CURRENT STUDIES IN COMPARATIVE EDUCATION, SCIENCE AND TECHNOLOGY, VOLUME 2, NUMBER 2, 2015, PP. 275-287

Phytochemical Composition and Mycological Evaluation of the Fruits of Dennettia Tripetala Baker f.

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ABSRACT *Dennettia tripetala* fruits obtained from six local sources were subjected to laboratory analyses to determine the proximate, mineral and anti -nutrient compositions in the Food Science and Technology Laboratory in Rivers State University of Science and Technology, Port Harcourt. The associated fungi responsible for the fruits rot were also evaluated in the Plant Pathology Laboratory. The fruits were sun dried for three days and thereafter smoked with low heat at 45^{o} C for another three days and stored in an air tight plastic container. The preserved samples were monitored for mould growth and palatability assessment at one month interval for six months respectively. It was observed that *D. tripetala* contains appreciable plant nutrients, minerals and anti-nutrient elements which distinguished it as an important tropical fruit. Mycological evaluation revealed that the fruits are prone to fruit rot caused by seven fungi with varying degrees of incidence. All the fungal isolates were found to be pathogenic to fresh fruits with some causing dry fruit rot while others caused soft fruit rot. Shelf life preservation by sun drying and smoking of the various fruit samples stabilized the fruit quality as evidenced by the inhibition of mould growth and the stable palatability records for six months. This study has shown that *D. tripetala* fruits are rich in essential plant nutrients required for healthy growth and development and as such should be consumed in large quantity. Several fungal organisms were capable of reducing the fruit quality. However, it has been found that sun-drying and smoking of the fruits at low temperature will preserve and prolong the shelf life of the fruits and at the same time inhibit mould growth and enhance palatability of the fruits.

Key words: Dennettia tripetala, Phytochemicals, Mycology, Shelf life

Introduction

Dennettia tripetala Baker f belongs to the family *Annonaceae*. It is a small tree well distributed throughout the tropical rainforest regions of the world. This tree is found in the tropical and some times in the savannah regions of Nigeria (Okwu, *et al*, 2000). It grows very luxuriantly at the onset of rain usually from April to June (Umoh, 1998). The fruits of the tree are edible and peppery as the name implies. In most localities among the Ikwerre ethnic people, the tender leaves are often eaten by children and leave a sweet fragrance in the mouth after consumption. The leaves are boiled in combination with mango leaves as pot herbs to treat minor fever (Nwinuka and Nwiloh, 2009). Some of the fruits extracts have been reported as active anti-fungal agents against *candida sp, Cryptococcus sp, Geotrichum sp, Rhizopus stolonifer, Aspergillus sp and Fusarium sp*. (Ejechi, *et al* 1999). The oil extracted from the seeds of this plant has been used effectively for the preservation of grains such as cowpea and maize without negatively affecting their viability (Akinwumi, 2011). *Dennettia tripetala* is usually propagated by seed (Mapongmetsem, 2007, Orwa *et al*, 2009, Udo, 2011). However, because of lack of availability of seeds due to infrequent fruiting, poor seed germination, slow seedling growth, and competition from wild animals, birds and humans vegetative propagation seems to be the most promising methods of propagation of this potent fruits (Osaigbovo *et al*, 2010). Like most tropical fruits, vegetables and nuts *D. tripetala* fruits are prone to fungal spoilage which affects the fruit quality. During the flush seasons the fruits are found in large quantity which must be consumed within a very short period after which they are found in large heaps in the markets as waste. Considering the various use into which the fruits can be put and the special preference it enjoys among the tropical and sub-tropical dwellers, it is however, important for the nutrient quality of fruits to be well understood. This work therefore investigated the nutrient compositions of the fruits of *D. tripetala* and the associated fungi that lead to the deterioration of the fruits. It also assessed sun drying and smoking of the fruits at low heat as measures of shelf life preservation. The knowledge of this will enable scientists foster the needed solutions that will encourage the production and consumption of the fruits and prevent this tree from extinction. It will also provide the needed apparatus for preventing fungal infections on the fruits and also increase the shelf life of the fruits.

Materials and method

Collection of samples

One basket full of freshly harvested fruits of *D. tripetala* was purchased from four local markets in Port Harcourt and transported to the Plant pathology laboratory for further studies. The fruits were sorted and the ones with blemishes were removed. Sampling was done at two weeks interval for two months during which the fruits were found in large quantity due to the seasonal nature of this fruits.

Determination of phytochemical compositions of Dennettia tripetala

Fresh fruits were collected from the sorted samples and taken to the Food Science and Technology Laboratory in the Rivers State University of Science and Technology Port Harcourt for proximate, mineral and anti-nutrient quality determination. These parameters were estimated using the AOAC (2005) methods of analysis.

