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Effect of Postharvest Period on Phytochemical Content and Brownish-black Rot Disease of Postharvest *Irvingia* Species Fruit Wastes

Tungbulu, G.
Department of Biological Sciences, Niger Delta University, Bayelsa State, Nigeria

Etebu, E.
Department of Biological Sciences, Niger Delta University, Bayelsa State, Nigeria. Correspondence Author: eetebu@gmail.com

Ezenwaka, J.
Department of Crop production, Niger Delta University, Bayelsa State, Nigeria

Target Audience: Postharvest plant pathologists, Food scientists, Natural resources managers

***Abstract* - *Irvingia* species are economically important fruit trees.Although the phytochemical qualities of its fruits are known, the relationship between postharvest disease and various phytochemicals composition are yet unknown. Hence in this research, the changes that occur in postharvest *Irvingia* fruit waste at different days after harvest (DAH) was assessed with respect to brownish-black rot disease and phytochemical contents (alkaloids, flavonoids, saponins and tannins). Results showed that whilst overall weighted mean severity of brownish-black rot diseasewas 36.57%, phytochemical contents per gram of postharvest *Irvingia* fruit waste were alkaloids (55.98mg), flavonoids (47.64mg), tannins (43.07mg), and saponins (24.98mg).**

Correlation/regression analyses showed that brownish-black rot disease was significantly ($P \leq 0.05$), positively related to tannins (Pearson coefficient = 0.73) and alkaloids (Pearson coefficient = 0.69). Brownish-black rot of *Irvingia* fruits potentially influences the quantitative content of phytochemical, and this work provides the prerequisite knowledge of when to maximally exploit the different phytochemicals inherent in *Irvingia* fruits wastes.

***Keywords* – Brownish-black Rot Disease, *Irvingia Species*, Phytochemicals.**

I. INTRODUCTION

There is a growing interest and awareness in the consumption of diets rich in fruits and vegetables because several studies have shown that secondary metabolites, generally called phytochemicals, occurring naturally in fruits and vegetables are able to significantly reduce the incidence of cardiovascular and some other chronic and degenerative diseases associated with oxidative damage and aging (Ames 1993; Tripoli *et al*, 2007; Block et al., 1992; Howe et al., 1992; Steinmetz and Potter, 1991, 1996; World Cancer Research Fund/American Institute of Cancer Research, 1997; Joshipura et al., 2001; Bazzano et al., 2002; Kris-Etherton et al., 2002).

Knowledge on the benefits of consuming diets rich in fruits and vegetables has elicited a corresponding response by governments and practitioners in the agro-industry of most countries which has led to increase in the production of fruits (Sanraraj *et al*, 2012). Sadly, the same response cannot be said of most developing countries such as Nigeria. In order to make similar progress in developing countries like Nigeria, there is the need to explore new options to improve fruit consumption patterns and thereby improve the health status and increase longevity amongst the populace. New options would most likely be readily acceptable by majority of locals in southern Nigeria if they are centered on fruits like *Irvingia* which they consume or are already familiar with.

Irvingia species is an under-utilized edible African fruit tree that grows in the humid lowland tropical forest zones of West and Central Africa (Harris 1996). Commonly called bush mango in Nigeria, it bears climacteric mango-like fruits which are green in color when unripe but become yellow when ripe (Etebu 2013; Harris, 1996). *Irvingia* is greatly valued especially amongst local farmers and traders who depend on it for their livelihood, both as source of food and means of income. The pulp of the fruit is usually eaten fresh mostly by harvesters as a snack whilst extracting the kernels (Ayuk *et al*, 1999). The Kernels are generally considered the most valuable part of the fruit because they contribute to the nutritional, economic and health status of consumers (Agbor *et al*, 2005; Ndoye *et al*, 1998; Ngondi *et al*, 2005). In particular, *Irvingia gabonensis* seeds contain phytochemicals with anti-oxidative properties, and have been associated with the prevention and or treatment of diabetes, and cardiovascular diseases. They have also been shown to aid the reduction of cholesterol levels in blood and in the control of obesity in Cameroon (Ngondi *et al*, 2005). The fleshy part of the fruit on the other hand is often discarded as waste and left to rot in dumps, water bodies and nearby bushes despite the fact that it constitutes over 80% of the fruit (Etebu, 2012, 2013), and could potentially serve as feed for pigs, and in the production of fruit drinks, wine, jam and other syrups (Ayuk et al., 1999; Akubor, 1996).

