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Chemical composition of 'Kiaat' (*Pteropcarpus angolensis*) bark and the effect of herb pastes on the quality changes in marinated cat fish during chilled storage

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ABSTRACT

Kiaat (*Pterocarpus angolensis*) bark, which is valued for its high medicinal effects, was subjected to proximate analysis, while the extract from its bark was examined for, phytochemicals and minerals compositions. Kiaat bark was mixed with garlic, to preserve minced fresh catfish and was stored at 4°C for 9 days. Physical and chemical characteristics were analysed. The results of proximate composition revealed that kiaat bark contains high crude fibre (30.67%), protein (17.33%) and low contents of fat (2.98%). Phytochemical screening showed that the kiaat bark has flavonoid (1.90%), tannin (5.79%) alkaloid (2.96%) and phenol (5.90%) contents. The mineral determination revealed that kiaat bark contained high calcium (24.50 mg/100g), iron (17.20 mg/100g) content with very small amount of lead (0.01 mg/100g). The pH (6.30) of the minced cat fish marinated with kiaat bark+garlic at day 0 tends to be more acidic with increase in storage days and was 4.80 at day 9. The total volatile nitrogen values (TVB) (0.30 mg/100 g-5.46 mg/100 g) were lower than the standard limit (<35 mg/100 g sample). Trimethylamine in minced fish preserved with kiaat paste+garlic was 0.36 mgN/100g which later decreased to 0.16 mgN/100g at day 9, while values for samples with only kiaat bark and the control ranged from 0.64 mgN/100g -0.8 mgN/100g at day 9. Results further showed that thiobarbituric acid reactive substances (TBARs) values raised in all marinated samples during storage, especially in control sample (2.1-5.0 mgma\100g), sample with kiaat paste (0.2-4.2mgma\100g) and fish treated with kiaat paste+garlic (0.24-3.67mgma\100g). Addition of garlic to kiaat bark paste may be a preffered method to increase shelf-life of fresh catfish during chilled storage.

KEYWORDS: Kiaat, marinade, phytochemical, TBARS, total volatile base nitrogen

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INTRODUCTION

Pterocarpus angolensis (kiaat) is indigenous to East and Southern Africa popularly known in Nigeria as *Madubiya* (North), *Apepe* (Ibo) and *Agbolosun* (Southwest) [1]. *P. angolensis* has been employed locally in the treatment of many ailments [2]. The bark of the tree has been reported to be useful in stimulation of lactation in nursing mothers, treatment of nettle rash, piles, stomach disorders, headaches, blood in theurine, earache and mouth ulcers. In addition, hot aqueous extract from the bark resulted in a red colour fluid used in treating skin lesions and ringworm.

The acetone extract of *P. angolensis* was found to be active against *Staphylococcus* aureus *and Enterobacter cloacae* [3]. GC-MS analysis of the hexane extract of bark of *P. angolensis* revealed that it contains some volatile phytoconstituents which include tetratriacontane, n-hexadecanoic7-dehydrodiosgeninstigmasta-3,5-dien-7-one, lupeol octadecanoic acid, friedelan-3-one, etc. [4].

Selected herbs and spices used in preparation of local medicinal soups have been investigated and reported. For instance, Thai green curry during chilled storage [5] and some local medicinal soups consumed in Nigeria [6,7] have been investigated for their antibacterial, phytochemical and antioxidant properties. The traditional application of extracts from *P. angolensis* may suggest its efficacy against free radicals and microbial infections. *P. angolensis* bark is locally employed in soup making and reported to serves as a good source of nourishment for the body. However, scanty information is available on the use of the bark of *P. angolensis* in the food industry. Therefore, we carried out this work to evaluate chemical characteristics of bark of *P. angolensis* and its application in marination.

MATERIALS AND METHODS

Samples collection

The raw materials for this study were fresh catfish, garlic (purchased from local market in Akure, Ondo State) and the bark of kiaat (*Pterocarpus angolensis*) tree was obtained from a farm in Oba-Akoko, Ondo state, Nigeria. They were transported to the laboratory killed, rinsed, and minced for further study. All chemical reagents used were of analytical grade.

