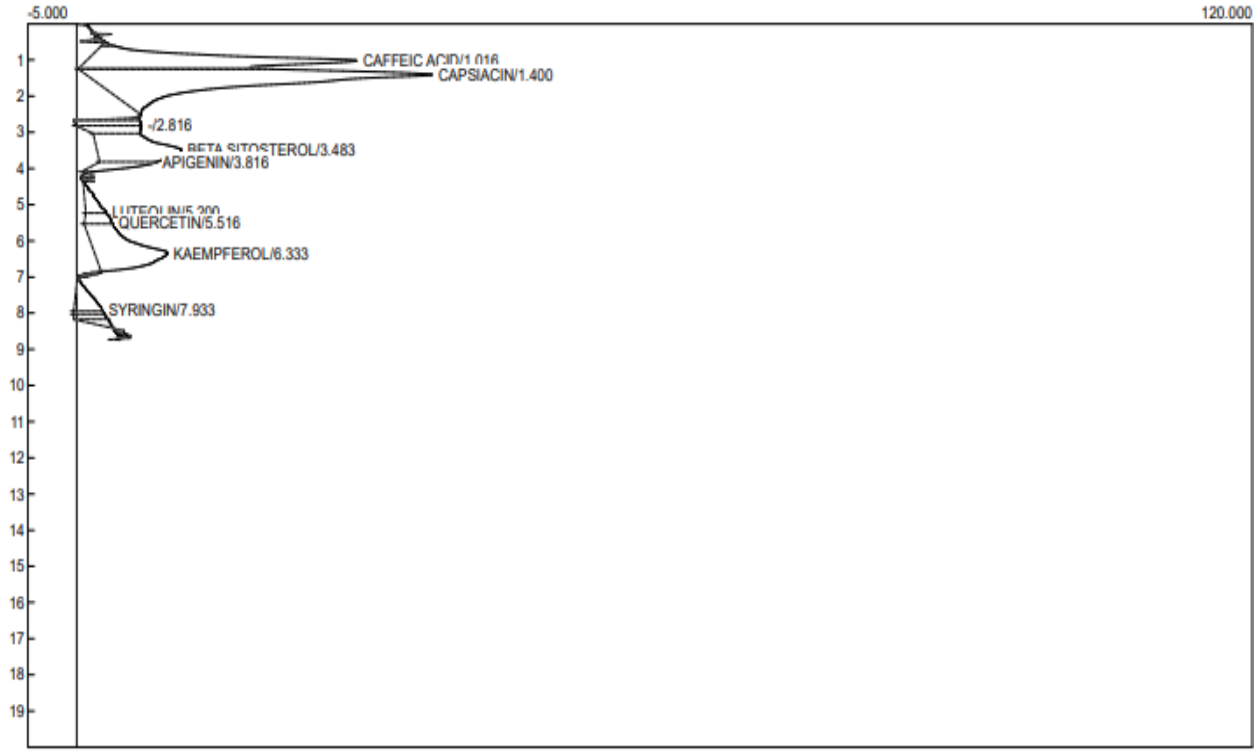


Fig 3 *Diameter of zones of inhibition (mm) of the fermented ripe fruits of M. paradisiaca against the test pathogens*

HPLC chromatogram identified eight constituents that were retained at varying times and indifferent peak heights (Fig 4). The highest constituent was capsaicin while the least was luteolin. HPLC method analyzed eight constituents in order of peak height: capsaicin, caffeic acid, beta-sitosterol, kaempferol, apigenin, syringin, quercetin, and luteolin. Kaemferol (KP) is a natural flavonoid compound commonly isolated from numerous vegetables and fruits. The presence of these flavonoid compounds in *M. paradisiaca* justifies their wide range of properties such as the antifungal and antibacterial activities of dihydroquercetin, dihydrokaempferol, and quercetin [46, 47], diabetes management potentials of KP by inhibiting cytokine expression and high glucose-induced ROS production [48]. Several studies have demonstrated the effects of syringin as hypoglycemic, anti-inflammatory [49], and antioxidative [50]. Kępa *et al*. [51] reported the antimicrobial activities of capsaicin. The antioxidant, antimicrobial, and anti-inflammatory properties of luteolin have been determined [52]. Apigenin and luteolin possess antimutagenic and anticarcinogenic effects [53]. Thus, the results of this study recorded the composition and antimicrobial potentials of fermented fruits of *Musa paradisiaca*. However, it is necessary to determine further *in vivo* toxicology analyses of the fermented sample to confirm the safety and dosage of the usage of *M. paradisiaca*.

4. Conclusion

It can be concluded from this study that just like the unripe fermented *Musa paradisiaca*, the fermented *M. paradisiaca* equally has an increased shelf life due to the acidic pH observed during the study. The low level of fat detected in this study signifies a health-promoting potential and the high carbohydrate content depicts *M. paradisiaca* as a prime source of energy. Hence, the nutritional components recorded in this study depict that the fermentation process converted the antinutritive components of the sample to the detected bioavailable compounds (tannin, alkaloids, flavonoids, glycoside, saponin, steroids, terpenoids, capsaicin, caffeic acid, beta-sitosterol, kaempferol, apigenin, syringin, quercetin, and luteolin) which have numerous biological activities such as antifungal, antibacterial, anti-inflammatory, antimutagenic, and anticarcinogenic activities of the fermented *M. paradisiaca*. Summarily, the considerable antimicrobial activities recorded in this study make the fermented ripe fruit of *M. paradisiaca* relevant as a suitable therapeutic food.



Component	Retention	Area	Height	External	Units
CAFFEIC ACID	1.016	544.4185	27.593	55.5523	ppm
CAPSIACIN	1.400	965.2720	35.380	108.1590	ppm
BETA SITOSTEROL	3.483	320.2495	8.656	32.0250	ppm
APIGENIN	3.816	69.6070	5.816	6.9607	ppm
LUTEOLIN	5.200	54.4370	2.056	5.4437	ppm
QUERCETIN	5.516	45.7120	2.865	0.0000	
KAEMPFEROL	6.333	365.8120	7.445	0.0000	
SYRINGIN	7.933	84.3725	2.912	0.0000	

Fig. 4 *HPLC Chromatogram showing different constituents in fermented ripe fruits of M. paradisiaca*

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

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