Whilst the presence of antioxidants and phytochemicals in *Irvingia* seeds have long been known (Agbor *et al*, 2005; Ndoye *et al* 1997; Ngondi *et al*., 2005) their presence in the fleshy pulp was investigated only recently (Etebu, 2012, 2013), and these studies showed that the fleshy exocarp equally possess alkaloids, saponins, tannins, flavonoids and glycosides.

Whilst not downplaying the importance of these studies (Etebu, 2012, 2013), it is worth noting that the assessment of the different groups of phytochemicals was only qualitative. No attempt was made to objectively quantify the different groups of phytochemicals inherent in the fruits' fleshy exocarp. Furthermore, amongst other reasons, phytochemicals in plants are generally reported to help the plant ward off pathogenic infections and other

forms of invasions (Ashihara *et al*, 2008; Wink, 1998). It would be interesting therefore to study the potential relationships between disease and different groups of phytochemicals in postharvest *Irvingia* fruits. Also, an objective and quantitative assessment of the phytochemicals of postharvest *Irvingia* fruit waste is paramount to unlocking the potential health, industrial and economic benefits of this fruit waste.

In this research, therefore, the changes that occur in postharvest *Irvingia* fruit waste after harvest was assessed with respect to brownish-black rot disease and phytochemical composition. Findings from this work would avail us the needed information to further extract and exploit phytochemicals of interest to maximize their health, economic and or industrial benefits.

II. Materials and Methods

A. Samples Collection and Experimental Design

Irvingia fruits were harvested from a natural forest situated in Amassoma town (Lat. 4°58′09″N Long. 6°06′34″ E) of Bayelsa state, Nigeria. A total of 600 fresh and green fruits were randomly selected and split with a machete to extract the kernel, and the exocarp which is usually considered waste by locals were thereafter separated into three replicates (200 fruits per replicate). A quadrant measuring about 3m × 1m having three equal compartments of 1m x 1m was constructed and fruits wastes from each replicate were spread in each of the three compartments of the quadrant respectively. The quadrant was bordered with net to exclude reptiles and the fruits were left to decay for 6 Days after harvest (DAH).

B. Postharvest Disease Assessment

At the onset (day 0) of the experiment 15 fruits were randomly selected each from all three replicates and their postharvest spoilage status were individually assessed based on brownish-black rot disease symptom as described by Etebu (2012). The average disease score of 15 fruit wastes (representing one replicate) was recorded as the score for each replicate. Assessment of postharvest spoilage status of the fruits was repeated on the 3rd and 6th day after harvest (DAH) respectively. Severity of postharvest spoilage was determined visually by the proportion of fruit area affected by brownish-black rot disease and expressed in percentage as prescribed by Etebu *et al*. (2003, 2009).

C. Qualitative Phytochemical Assessment

All fruits selected for assessment of postharvest disease on the 0th, 3rd and 6th day after harvest were separately washed in running tap water at room temperature. Thereafter, unquantified amount of the fruits' fleshy exocarp was thereafter sliced out and blended with household blender for 30secs under aseptic conditions. One hundred (100) gram of the resultant slurry was treated in various ways to assess the different types of phytochemicals. Screening for phytochemicals was done according to Harbone (1973), Sofowora (1993) and Trease and Evans (1989).

Alkaloids: Five (5) millilitre of 2% hydrochloric acid was added to 2 ml of *Irvingia* fruit pulp in a test tube and boiled in a water bath for 5 minutes. The mixture was left to cool and thereafter filtered. To 2 ml of filtrate was added 1ml of Mayer's reagent. The formation of a creamy white precipitate indicated the presence of alkaloids.

