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Proximate Composition, Mineral Content and Phytochemical Evaluation of Different Solvent Extracts of Pineapple (*Ananas comosus*) Stalk

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ABSTRACT: The objective of this study was to evaluate the proximate composition, mineral content and phytochemical constituents of *Ananas comosus* stalks using distilled water, methanol, ethanol, chloroform and petroleum ether as extraction solvents. Analysis of minerals present in the different extracts of the stalk of *A. comosus* revealed that the aqueous extract had the highest concentration of sodium, magnesium, zinc, tin, molybdenum and manganese while the lowest was found in petroleum ether extract. Methanol extract had the highest concentration of potassium, iron and copper. The result of the physicochemical analysis of the stalk of *A. comosus* revealed that the stalk was high in nitrogen free extract (58.74±1.13 %). However, ether extract had the least value (1.12±0.20 %). Qualitative phytochemical screening of *A. comosus* stalk extract revealed the presence of saponins, alkaloid and terpenes. Quantitative evaluation of the phytoconstituents revealed that flavonoids and phenol were highest in petroleum ether extracts (2.74±0.02 mg/kg and 2.73±0.02 mg/kg) while alkaloids concentration was highest in the ethanol extract (1.78±0.01 mg/ml). The study found that *Ananas comosus* stalk extracts had a good amount of micronutrients according to the various criteria examined; the high ash content also supports this finding. The study showed that the stalk of *A. comosus* is a good source of important phytochemicals.

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Pineapple (*Ananas comosus*) is a tropical fruit with a unique aroma and sweet taste. It contains complex mixtures of several compounds in small amounts. Pineapple is also a rich source of minerals and vitamins that offer several health benefits (Maimunah *et al*., 2020). It plays a vital role in the digestive system, maintaining weight and balancing nutrition (Chaudhary *et al*., 2020). Pineapple has a high nutritional value and serves as a source of vitamins A, B and C together with minerals such as calcium, phosphorus and iron. It has also been reported to have some antioxidant activities (Amzad and Rahman, 2011). Pineapples also contain a compound known as bromelain which plays a vital role in maintaining health. It has been reported as one of such fruits that can be used to maintain dental health because it contains vitamin C and flavonoids which have antimicrobial effects (Yong *et al*., 2018). Pineapple consists of adequate amounts of bioactive compounds, dietary fibre, minerals, and nutrients. Hitherto, it has been proven that pineapples have certain health benefits such as; anti-inflammatory, antioxidant activity, monitoring nervous system function, and improving bowel movement (Maimunah *et al*., 2020). Processed pineapples are consumed worldwide and pineapple processing industries are exploring new technologies to retain the nutritional quality of the pineapple fruit in order to provide healthy and nutritious products for consumption (Chaudhary *et al*., 2020). The pineapple processing industries produce

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large quantity of pineapple waste (peels, stalks, leaves and pulps) which are potentially unutilized raw materials. The ongoing quest for new therapeutic agents requires the maximum utilization of all parts of plants. As a supposed waste product, pineapple stalk could be a potential source for the extraction of bioactive compounds with the sole aim of discovering and developing new therapeutic agents. Several studies have investigated the nutritional composition, phytochemical constituents and antioxidant activities of pineapple pulp, seeds and peels. However, there has been no report regarding the stalks, which is usually more often discarded as waste. The objective of this study was to evaluate the proximate composition, mineral content and phytochemical constituents of *Ananas comosus* stalks using distilled water, methanol, ethanol, chloroform and petroleum ether as extraction solvents

MATERIALS AND METHODS

Collection of Plant Material and Identification: Ananas comosus (L.) Merr. stalk was collected from a pineapple farm in Benin, Edo State in the month of December, 2021. The studied plant specimen was identified and authenticated by Mr. Michael O.E. at the Department of Botany, Delta State University, Abraka, Delta State, Nigeria. A voucher specimen (DELSUH122) was given and deposited in the herbarium for reference purpose.

Extraction of Ananas comosus Stalk: The collected *Ananas comosus* stalks was washed, shade dried, pulverized and stored in air-tight bags for future use. One hundred gram (100g) of coarsely powdered pineapple stalk was extracted with 500ml of various solvents [distilled water, methanol, chloroform, ethanol and petroleum ether (95%v/v)] using cold maceration for 48hours with constant agitation (Duraipandiyan, 2006). The extracts were then filtered through a cheese cloth with fine pore, and the filtrate was filtered for the second time using Whatman No. 1 filter paper. The resulting extracts were concentrated at 50ºC in a rotary evaporator for 2hours and were subjected to dryness in a water bath maintained at 50ºC and evaporated to dryness. The obtained extract was stored at 4ºC until when required for use.

*Proximate Analyses**:*** Proximate composition analysis refers to the determination of the major constituents of feed and it was used to assess if a feed is within its normal compositional parameters or somehow been adulterated. This method partitioned nutrients in feed into 6 components: water, ash, crude protein, ether extract, crude fibre and Nitrogen Free Extract (NFE). Proximate analysis was carried out using the methods described by (Isikhuemen *et al.*, 2020) with little modification.

