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PROXIMATE AND PHYTOCHEMICAL COMPOSITION OF *PIPER METHYSTICUM* G. FORST. LEAVES EXPOSED TO ALPHA-SPIN NANOPARTICLE

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ABSTRACT

Despite the fact that pre-harvest treatments are known to have an impact on the nutritional and phytochemical makeup of medicinal plants, alpha-spin nanoparticles' effects on *Piper methysticum* are still unknown. Comprehending this effect is essential to maximising its therapeutic and nutritional benefits. This study evaluated the proximate and phytochemical composition of *Piper methysticum* exposed to alpha-spin nanoparticles. Seeds were treated for 10 and 20 minutes and planted in the field using a randomized complete block design (RCBD) with three biological replications and maintained under standard cultural practices. Standard laboratory methods were employed for proximate and phytochemical analysis. Data from proximate analysis were statistically analyzed using a paired-sample t-test with significance determined at $P \leq 0.05$. Proximate composition differed significantly among treatments. Leaves from 20-minute exposure exhibited the highest levels of ash (14.73±0.42), crude protein (19.42±0.53), crude fiber (3.67±0.37), carbohydrates (46.56±0.78), moisture content (15.65±0.22), and fat (13.81±0.33). In contrast, 10-minute exposure yielded lower values across all parameters. Phytochemical screening, conducted using qualitative methods, indicated the presence of alkaloids, saponins, glycosides, terpenoids, phenols, and tannins in leaves exposed to both treatments, whereas flavonoids were absent. Control samples lacked alkaloids, terpenoids, and flavonoids. The findings suggest that 20-minute exposure to alpha-spin nanoparticles enhances the nutritional and phytochemical *profile* of *Piper methysticum*. This study highlights the potential of alpha-spin nanoparticle treatment in improving the nutritional quality of medicinal plant.

Keywords: Alpha Spin, Nano Particles, *Piper methysticum*, Phytochemical, Proximate, Awa

INTRODUCTION

Piper methysticum G. Forst, commonly known as kava, is an edible medicinal shrub of the family Piperaceae and genus Piper (Ujvary, 2014). Kava has long been prized for its cultural and therapeutic significance; it has been used for centuries in social gatherings, religious ceremonies, and as a natural remedy for its sedative and calming effects (Cassileth, 2011; Showman *et al*., 2015; White, 2018; Aporosa *et al*., 2020). The plant's roots are commonly prepared as water infusions to soothe nerves, promote relaxation and sleep, alleviate fatigue, and aid in weight reduction (Backleh *et al*.,2003; Xuan *et al*., 2008).

Phytochemical investigations have identified over 56 compounds in *Piper methysticum*, categorized into kavalactones and flavokavins (Sarris *et al*., 2011). Among these, 29 kavalactones—including methysticin, dihydromethysticin, and kavain—are the major bioactive constituents responsible for its pharmacological effects (Xuan *et al*., 2008). These effects include anxiolytic, antidepressant, anticonvulsant, antipsychotic, and neuroprotective properties (Wang *et al*., 2020; Krum *et al*., 2021). Previous studies on the proximate and phytochemical composition of kava leaves reported moisture (36.80%), protein (0.03%), ash (30.05%), fiber (28.60%), fat (3.35%), and carbohydrates (1.27%), along with the presence of terpenoids, flavonoids, tannins, cardiac glycosides, and alkaloids (Uzomba *et al*., 2020).

Nanotechnology has emerged as a transformative tool in agriculture, with nanoparticles (NPs) ranging from 1 to 100 nm offering enhanced physicochemical properties (Kwong-Ndung *et al*., 2019). Research has demonstrated that NPs can influence plant growth by improving nutrient uptake and yield (Vázquez-Núñez *et al*., 2018). Notably, studies have shown their positive effects on crops like *Moringa oleifera* (Kwong-Ndung *et al*., 2019) and tomatoes, *Solanum lycopersicum*; (Terna and Oshinowo, 2019). However, despite the known benefits of nanoparticles, there is limited information on their effect on *Piper methysticum*.

This study aims to investigate the impact of alpha spin nanoparticles on the nutritional and phytochemical composition of *Piper methysticum* leaves, providing new insights into the application of nanotechnology in enhancing the nutritional quality of medicinal plants.