***Flavonoids*:** Eight (8) millilitre of ethyl acetate was added to 2 ml of *Irvingia* pulp in a test tube and brought to boiling for 1min in a water bath. It was thereafter left to cool and filtered. 4 ml of the resultant filtrate was then mixed with 1ml of 1% Aluminium chloride solution. The formation of a yellow upper layer which persisted in the presence of 1ml dilute ammonia solution indicated the presence of flavonoids.

Saponins: Five (5) milliliter of distilled water was added to 1 ml of *Irvingia* fruit waste extract and boiled in a water bath for 5mins. The mixture was then filtered while hot. To 1 ml of filtrate 2 drops of olive oil was added in a test tube. The tube was thereafter corked and shaken vigorously for about 60 seconds. The formation of a stable frost indicated the presence of saponins.

***Tannins*:** Two (2) milliliter of *Irvingia* fruit extract was added to 10ml of distilled water in a test tube and boiled in a water bath for 5 minutes. The resultant mixture was filtered while hot and allowed to cool. To 1 ml of filtrate was added 1ml of ferric chloride. A bluish-black or brownish-green precipitate indicated the presence of tannins. A relative increase in the amount of precipitate indicated a corresponding higher amount of tannins present.

D. Quantification of Phytochemicals

One hundred (100) gram of slurry obtained from qualitative assessment procedure as described above was evaporated in a water bath at 70°C until dried to about 20g. The dried residues were thereafter treated in various ways to assess and quantify the different groups of phytochemicals (alkaloids, flavonoids saponins and tannins).

***Alkaloids*:** Alkaloids were quantitatively determined according to the method of Harborne (1973). Five gram of *Irvingia* residue (equivalent to 25grams of *Irvingia* fruit waste slurry prior to evaporation of moisture) was first dissolved in 50 ml of 10% acetic acid and made up to 100ml with ethanol. The mixture was filtered after 4hrs and then concentrated in a water bath to a quarter of its original volume. Concentrated ammonium hydroxide was thereafter added dropwise to precipitate alkaloids out of the solution. The process was continued until the precipitation was completed. The filtrate was then filtered onto a pre-weighed Filter paper. The collected precipitates were further washed with 1% ammonium hydroxide, and the alkaloid residue together with the pre-weighed filter paper was dried at 60°C for 1 hr and re-weighed. The percentage alkaloid content was then determined using the formula, and thereafter recorded in mg/gram of *Irvingia* fruit waste slurry.

$$\% \text{ Alkaloids} = \frac{W_2 - W_1}{W} \times 100$$

Where:
W = Initial weight of sample (*Irvingia*)
W_1= Weight of Filter paper
W_2= Weight of Filter paper + alkaloid precipitate

***Flavonoids*:** Flavonoids were quantified according to Harborne (1973) and Zhishen et al., (1999) with slight modifications. Half a gram of dried *Irvingia* fruit granules were boiled in 2M HCl for 30mins under reflux and filtered after cooling. Thereafter flavonoids were extracted with 6ml of ethyl acetate. The ethyl acetate was then evaporated in a water bath. To the flavonoid residue was added 10% Aluminium Chloride dropwise until a yellow color was developed indicating flavonoid presence. Thereafter absorbance was measured at 510nm with a spectrophotometer (Genway, model 6505, USA). Rutin was used as a standard for the construction of a calibration curve from which flavonoid content of the *Irvingia* fruit wastes was determined in mg/g.

***Saponins*:** Half a gram of *Irvingia* samples as described above was added boiled in 1N HCl for 4 hrs and filtered after cooling. Thereafter, 50mls of petroleum ether was added to the filtrate and shaken vigorously to produce the ether layer. Afterwards, the aqueous layer was recovered and ether evaporated. To the residue, 5mls of ethanol-acetone mixture (1:1) was added. Six milliliter of Ferrous Sulphate was then added to 0.4mls of the resultant solution followed by 2mls of concentrated H_2SO_4. Spectrophotometric absorbance reading was taking at 490nm. Meanwhile standards of different concentrations of Bioscin (Sigma, UK) had been earlier on prepared. The absorbance of saponins of the bioscin standard solutions as well as sample was measured after 490 nm using a spectrophotometer (Genway, model 6505, USA). The saponin content was expressed as mg/g of *Irvingia* pulp.