Mineral Analysis: Different minerals in the various solvent extracts of *A. comosus* were determined using Atomic Absorption Spectrophotometry (AOAC, 1984).

Qualitative Phytochemical Analysis: Preliminary phytochemical screening of the various solvent extracts of *A. comosus* stalk was carried out using standard methods to determine the presence of various phytochemicals such as alkaloids, terpenoids, phenolics, steroids, saponins and tannins as described by Borokini and Omatayo (2012) and Njoku and Obi (2009).

Estimation of total phenols and total tannin:A. comosus stalk extracts (methanol, chloroform, petroleum ether and aqueous) of 1.0 mL volume was mixed with 1.0mL of Folin- Ciocalteau's phenol reagent, and after 3 min, 1.0 mL of saturated $Na_2CO_3$was added to the mixture and the volume was made up to 10 mL by adding distilled water. The reaction mixture was kept in dark for 90 min, after which its absorbance was read at 725nm. A calibration curve was constructed with different concentrations of gallic acid and tannic acid (20 - 100μ/g ml) for phenol and tannin respectively. The results were expressed as mg of gallic acid (GAE), equivalents/g extracts and mg of tannic acid (TAE) equivalents/g extracts respectively *(Singleton and Rossi, 1965)*.

Estimation of total flavonoids:A. comosus stalk extracts of 0.5 mL was added into test tubes containing 1.25mL distilled water and 0.075 mL of 5 % $NaNO_2$ solution. The tubes were allowed to stand for 5 min. 0.15mL of 10 % $AlCl_3$ was added and after 6 min 0.5mL of 1.0 M NaOH was also added and the mixtures were diluted with 0.275 mL of distilled water. The absorbance was measured immediately at 510 nm. Catechin was used as the standard (20 - 100μg/ml). The total flavonoids content was expressed as milligram of catechin (CAE) equivalents/g extracts (Jia et al., 1999).

Quantitative Estimation of Alkaloids: To 1ml of *A. comosus* stalk extracts, 5 ml pH 4.7 phosphate Buffer was added and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents (40 - 120µg/ml)(Shamsa et al., 2008).

Statistical Analysis: All data were subjected to statistical analysis. Values were reported as Mean ± Standard deviation while one-way ANOVA was used to test for differences between the various solvents. The results were considered significant at p-values of less than 0.05, that is, at 95% confidence level ($p<0.05$). Turkey HSD post- Hoc tool was used for basis of statistical comparison.

RESULTS AND DISCUSSION

The physicochemical properties of agricultural goods are crucial for preservation techniques (Ramirez et al., 2021). The result of the moisture content of the stalk of A. *comosus* shows that the moisture content was relatively low after drying (Table 1).

Table 1: Proximate Composition of the Stalk of *A. comosus*

Parameters	Sample
Moisture content (%)	8.50±1.14
Crude protein	4.25±0.14
Ash content (%)	8.54±0.17
Ether extract (%)	1.12±0.20
Crude fibre	19.73±0.67
Nitrogen free extract	58.74±1.13

Therefore, the plant stalk is not prone to microbial and fungus growth as a result of the low moisture content, and thus has a long shelf life on storage as high moisture content promotes mould survival and proliferation, according to Garg *et al* (2012). The values obtained in this study contradicts that reported by Jasmine and Estherlydia (2014) and Koffi *et al* (2021) who reported a high moisture content in the fruit of *A. comosus*. The percentage ash contents of the pineapple stem was moderately high. According to Cissé (2012) and Ouattara *et al* (2016), the high mineral content of the pineapple fruit stalks investigated (Table 2), particularly micronutrients, may help to explain the ash concentration.

These authors stated that pineapple fruit ash level would be a good predictor of the fruit's mineral element richness. The ash values of the stalk are significantly greater than those found in the fruit of *A. comosus* (Koffi *et al*., 2021).

In contrast to the study of Olayinka and Etejere (2013) on the fruit of *A. comosus*, crude fibre of the stalk content was relatively high. This could be attributed to the component being studied. Given that a shortage of fibre in the body increases the risk of constipation and intestinal disorders, the crude fibre content found in this study would be beneficial for regular intestinal digestion as stated by Olayinka and Etejere (2013). The body needs fibre to function properly. In fact, consuming a lot of crude fibre helps prevent the absorption of extra cholesterol as well as regulating intestinal transit, constipation, diabetes, colon, and breast cancer (Okonwu *et al*., 2018).