MATERIALS AND METHODS

Study Area and Experimental Site

This study was conducted in Lafia, Nasarawa State, located at latitude 08.33N and longitude 08.32E, covering a total land area of 2,711.km^2. According to the 2006 census, Lafia has a population of 330,712, with agriculture being the primary economic activity. Proximate analysis was performed at Faculty of Agriculture, Shabu-Lafia Campus, Nasarawa State University, Keffi. Phytochemicals analysis was conducted at the Department of Chemistry, Federal University of Lafia.

The leaf samples' proximate composition was determined using the standard procedures of analysis of the Association of Official Analytical Chemists (AOAC, 2016). The following proximate compositions were determined: crude protein, crude fats, moisture content, total ash, crude fibre and carbohydrates. Three replicates of each proximate constituent were made. The samples' moisture content was ascertained using the air-oven dry method, which involved weighing 5 g of the sample in moisture, placing it in a container or dish, and drying it in a dry oven at 105˚C until its weight remained constant. Moisture content% = weight of fresh sample – weight of dry samples/weight of fresh samples × 100. The total amount of ash was measured using a muffle furnace that was kept between 550 and 600˚C for four hours until grey ash

was produced. Ash% = Weight of ash/weight of sample × 100. The Soxhlet extraction method, which uses ether as the extraction solvent, was used to determine the crude fat. Crude fat (% of DM) = weight of fat/weight of sample × 100. The micro-Kjeldahl technique was used to evaluate the samples' crude protein content. Crude Protein (CP)% = Total Nitrogen (N_T) × 6.25 (Protein factor). The difference between the total of all the proximate components from 100% was used to calculate the carbohydrate contents. That is: 100% – (Fat% + moisture% + ash% + protein%). Determination of crude fiber involved the digestion of the sample with 100 ml of 1.25% (v/v) sulphuric acid and 100 ml of 1.25% (w/v) sodium hydroxide. This was followed by incineration in muffle furnace. The fiber content was calculated from the loss of weight on ignition. Qualitative phytochemical screening was done using the methods of Mumtaz *et al.* (2014) and Muddapur *et al*. (2023). Phenols: Two millilitres of the extract was placed in a beaker. A ferric chloride solution of 2ml was then added. A deep bluish green solution suggested the presence of phenols.

Terpenoids: Two millilitres of chloroform were combined with 5ml of aqueous extract. Then, to create a layer, 3ml of sulphuric acid concentrate was applied. The presence of terpenoids was revealed by the interface's reddish brown colouration. Saponins: After boiling 2 g of the powdered sample in 20 ml of distilled water in a water bath, the solution was filtered. Next, 5 ml of distilled water was added to 10 ml of the filtrate, and the mixture was shaken rapidly to create a stable, long-lasting foam. After adding three drops of olive oil to the foam and shaking rapidly, an emulsion formed, indicating the presence of saponins.

Flavonoids: For five minutes, 1 g of the powdered sample was heated with 10 ml of ethyl acetate in a steam bath set between 40 and 50°C. One millilitre of diluted ammonia was added to the filtrate. A positive flavonoid test result was shown by a yellow colouration.

Alkaloids: 1 g of the powdered sample was extracted using 5 ml of methanol and 5 ml of 2N hydrochloric acid. The filtrate was then subjected to Wagner's and Meyer's reagent treatments. The samples were scored positive on the basis of turbidity. Glycosides: One millilitre of glacial acetic acid was added to two millilitres of filtrate. Then, 1 millilitre of concentrated sulphuric acid was added along with 1 millilitre of ferric chloride. The solution's green-blue colouration suggested the presence of glycosides.

Tannins: Two to three millilitres of methanolic extract (1:1) were mixed with a 10% alcoholic ferric chloride solution. The solution's dark blue colouration development suggested the presence of tannins.

Sample collection

The seeds of *Piper methysticum* used for the experiment was obtained from the farmers of Nomeh village, Nkanu LGA, Enugu State. Nigeria. The alpha-spin nanoparticles used was obtained from the Department of Plant Science and Biotechnology of Federal University of Lafia.

Experimental Design

The seeds collected were exposed to alpha-spin nanoparticles at two (2) different time period of 10 and 20 minutes respectively, and along with the untreated (control) laid out in a Randomized Complete Block Design (RCBD) with three replications. Each experimental plot consisted of three pots, each measuring 1m × 1m, with a 2m walkway separating the plots. A total of 36 seeds were distributed evenly across the nine pots.