***Tannins*:** Tannin was quantified according to Swain (1979). One gram of *Irvingia* residue was added to 20ml of 50% methanol. This was shaken thoroughly and heated at 80 for 1 h in a water bath to ensure homogeneity of the mixture. The extract was thereafter filtered, followed by addition of 20 ml of distilled water, 2.5 ml of Folin-Denis reagent and 10 ml of 17% aqueous Na_2CO_3 was also added and thoroughly mixed together. The mixture was made up to 100 ml with distilled water, then mixed and allowed to stand for 20 min. Meanwhile standards of different concentrations of Tannic acid ranging from 0-10 ppm had been earlier on prepared. The absorbance of the tannic acid standard solutions as well as sample was measured after color development at 760 nm using a spectrophotometer (Genway, model 6505, USA). The tannin content was expressed as mg of tannic acid equivalent/g of *Irvingia* pulp.

***Data analysis*:** Data on brownish-black rot disease severity were first subjected to arcsine transformation while data on phytochemical contents were square root transformed to fulfill the assumptions of ANOVA according to Gomez and Gomez (1984). The transformed data were thereafter subjected to ANOVA using Generalized Linear Model of Minitab version 14.0 Statistical software. Correlation/regression analyses were performed between the transformed data on brownish-black rot disease of *Irvingia* fruits and the different phytochemical components. Mean disease and phytochemical content data were further subjected to Tukey's mean separation test. Mean transformed set of data were thereafter de-transformed (weighted) and discussed hereunder.

III. Results and Discussion

Effect of Postharvest days on Brownish-black Rot Disease and Phytochemical Content of Irvingia Fruit Wastes

Postharvest *Irvingia* fruit wastes showed a rapid spread of brownish-black rot disease symptoms in 6 days after harvest in an open field. Severity of postharvest disease was observed to progress significantly as the number of days after harvest (DAH) increased (Table 2). Mean weighted brownish-black rot disease scores on 0^{th}, 3^{rd} and 6^{th} DAH were 1.74%, 22.16% and 89.24% respectively. Findings from this present work were comparable to results of previous works, and postharvest disease symptoms largely followed the same pattern of occurrence previously observed in some earlier works (Joseph and Aworh 1991, 1992; Etebu, 2012, 2013; Etebu and Tungbulu, 2015, 2016). Earlier works have shown that specific species of fungi and bacteria are associated with decaying *Irvingia* fruit wastes. These include fungal species such as *Aspergillus*, *Penicillium*, *Botrytis*, *Rhizopus*, and *Mucor* (Joseph and Aworh, 1991; Etebu, 2012, 2013) and bacterial species such as *Bacillus*, *Enterobacter*, *Oceanobacillus*, and *Staphylococcus* (Etebu and Tungbulu, 2015).

All four groups of phytochemicals (alkaloids, flavonoids, saponins and tannins) investigated in this work were observed to occur in postharvest *Irvingia* fruit wastes. Overall weighted mean contents per gram of fresh *Irvingia* waste pulp for phytochemicals assessed were alkaloids (55.98mg), flavonoids (47.64mg), tannins (43.07mg), and saponins (24.98mg) (Table 1). The presence of phytochemicals in fruits, including *Irvingia* fruits, is widely known and reported by several workers (Schreiner and Huyskens-Keil, 2006; Etebu, 2012, 2013). Phytochemicals in general have been variously reported to play different significant roles in the survival of their host plants, ranging from deterring herbivores, protecting host plants against pathogens or various abiotic stresses, functioning as antioxidants or signaling molecules (Schreiner and Huyskens-Keil, 2006).