Table 2: Mineral content of various extracts of *A. comosus* stalk

Nutrients (mg/kg)	Extracts				
	Aqueous	Methanol	Ethanol	Pet. Ether	Chloroform
Na	$0.23±0.01^a$	$0.19±0.01^a$	$0.14±0.01^b$	$0.12±0.01^b$	$0.16±0.01^a$
Mg	$0.28±0.01^a$	$0.25±0.01^b$	$0.23±0.01^a$	$0.14±0.01^c$	$0.23±0.01^a$
K	$5.13±0.01^a$	$5.93±0.04^b$	$4.66±0.01^c$	$1.27±0.01^d$	$4.84±0.00^c$
Fe	$6.22±0.01^a$	$7.85±0.01^b$	$6.57±0.01^c$	$4.21±0.01^d$	$6.04±0.01^a$
Zn	$22.63±0.07^a$	$11.43±0.03^b$	$12.13±0.05^b$	$3.46±0.01^c$	$5.38±0.01^c$
Cu	$2.60±0.01^a$	$2.81±0.01^b$	$2.72±0.01^b$	$0.34±0.01^d$	$0.91±0.01^d$
Mn	$2.02±0.01^a$	$1.43±0.01^b$	$0.88±0.01^c$	$0.20±0.01^c$	$0.56±0.01^c$
Sn	$2.02±0.01^a$	$0.61±0.01^b$	$1.16±0.01^c$	$0.30±0.01^d$	$0.53±0.01^b$
Co	$1.02±0.01^a$	$0.65±0.01^b$	$1.24±0.11^c$	$0.45±0.01^d$	$0.67±0.01^b$
Mo	$0.87±0.01^a$	$0.55±0.01^b$	$0.52±0.01^b$	$0.60±0.01^b$	$0.72±0.01^a$

**P<0.05 values with different letter are not statistically significant*

Table 3: Qualitative Phytochemical Screening of the Extracts of *A. comosus*

Phytochemicals	Extract				
	Aqueous	Methanol	Ethanol	Petroleum ether	Chloroform
Saponins	-	+	++	+	+
Phlobatannins	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-
Flavonoids	+	+	+	+	-
Tannins	+	+	+	+	+
Alkaloids	+	+	+	+	-
Terpenes	+	+	+	-	-
Phenol	+	+	+	+	+
Steroids	-	-	-	-	-
Thiols	-	-	-	-	-

Key: *+ = Present; ++ = Moderately present; +++ = Highly present; - = Absent*

Table 4: Quantitative Evaluation of Phytochemicals of the Extract of *A. comosus*

Phytochemicals	Extract				
	Aqueous	Methanol	Ethanol	Petroleum ether	Chloroform
Phenol (mg/gGAE)	1.19±0.01[a]	1.21±0.01[b]	1.53±0.01[c]	2.74±0.02[d]	1.28±0.01[b]
Flavonoids (mg/gCAE)	1.03±0.00[a]	0.90±0.01[a]	2.21±0.01[b]	2.73±0.02[c]	1.19±0.01[d]
Tannins (mg/gTAE)	1.19±0.01[a]	1.21±0.01[b]	1.53±0.01[c]	2.74±0.02[d]	1.28±0.01[b]
Alkaloids (mg/gATE)	0.58±0.00[a]	1.54±0.05[b]	1.78±0.01[c]	1.76±0.02[c]	1.37±0.01[b]

**P<0.05 values with different letter are not statistically significant*

Phytochemicals, commonly referred to as phytonutrients, are molecules that exist naturally in plants. These chemicals have been shown to exhibit antioxidant action in addition to being helpful to human health (Gupta *et al*., 2014). Phytochemicals may have anti-inflammatory and antioxidant properties. It is crucial in the body's detoxification from harmful and damaging chemicals (Gupta *et al*., 2014). The extracts of *A. comosus* stalk contains appreciable amount of phytochemicals (Tables 3 and 4). In fact, fruits and vegetables have been reported to contain phytochemicals such as saponin, flavonoids, glycosides, and tannins that are important in reducing the severity of disease (Ikeyi *et al*., 2013). This is predicated on the fact that saponin may have anticancer and antimutagenic properties that reduce the chance of developing human cancer. Similarly, alkaloids are complex organic nitrogenous substances that are mostly basic in nature (El-Olemy *et al*., 2010). Due to their analgesic, antispasmodic, and antibacterial properties, they are found as salts in organic acids and are used as medications (Choe et. al., 2012). Hence the presence of these phytochemicals in *A. comosus* makes it a candidate for drug production (Trease and Evans, 2011). Also, flavonoids may play a part in the prevention of cancer and heart disease (Carlsen *et al*., 2010). They could activate processes that have an impact on cancer cells and prevent tumour invasion (Gupta *et al*., 2014). Tannins have been discovered to form reversible complexes with proteins high in proline. Astringent plants with tannins as their primary constituents are used to treat gastrointestinal illnesses like diarrhoea and dysentery (Jasmine and Estherlydia, 2014). Fruits and vegetables contain phytochemicals that can influence oxidative agents, immune system stimulation, hormone metabolism, gene expression in cell proliferation and death, as well as bacterial and viral activities (Jasmine and Estherlydia, 2014). The study, therefore revealed that *A. comosus* stalk contains a good quantity of these phytoconstituents which is the reason for its medicinal value as published previously (Maimunah *et al*., 2020).

Conclusion: The pineapple stalk has a reputation for being waste, but if improperly used, it can lead to environmental contamination issues. This work has made it possible to turn pineapple waste, like the stalk, into a value-added product. This is because *Ananas comosus* stalk extracts had a good amount of micronutrients and phytochemicals that can be harnessed

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