Processing of kiaat bark

The bark of kiaat was brought into the laboratory, sorted, and washed under running water to remove sand and foreign particles. It was dried in the oven at 50°C for 72 h. After drying, the sample was milled using the milling machine to pass through the 200 μm sieve size.

Preparation of *P. angolensis* bark extract

About 2.0 g of powdered sample of kiaat bark was mixed with methanol (80%) in a ratio of 1:25 and extracted in a Soxhlet apparatus for 5 h. This process was repeated for 3 times and all extracts were added together. The extract was concentrated using rotatory evaporator and was used for estimation of phytochemical and antioxidant properties of the bark.

Kiaat paste preparation

The kiaat paste was a combination of kiaat bark (75 g) and water (25 ml). The kiaat-garlic paste consisted of 50% garlic, 40% kiaat bark and 10% ml of water. These were blended together using a Philip blender.

Preparation of fish marinade

The herb paste (100 g) prepared as mentioned above was added to minced catfish (250 g) thoroughly stirred and packed in plastic bags of 5.0mm thickness. The marinated fish were stored in a refrigerator at 4°C for 9 days and samples were taken for analysis every three days to determine the chemical, physical and microbiological changes.

Proximate composition of *P. angolensis* bark

The method of AOAC [8] was used to determine the percentages of moisture, protein, crude fibre and fat in the bark sample while the carbohydrate content was obtained by difference.

Determination of phytochemical present in the bark extract

Alkaloid was determined using standard method [9]. The quantitative estimation of tannins was performed by standard method [10]. Phytate was determined following the method of Wheeler and and Ferrel [11]. Saponin was analysed based on the standard method [12]. The total phenol content of the sample was determined by the Folin-Ciocalteu assay as described [13]. The total flavonoid content of the extract was determined using a colourimeter assay [14].

Mineral analysis of kiaat bark

The mineral elements of the fine powdered bark were determined using dry ash methods as described in [15]. About (1.0 g) was weighed into crucibles and transferred to a muffle furnace and ash at 550°C until all the carbon was burnt off and the crucibles plus ash were transferred into different desiccators to cool after which 0.1M HCl solution (10 ml) was added to the crucible to break up the ash and leach the metals. The crucible was washed three times with 0.1M HCl and made up to 100 ml with deonized water.

The mineral contents of the samples under study were carried out using *Atomic Absorption Spectroscopy (AAS)*. The following elements were analysed calcium, magnesium, potassium, zinc, iron and lead. Standard stock solutions were prepared for each metal using suitable metal salts of each metal to prepare a standard curve.

Sodium: Flame photometer was used to determine the concentrations of the elements in the sample. The standard solutions were prepared using sodium chloride for sodium determination respectively. The standard solutions were measured from the flame photometer and the value obtained was plotted against the strength of various solutions. The digests were determined from the flame photometer. The values were plotted in the respective standard value to read the original values of the concentration of the elements.

pH determination of *P. angolensis* bark

Ten grams of each sample was homogenized with 10 ml of distilled water before being measured with a pH meter (Thermo Orion, Model 230).

Determination of total volatile base-nitrogen (TVB-N) of marinated fish

TVB-N was determined based on an adaptation of the current official European steam-distillation method as described by European Union/European Commission [16].

Determination of trimethylamine-nitrogen (TMA-N) of marinated fish

Trimethylamine- Nitrogen (TMA-N) of marinated fish was determined by using the method of [17]. TMA-N contents were expressed as mgN/100 g.

Determination of thiobarbituric acid-reactive substances (TBARS) of marinated fish

Modified TBARS of fish samples treated with kiaat bark extract was carried out following the method described [18].

Statistical analysis

The mean and standard error of means of the triplicate analyses were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means, while the means were separated using the new Duncan multiple range test.