Results from this present work further showed that apart from saponins and flavonoids, the concentration of the other phytochemicals in *Irvingia* fruits were significantly influenced by post-harvest period. Fresh *Irvingia* fruit wastes were observed to possess alkaloids, and their abundance was noted to be dependent on postharvest period. Whilst fresh fruits (DAH = 0) had 47.89mg/g as weighted mean occurrence of alkaloids, the amount increased significantly ($P \leq 0.05$) to 61.31mg/g at the 6^{th} day after harvest. The alkaloid contents at the 3^{rd} day after harvest (59.29mg/g) was not significantly different from those at 0 or 6^{th} day after harvest (Table 2). Contrary to the findings of this present work, Etebu (2012) in his subjective ratings adduced that alkaloid content of *Irvingia* fruits decreased as postharvest period increases. Photolysis was potentially branded as being responsible for the

dissipation and degradation of alkaloids, but earlier reports had shown that most alkaloids are neither easily biodegraded, hydrolyzed nor volatilized (McGee, 2004). Furthermore, Adebayo *et al* (2010) showed that the amount of total phenol content of pepper fruit (*Dennettia tripetala*) increased as storage time of the fruits increased. These earlier findings buttresses and substantiates the correspondence increase in alkaloid content with increase in postharvest period, as observed in this present work with respect to *Irvingia* fruit wastes in storage.

Similar to other phytochemicals, alkaloids are known to defend plant, wherein they are produced, against herbivores, fungi, bacteria, viruses and other competing plants (Ashihara *et al*, 2008; Wink, 1998). The antimicrobial activity of most botanicals are attributed mainly to alkaloids which they possess (Okigbo and Ajalie, 2005). Interestingly, findings from a fairly recent work showed that *Irvingia* fruit waste extract significantly inhibited the growth of some postharvest spoilage fungi such as *Aspergillus, Fusarium* and *Mucor* species (Etebu and Benjamin, 2014). Two species of *Irvingia* are reported to occur in Nigeria, *I. gabonensis* and *I. wombolu* (Etebu, 2013; Etebu and Tungbulu, 2016). Whilst *I. gabonensis* is sweet and edible, the other is bitter and inedible (Harris, 1996). Findings from a comparative study between these two species of *Irvingia* suggest that *Irvingia wombolu* (the bitter species) fruits stored for 2 days after harvest potentially possess a higher amount of alkaloids than their counterparts of the *I. gabonensis* (sweet species) stock (Etebu, 2013). Although this present work did not take into account the potential variability in phytochemical contents between the two species, it nonetheless showed the fate of the various phytochemicals over time after harvest, and alkaloids were observed to occur in considerable high amounts in *Irvingia* fruit waste all through the 6 day period under investigation (Table 2).

The abundance of tannins in postharvest *Irvingia* fruits was also observed to be influenced by postharvest period. Whilst pulp of freshly harvested (DAH = 0) *Irvingia* possessed 25.70mg of tannins per gram, the concentration increased significantly ($P \leq 0.05$) to 51.84mg/g on the 3rd day after harvest (Table 2). Difference in occurrence of tannins in the fruits on the 3rd and 6th days after harvest was not significant at 5% probability level.

Tannins are polyphenolic compounds, known to antagonize microbial activities through inhibition of protein synthesis (Ayepola and Adeniji, 2008). Their antimicrobial potency are being exploited in herbal medicine where plants possessing them are deployed in curing wounds, various ulcers, haemorrhoids, inflamed mucous membranes frost bites and burns (Eleazu, *et al*, 2012). Tannins have also been associated with various pharmaco antioxidants (Koleckar *et al.*, 2008), antihelmintics (Ketzis 2006), and in cancer chemotherapy (Chung et al., 1998) Although reports on the use of *Irvingia* fruit extract in the aforementioned aspects of herbal medicine are non-existent, to the best of our knowledge, *Irvingia* fruits are apparently potential raw materials waiting to be exploited for their tannin content. Also, tannins are known in the food industry to cause the astringency and bitter taste of foods and drinks (McRae and Kennedy, 2011). This again portends that the fleshy pulp of *Irvingia* fruits, often discarded as wastes could be used in our food industries where the end product is expected to have bitter taste.