Practices

Top soil was mixed in the ports and preparation was manually on 28th of march 2022 and planting was done in the field on 4th April, 2022. Weeding was carried out manually once at two weeks intervals after planting using hoe. Four seeds were planted in each pots directly and watered daily. *Piper methysticum* leaves were harvested after 8 weeks of planting (Prasad, 2018).

Preparation of Leaves The leaves were properly washed with tap water, rinsed with sterile distilled water, shade-dried for two weeks and pulverize into fine powder using a mortar and pestle and then sieved to obtain finer particles with higher surface area for extraction. (Sulaiman *et al.* 2019).

Preparation and Analysis of Plant Extract

The aqueous extract of the sample was prepared using a triple maceration procedure. The sample was immersed with 10% aqueous-ethanol in amber glass bottles for 24 hours, filtered through a Whatman-1 filter paper, and repeated twice. The filtrates were then evaporated using a rotary evaporator, and the crude extract was lyophilized to remove moisture. The dried extract was transferred to amber glass jar and stored at −4 °C in a refrigerator (Mumtaz *et al*. 2014). The dried extract was used for phytochemical screening and proximate analysis.

Phytochemical analysis was conducted using the qualitative screening method described by Mumtaz *et al*. (2014) and Muddapur *et al*. (2023), assessing the presence of phenol, saponin, alkaloid, flavonoid, tannin, terpenoid and glycoside. For proximate analysis, the leaf samples were evaluated for crude protein, ash, crude fat, moisture content, and carbohydrates according to methods of the Association of Official Analytical Chemists (AOAC, 2016).

Statistical Analysis

The proximate analysis results were statistically analysed using a paired-samples t-test in the Statistical Package for the Social Sciences (SPSS) version 21. P values were determined using the paired-samples t-test at a 5% confidence interval. Data were presented as means ± standard deviation (SD) to indicate variability within each treatment group.

RESULTS AND DISCUSSION

Results

The results of the proximate analysis (Table 1) showed that *Piper methysticum* leaves exposed for 20 minutes had the highest percentages of moisture (10.93±0.27) and crude protein (19.42±0.53), which differed significantly from the control (15.65±0.22 and 16.58±0.43, respectively). The lowest moisture (10.74±0.31) and crude protein (10.56±0.36) contents were recorded in leaves exposed for 10 minutes, which also differed significantly from the control (15.65±0.22 and 16.58±0.43).

The ash content was highest (14.73±0.43) in leaves exposed for 20 minutes, which significantly differed from the control (12.57±0.43). However, the lowest ash content (12.65±0.41) in leaves exposed for 10 minutes did not differ significantly from the control (12.57±0.43).

Leaves treated with Alpha Spin Nanoparticles for 20 minutes had the highest fat (13.73±0.42%) and crude fiber (3.67±0.37) contents, which did not differ significantly from the control (13.81±0.33% and 3.43±0.51). However, the lowest fat (13.02±0.18) and crude fiber (3.42±0.54%) contents were recorded in leaves exposed for 10 minutes, and these values differed significantly from the control.

The carbohydrate content was highest (46.56±0.75) in leaves exposed for 20 minutes, which significantly differed from the

control (38.66±0.46). The lowest carbohydrate content (41.45±0.44) was observed in leaves exposed for 10 minutes, which also significantly differed from the control (38.66±0.46) .

The qualitative phytochemical analysis of *Piper methysticum* leaves treated with Alpha Spin Nanoparticles for 10 and 20 minutes (Table 2) revealed the presence of alkaloids, saponins, glycosides, terpenoids, phenols, and tannins, while flavonoids were not detected in any treated samples. The control sample tested positive for saponins, glycosides, phenols, and tannins but negative for alkaloids, flavonoids, and terpenoids.

Table 1: Proximate Composition of *Piper methysticum* Leaves Exposed to Alpha Spin Nanoparticles

TIME	M.C	ASH	C.P	E.E	C.F	NFE
10	10.74±0.31	12.65±0.41	10.56±0.36	13.02±0.18	3.42±0.54	41.45±0.44
20	10.93±0.27	14.73±0.42	19.42±0.53	13.73±0.42	3.67±0.37	46.56+0.78
CONT	15.65±0.22	12.57±0.43	16.58±0.45	13.81±0.33	3.43±0.51	38.66±0.46
LSD	2.776					
D.F	4					

Statistical analysis was performed using a paired-samples t-test at a 95% confidence interval ($P \leq 0.05$). Values are presented as mean ± standard deviation (SD).