RESULTS AND DISCUSSION

Proximate composition of kiaat bark

The result obtained from proximate analysis (Table 1) of kiaat bark showed that the reduced moisture (5.93%) of the bark can reduce the pathogen growth and to prolong the storage life [19]. Kiaat bark contains ash content (27.50%), crude protein (17.33%), crude fat content (2.98%) and high content of crude fibre (30.67%) which is higher than the 4.28% previous reported [20] for soybean. Comparatively the protein content of the herb is higher than the maximum standard (3.3%) suggested by the USDA Nutrient Database for Standard Reference [21]. The protein value of kiaat as observed in this study conferred on it to be more advantageous as rich source of herbal protein over some reported previously [22]. The enriched proximate composition of *P. angolensis* might describe its utilization as a source of nourishment to the local lactating women during child feeding.

Table 1: Proximate composition of kiaat bark

Characteristics (%)	Kiaat bark
Moisture	5.93±0.02
Ash	27.50±0.02
Crude protein	17.33±0.01
Crude fat	2.98±0.00
Crude fibre	30.67±0.01
Carbohydrate	15.59±0.00

Table 2: Phytochemical composition of *P. angolensis* bark

Characteristics (%)	Kiaat bark
Alkaloids	2.96±0.02
Tannins	5.79±0.02
Saponin	3.31±0.01
Flavonoids	1.90±0.01
Phytate	1.90±0.02
Phenol	5.90±0.02

Table 3: Mineral composition of *P. angolensis* bark

Characteristics (%)	Kiaat bark
Sodium	2.40±0.03
Calcium	24.50±0.00
Iron	17.20±0.00
Potassium	16.50±0.06
Zinc	0.064±0.00
Lead	0.001±0.01
Magnesium	0.526±0.00

Phytochemical composition of kiaat bark

Table 2 shows the results of the phytochemical screening test of kiaat bark. It was observed that kiaat contains bioactive compounds required in various herbs used for medicinal purposes. Kiaat bark possessed phenol (5.90%) with tannin (5.79%) saponin (3.31%), alkaloids (2.96%), Flavonoids (1.90%) and phytate (1.90%). Phenols can remove bacteria [23]. Flavonoids are antioxidants and protect from cell damage [24]. The importance of these phytochemicals in various antibiotics used in managing common pathogenic strains has recently been reported by [25].

Mineral analysis of kiaat bark

Table 3 shows the mineral analysis results of the kiaat bark. It was observed that kiaat bark has high content of calcium (24.50 mg/100g), iron (17.20 mg/100g), potassium (16.50 mg/100g), low sodium (2.40 mg/100g), magnesium (0.52 mg/100g), zinc (0.06 mg/100g) and lead (0.001 mg/100g). Some inorganic element such as; potassium, zinc and calcium are important in maintenance of normal glucose tolerance [26]. High concentration of calcium is indispensable for all organisms due to its role in blood coagulation and essential for desirable functioning of the muscle and nervous systems [27]. Magnesium is a constituent of bones, teeth and enzyme cofactor in which it will be absorbed in the intestines and then transported via the blood to cells and tissues [28]. Zinc is indispensable to all organisms and has an important function in metabolism, growth, development and general well-being, as well, thus becoming a fundamental co-factor for numerous enzymes in the body [29, 30]. Recommended limit of zinc in medicinal flora as reported by World Health Organization is 50 mg/kg [31], while its consumption in food is 11 mg/day [32]. Thus, the concentration level of zinc obtained in the current study was within the safe limit. Iron is vital for the formation of hemoglobin and played a crucial role oxygen and electron transfer in a human body [29]. WHO has recommended an intake of iron 10-20 mg/day for an adult and the permissible level of iron in medicinal plants was 20 mg/kg [31]. The iron content of *P.angolensis* may contribute to the reason why the local users engage this herb in treatment of anemic conditions and as blood enhancer to women who just gave birth. Notably, Na/K ratio less than one is recommended by [33] in the diet of people living with hypertension.

Change in pH during marination

The change in pH of the marinated catfish during storage at 4°C for 9 days is shown in Figure 1. The pH of the sample with kiaat+garlic (6.30) at day 0 tends to be more acidic as the number of days increased and finally to 4.80 at day 9. High pH inhibits many microorganisms during storage [34]. There was an increase in the pH of the control sample from 6.4 at day 0 to 6.8 at day 9. Endogenous proteolytic activity may be responsible for alterations in pH. The increase in pH values after day 6 may be an indication, suggesting a quality loss of fish muscles and in accordance with previous study [35].