It is pertinent to also state that apparent negative reports also exist in scientific literature about tannins. For example, tannins are thought to greatly impact on animal nutrition, including inhibition of growth rate and digestive enzymes but these same properties have also been reported as being useful in the prevention of certain diseases (Bennick, 2002). In particular, tannins have been reported to interfere with the absorption of dietary iron and the inhibition of digestive enzymes at high concentrations (Eleazu et al, 2012). However, results from this work showed that tannin concentration in *Irvingia* fresh fruit is relatively low hence consumption of fresh fruits would not interfere with iron absorption nor inhibit digestive enzymes. Whilst not downplaying these potential concerns, no adverse report of known medical significance has been heard from or about locals arising from ingestion of *Irvingia* fruits.

Unlike the aforementioned phytochemicals whose relative abundance was dependent on postharvest period, saponin content of *Irvingia* fruit wastes were not significantly ($P=0.05$) affected by postharvest period. Weighted saponin content per gram of *Irvingia* fruit wastes on 0th, 3rd and 6th days after harvest were 20.07mg, 25.70mg and 29.70mg respectively (Table 2). Like tannins, saponins have been demonstrated to affect animals in both positive and negative ways. Studies have indicated that saponins have haemolytic properties, being able to precipitate and coagulate red blood cells (Eleazu *et al*, 2010). Also, some saponins have been reported to impair the digestion of protein and the uptake of vitamins and minerals in the gut; causes hypo-glycaemia in animals. These negative properties, notwithstanding, some saponins are also known to play very important roles in human and animal nutrition. In particular, saponins bind with bile salt and cholesterol in the digestive tract, and that prevents cholesterol from being reabsorbed into the body. The ability of dietary saponins to lower blood cholesterol is of particular interest in human nutrition (Chandel and Rastogi, 1980). Ngondi and Associates (2005) whilst working with the kernels of *Irvingia* fruits showed that the kernels were able to aid the reduction of cholesterol levels in blood and in the control of obesity in Cameroon. Although saponins were not specifically mentioned as being responsible for this welcome health feat, it is interesting to note that saponins, observed in fleshy *Irvingia* fruit of this present work, could lower cholesterol levels in humans.

Furthermore, saponins are known to also react with cholesterol rich membranes of cancer cells, preventing these cells from growing (Francis *et al.*, 2002). Like other groups of phytochemicals, saponins have been reported to possess antioxidative, antiviral and antibacterial properties (Mert-Türk, 2006; Simões et al., 1999). In the brewing industry, saponins are used in the production of beer and other beverages because of their bitter taste (Sodipo *et*

al.,2000) and foaming properties in aqueous solutions (Eleazu *et al.*, 2010). From the foregoing, *Irvingia* fruit wastes, often regarded as wastes, could be further explored for their saponin content and channeled into other productive uses, especially against the backdrop of its content being relatively unaffected by postharvest period. There is need to further study the different specific saponins inherent in *Irvingia* fruits to ascertain the benefits and potential medical or health concerns.

Similar to saponins, the relative amount of flavonoids was not significantly (*P≤0.05* affected by postharvest period. Weighted flavonoid content of the fruits on 0th, 3rd and 6th day after harvest were 46.79mg, 56.25mg and 40.45mg per gram respectively (Table 2). Earlier qualitative works had shown the presence of flavonoids across both in *I. gabonensis* and *I. wombolu* in Nigeria (Etebu, 2012, 2013). Medical benefits of flavonoids have been reported to include protection of the gastrointestinal (GI) tract, antispasmodic, antidiarrhoeal, antibacterial, antisecretory and antiulcer properties (Di Carlo *et al.*, 1993; La Casa *et al.*, 2000; Sunairi *et al.*, 1994; Isomoto *et al.*, 2005; Rice-Evans *et al.*, 1997). Flavonoids are particularly known for their strong antioxidant capacities which positions them as effective key players in plant based preventive therapies (Lotito and Free, 2011; Eleazu *et al*, 2012; Williams *et al*, 2012).