M.C= Moisture Content C.P= Crude Protein, E. E= Fats, C.F= Crude Fibre

NFE= Carbohydrate, D.F= Degree of Freedom

Table 2: Phytochemical Composition of Piper *methysticum* Leaves Exposed to Alpha Spin Nanoparticles

Phytochemicals	10 Minutes	20 Minutes	Control
Alkaloids	+	+	ND
Saponins	+++	+++	+++
Glycosides	+	+	+
Terpenoids	+	+	ND
Flavonoids.	ND	ND	ND
Tanin	+	+	+
Phenol	+	+	+

KEY: "+" = Present, "+++" = Strongly Present, "ND" = Not Detectable

Discussion

The proximate parameters analyzed, including moisture content, ash, crude protein, crude fiber, fat, and carbohydrates, are crucial indicators of the nutritional quality of *Piper methysticum*.

The moisture content of *Piper methysticum* was highest at 20 minutes exposure (10.93±0.27), followed by 10 minutes exposure, while the control sample had the highest moisture content (15.65±0.22). This implies that exposure to Alpha Spin nanoparticles reduced moisture content. Moisture content is a key factor in determining freshness and shelf life, as high moisture levels can increase microbial spoilage and deterioration (Adepoju and Oyewole, 2008).

The crude protein content was highest at 20 minutes exposure (19.42±0.53), followed by 10 minutes exposure, indicating that longer exposure enhances protein content. However, the control had higher protein content than the 10-minute exposure, suggesting that further optimization of exposure time is needed to maximize protein retention. Protein is an essential nutrient for growth, tissue repair, and enzyme production, and its deficiency can lead to growth retardation, muscle wasting, and edema.

The ash content, which points out the mineral composition, was highest at 20 minutes exposure (14.73±0.42), followed by 10 minutes exposure. This reveals that prolonged exposure to Alpha Spin nanoparticles enhances the mineral content of *Piper methysticum*. A higher ash content is beneficial, as minerals play a vital role in blood coagulation, wound healing, and metabolic processes.

The crude fiber content was highest at 20 minutes exposure (3.67±0.37), followed by 10 minutes exposure. This indicates that prolonged exposure might enhance fiber content. Dietary fiber is important for gut health and digestion, and it has been reported to aid in the removal of carcinogens, xenobiotics, and bile acids, thus promoting overall health benefits (Ayoola and Adeyeye, 2010).

The fat content was highest in the control sample (13.81±0.33), followed by the 20-minute exposure. This suggests that Alpha Spin exposure may slightly reduce fat content. Fat is important for energy storage, insulation, and cell membrane integrity, and its reduction might affect flavor stability due to oxidation. Future studies should examine the impact of longer exposure times on lipid stability.

The carbohydrate content was highest at 20 minutes exposure (46.56±0.78), followed by 10 minutes exposure. This suggests that Alpha Spin exposure enhances carbohydrate content, which is beneficial as carbohydrates are the primary source of energy for the body.

The phytochemical analysis revealed that alkaloids and terpenoids were present in leaves exposed for 10 and 20 minutes, but absent in the control, suggesting that Alpha Spin nanoparticles influence the synthesis or availability of these compounds. Alkaloids have been reported to possess neuroactive, analgesic, and antimicrobial properties, while terpenoids contribute to antioxidant and anti-inflammatory activities.

Flavonoids were not detected in any of the samples, indicating that Alpha Spin exposure had no effect on flavonoid content. Saponins were detected in abundance across all samples, indicating that Alpha Spin did not alter saponin levels. Saponins are known for their antimicrobial, anti-inflammatory, and cholesterol-lowering properties. Furthermore, Glycosides, phenols, and tannins were present in all samples, with slight variations in intensity. This suggests that a longer exposure time may influence the qualitative composition of these phytochemicals.

CONCLUSION

The findings of this study indicate that *Piper methysticum*'s exposure to Alpha Spin nanoparticles for 20 minutes resulted in higher nutrient retention compared to 10 minutes exposure and the control. The higher levels of crude protein, ash, fiber,

and carbohydrates at 20 minutes exposure reveal that Alpha Spin nanoparticles beneficially affect the nutritional quality of *Piper methysticum*. Furthermore, the detection of alkaloids and terpenoids in treated samples implies that Alpha Spin exposure alters the phytochemical composition, which may have potential health benefits. Alpha Spin nanoparticles have a positive impact on *Piper methysticum*, but additional researches are necessary to find the optimal exposure duration for maximizing nutrient retention and enhancing phytochemical content.

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