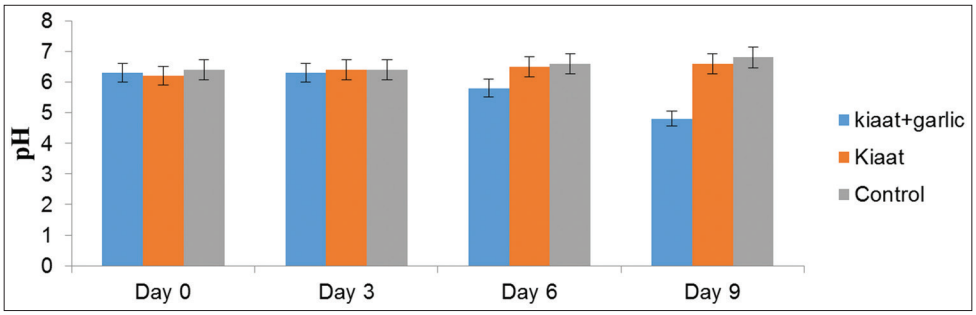

Figure 1: Effect of the herb pastes on pH values of minced marinated cat fish stored at 4°C. Bars have mean values that are significantly different ($p<0.05$)

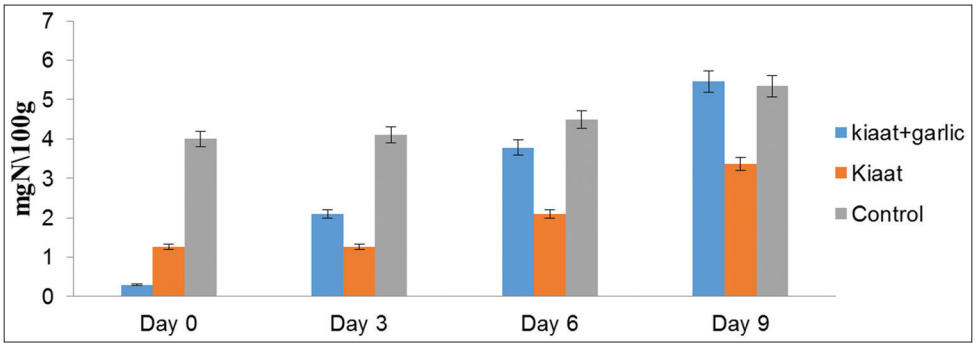

Figure 2: Effect of the herb paste on total volatile base nitrogen (TVB-N) values in minced marinated cat fish stored at 4°C for 9 days. Bars have mean values that are significantly different ($p<0.05$).

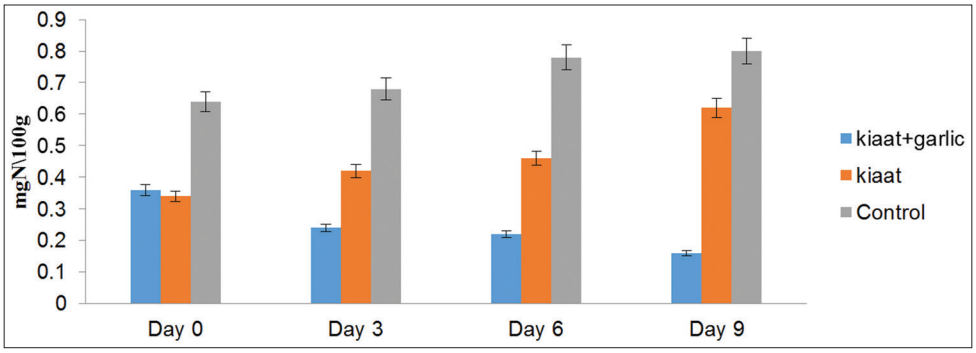

Figure 3: Effect of the herb paste on trimethylamine (TMA) values in minced marinated cat fish stored at 4°C for 9 days. Bars have mean values that are significantly different ($p<0.05$)