Irvingia fruits could be explored as potential remedy against allergies, inflammations, microbes, viruses and tumors. This speculative assertion is hinged on the fact that flavonoids are potent free radical scavengers which prevent oxidative damage and protect against carcinogenesis (van Acker, 1995), and *Irvingia* fruit wastes, in this study, were observed to possess relatively high amounts of flavonoids whose abundance was not significantly (*P=0.05*) influenced by postharvest period (Table 2). The relative considerable amount of flavonoids in *Irvingia* fruit wastes would doubtless add to the potential benefit for their consumption both as food for humans or feed for animals.

Inter-relationship between Brownish-black Rot Disease and Phytochemicals of Postharvest Irvingia Fruit Wastes

The potential relationship between brownish-black rot disease and the different groups of phytochemicals were assessed in this work because some earlier works on other fruits had shown that the abundance or otherwise of specific phytochemicals in postharvest fruits is significantly dependent on postharvest storage period and conditions (Schreiner and Huyskens-Keil, 2006). Results from this study showed that brownish-black rot disease of *Irvingia* fruit wastes were significantly (*P≤0.05*), directly correlated to alkaloids (Pearson Correlation coefficient = 0.69) and tannins (Pearson Correlation coefficient = 0.73) (Table 3). Results of correlation/regression analyses between brownish-black rot disease and saponins or flavonoids were not significant (*P≤0.05*). Whilst saponins were observed to significantly, negatively related to flavonoids (Table 3), Somit *et al.*, 2013) working on phytochemicals of leaves of *Croton bonplandianum*, on the contrary, showed that saponins are positively related to flavonoids. Also, whilst Somit et al., (2013) observed a positive relationship between saponins and tannins in the plant they studied, findings in this work showed no significant relationship between tannins and saponins at the 5% probability level. This shows that the amount and potential relationship between different plants and plant parts may vary.

The separate positive relationships between brownish-black rot disease with alkaloids and tannins may be linked to the overall inter-relationship between the *Irvingia* fruit and disease causing pathogens. Alkaloids and tannins are known to protect their host plants from microbial invasion (Ashihara *et al*, 2008; Wink, 1998). Furthermore, brownish-black rot disease of *Irvingia* species is significantly, positively related to number of fungi associated with diseased fruits (Etebu, 2013). Going by these submissions, it would suffice to suggest that the increase in alkaloids and tannins (Table 2) could have been in response to increase in severity of brownish-black rot disease of the fruits, apparently to inhibit the activities of disease causing microorganisms.

The result of this present work also showed that the relative amount of alkaloids is highly significantly (*P≤0.01*), positively related to those of tannins (Table 3). This showed that an increase in alkaloids led to a correspondingly increase in tannins. Meanwhile Janzen and Waterman (1984) reported a negative correlation between alkaloid and tannin contents in a dry forest in Costa Rica. This earlier finding notwithstanding, the highly positive relationship between alkaloids and tannins could be a phenomenon beyond antagonizing the proliferation or degradative activities of microorganisms invading *Irvingia* fruits as earlier posited going by their respective significant positive relationship with brownish-black rot disease (Table 3). The highly positive relationship between the two phytochemicals may be nature's way for each phytochemical to counterbalance the negative consequences of the other and vice versa. The apparent credibility of this position is strengthened by an earlier report of Freeland and Janzen (1974). These workers maintained that alkaloids and tannins form insoluble alkaloid-tannates in herbivore guts to negate the effects of each other. Furthermore, tannins are commonly used in human medicine as an antidote to alkaloid poisoning because, as with proteins, they are able to form complexes with alkaloids (Hagerman, 2002). The corresponding increase in tannins in direct relationship to increase in alkaloids could therefore be a natural mechanism aimed at dissipating the apparent toxic properties of alkaloids to ensure that consumption of fresh *Irvingia* pulp is safe for humans and other animals. Further research would be required to ascertain the veracity or otherwise of this informed speculation.

IV. CONCLUSION

Although the bioavailability of phytochemicals in plants has been reported to be influenced by a variety of factors including, but not limited to, genotype, maturity, environment and postharvest conditions (Manach *et al.*,

2004; Boyer and Liu, 2004;Schreiner and Huyskens-Keil, 2006), results obtained from this work showed that the amount of the various groups of phytochemicals are considerably significant especially when the fruits are fresh.

Furthermore, results from this work show that phytochemicals which are important to and of health benefit to the gut of animals persisted in postharvest *Irvingia* fruit wastes up to the 6th day after harvest in spite of a corresponding increase in severity of brownish-black rot disease. Findings from this present work therefore portends that fresh *Irvingia* fruit pulp would serve as a potential raw material for the production of *Irvingia* fruit juice, beverage or snack whilst *Irvingia* fruit waste at advanced stage of spoilage may be processed into animal feed as proposed by some earlier workers.

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Table 1. Cumulative mean severity and occurrence of disease and phytochemicals in Post-harvest *Irvingia* fruit wastes

Variable	Transformed Mean ± S.E.	Weighted Mean
Brownish-black rot disease	35.50 ± 9.45	36.57
Alkaloids	7.48 ± 0.18	55.98
Tannins	6.56 ± 0.41	43.07
Saponins	5.00 ± 0.28	24.98
Flavonoids	6.90 ± 0.29	47.64

Table 2. The effect of postharvest period on disease and phytochemical contents of *Irvingia* fruit waste

Days after harvest	**Brownish-black rot disease**		**Alkaloids**		**Tannins**		**Saponins**		**Flavonoids**	
	T-mean^{x}	W-mean	T-mean^{z}	W-mean	T-mean^{z}	W-mean	T-mean^{z}	W-mean	T-mean^{z}	W-mean
0	7.58^{a}	1.74	6.92^{a}	47.89	5.07^{a}	25.70	4.48^{a}	20.07	6.84^{a}	46.79
3	28.08^{b}	22.16	7.70^{ab}	59.29	7.20^{b}	51.84	5.07^{a}	25.70	7.504^{a}	56.25
6	70.85^{c}	89.24	7.83^{b}	61.31	7.42^{b}	55.06	5.45^{a}	29.70	6.36^{a}	40.45

T-mean^{x} = Arcsine transformed means
T-mean^{z} = Square root transformed means
W-mean = Weighted (de-transformed) mean

Table 3. Correlation matrix of brownish-black rot disease and phytochemicals of postharvest *Irvingia* fruit wastes

Parameters	Disease	Alkaloids	Tanins	Saponins
Alkaloids	0.69*			
Tanins	0.73*	0.91**		
Saponins	0.36^{ns}	0.19^{ns}	0.33^{ns}	
Flavonoids	-0.21^{ns}	0.05^{ns}	0.00^{ns}	-0.71*

*=Significant at *P=0.05*
**=Significant at *P=0.01*
ns=Not significant at *P=0.05*

AUTHORS' PROFILES

Gloria Tungbulu has a MSc. Degree in Industrial Microbiology from the University of Port Harcourt, Nigeria. She is a doctoral student of the Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Ebimieowei Etebu is an Associate Professor of Molecular and Applied Microbiology in the Department of Biological Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. He has a PhD degree in Molecular Microbiology from the University of Sheffield, United Kingdom. His main research interests are in the area of biological sciences, in particular the area of agricultural and environmental microbiology.
*Correspondence Author: eetebu@gmail.com

Mr Jasper Ezenwaka has a MSc. Degree in Natural resources management and community development from Cranfield University, UK. He is a Lecturer with the Niger Delta University, a Tropical Forester, interested in issues of sustainability of rural livelihoods, rural community development and stakeholder participation in the management of forest resources.