Total volatile base nitrogen values of marinated cat fish

In this study, the volatile amines of all treatments increased with increase in storage time as shown in Figure 2. Result showed that the highest value of TVB-N (Total volatile base nitrogen) was recorded from the sample with herb + garlic (5.46 mgN/100) at day 9 followed by the control (5.34 mgN/100) while the marinade with the herb paste (3.36 mgN/100) had the least. However, all the values were lower than 30 mg N/100 g which was considered to be stale, whilst regarded as unfit for consumption at level of 40 mg N/100 g [36]. It was observed that the sample without garlic paste had the lowest TVB-N value during storage, which might be due to some phytochemicals present in the herb paste used in the marination. These present findings agreed with previous report [37]. Therefore, the TVB-N value may not a good indicator for quality determination of marinated food [37].

Trimethylamine (TMA) values of marinated cat fish

Figure 3 shows the TMA values of minced marinated cat fish stored at 4 °C for 9 days. Trimethylamine is a chemical index of spoilage of fish and it is responsible for the off odor. At day 0, the TMA in sample with kiaat+garlic was 0.36 mgN/100g and later decreased to 0.16 mgN/100g as the number of days increased at day 9, which may be due to the synergistic effect of garlic with kiaat paste in the sample. There was a significant increase in the sample without garlic (0.34 mgN/100g -0.62 mgN/100g) and the control sample (0.64 mgN/100g -0.8 mgN/100g) which may be due to the pH value of <6 as the odor of TMA increases substantially at pH value >6.5 [38]. These values are lower compared with the acceptance limit (10-15mg-N/100g) for TMA as proposed [39].

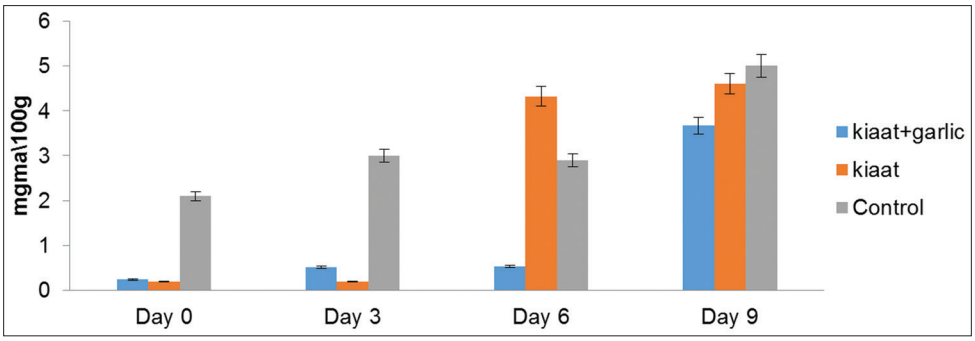

Figure 4: Effect of the herb paste on 2-thiobarbituric acid reactive substance (TBARS) values in minced marinated cat fish stored at 4°C for 9 days. Bars have mean values that are significantly different ($p<0.05$).

Lipid oxidation of the marinated fish

The effect of the herb pastes on the TBARS values of fresh catfish refrigerated for 9 days is shown in Figure 4. The TBARS values in the control sample (2.1-5.0 mgma\100g) and the sample without garlic paste (0.2-4.2 mgma\100g). The TBARS values of the control and the fish with *P. angolensis* pastes were significantly higher than the fish treated with herb+garlic paste (0.24-3.67mgma\100g). The ability of the herbal paste that contained garlic to reduce the TBARs may be explained by the bioactive compounds, alliin, diallylsulphide, allyl-sulphide and propyl-sulphide derived from garlic. This result revealed that addition of garlic paste to *P. angolensis* bark paste retarded lipid oxidation during the 9 days' storage. This result is in agreement with [40].

CONCLUSION

The results from the chemical composition of *P.angolensis* revealed that it is rich in protein, ash, crude fibre, calcium, iron and potassium. The bark was found to contain some phytochemicals which may be responsible for its medicinal effect. On application of the paste from *P. angolensis* as a marinade in cat fish, it was observed that the herb together with garlic synergistically reduced lipid oxidation better than when only the herb was applied. However, further work should be carried out to study the effect or contribution of the red pigment in the bark on the properties demonstrated by the bark.

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