Mycological studies

Sterilization of conical flask, slides, Petri dishes and all the equipment needed for the experiment was carried out in the laboratory. While the glass wares were sterilized in the oven at 120^{o} C for hours after washing them with soap, the other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Agrios, 2005). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used for the isolation was Sabouraud Dextrose Agar which was prepared using the standard method in a conical flask. The mouth of the flask was plugged with non-absorbent cotton wool and wrapped with aluminium foil. The conical flask with its contents was autoclaved at 121^{o}C and a pressure of 1.1 kg cm^{-3} for 15 minutes. The molten agar was allowed to cool to about 40^{o} C and then dispensed into Petri dishes at 15 mls per plate and allowed to further cool down and solidify.

Isolation of fungi

Fruits showing symptoms of rot were washed with tap water and rinsed with distilled water. The fruits were surface sterilized with 5% sodium hypochlorite. Thin slices of the partially rotted fruits were cut starting from the healthy portions up to the points were the rot had established with sterile scalpel and inoculated onto sabouraud dextrose agar in Petri dishes and incu-

bated for 5days at ambient temperature of 25^{o}C ± 3^{o} C (Baudoni, 1988, Chuku, 2009, Samson *et al,* 1988). The entire set up was observed for seven days until the organism was fully grown. Pure cultures of isolates were obtained after a series of isolations.

Identification of fungi of D. Tripetala

The microscopic examination of fungal isolates was done by needle mount method (Cheesebrough, 2000). The spores were properly teased to ensure proper spread of the spores for perfect visibility. The well spread spores were stained with cotton blue in lacto phenol and examined microscopically under low and high power objective. The fungi were identified based on their colonial morphology, mycelia structure, nature of spores and associated structures according to the keys of (Samson *et al,* 1988, Olds, 1983, Barnett and Hunter, 1972).

Pathogenicity studies

Pathogenicity test was carried out to determine if the fungi responsible for the rot of *D. tripetala* were host specific. The procedure described by Agrios, (2005) and Trigiano, *et al* (2004) was basically followed. Freshly harvested fruits were purchased from the same sources in Port Harcourt. The fruits were surface sterilized with 70% ethanol and "V" shaped wound was created on the fruits and each of the fungal isolates was transferred into the fruits in triplicate and sealed with Vaseline to avoid microbial contamination. The set up was incubated for 7 days at room temperature of 25^{o}C ± 3^{o}C.

Shelf life preservation of D. Tripetala

Dennittia tripetala like most tropical fruits is prone to deterioration by microorganisms. The unavailability of good storage facilities further compounds this problem and as such most of the fruits are wasted during the flush seasons and become relatively scarce thereafter (Okaka, 1997, Chuku, 2013, Onuegbu, 2002). Healthy mature green fruits, half ripe fruits and fully ripen fruits were acquired from the same sources, washed in tap water and sundried for three days. The sundried fruits were further put in a squared shape metallic sieve and placed over oven on low heat (45^{0} C) and smoked for three days. At the end of the third day, the fruits were placed in a clean dry plastic container and monitored for mould growth for six months. Palatability assessment using a hedonic scale of 1 – 5 was also carried out where 5 = 100% acceptability, 4 = 80%, 3 = 60%, 2 = 40%, 1 = 20%. The major parameter considered was the peppery quality of the fruits. The scale of 1-5 was used considering the sample size even though the standard hedonic scale

of 1-10 is appropriate.

Results

Results of the phytochemical composition of *D. tripetala* are shown in Table 1. The proximate composition consisting of moisture, ash, fibre, fat, carbohydrate and protein were found in appreciable amount indicating that the fruit is rich in vital nutrient elements required for normal body growth. Calcium, phosphorus, potassium, sodium magnesium and iron were the mineral components of *D. tripetala*. The minerals were also present in reasonable quantity especially calcium, potassium and magnesium. Tannins, saponins, oxalates and cyanogenic glycosides were also found in the fruits of *D. tripetala.* The presence of these phytochemicals in the fruits of *D. tripetala* indicates that it is a very important fruit both for consumption and for pharmaceutical industries.

Table 1: Phytochemical components of Dennettia tripetala

Parameters	**Values**
Proximate composition (%)	
Moisture	42.3 ± 0.001
Ash	5.6 ± 0.001
Fibre	3.9 ± 0.002
fat	18.3 ± 0.001
carbohydrate	13.6 ± 0.002
protein	16.6 ± 0.003
Mineral contents	
Calcium (%)	20.4 ± 0.001
Phosphorus (%)	0.35 ± 0.001
Potassium (mg/100g)	1.66 ± 0.002
Sodium (mg/100mg)	0.25 ± 0.001
Magnesium (mg/100mg)	4.5 ± 0.004
Iron (mg/100mg)	0.28 ± 0.002
Anti- Nutrient (%)	
Tannin	0.005 ± 0.001
Saponin	1.78 ± 0.002
Oxalate	3.24 ± 0.001
Cyanogenic glycosides	22.37 ± 0.003

Values are means of triplicate determination ± standard error
Legend
± = Standard error.

The mycological evaluation of the fruits of *D. tripetala* is presented in Table 2. Seven fungi with varying degrees of incidence were found to be associated with the rot of the fruits of *D. tripetala.* The fungi were *Fusarium rolfsii, Rhizopus stolonifer, Cryptococcus neoformans, Aspergillus niger, Aspergillus flavus and Muccor mucedo. F. rolfsii, R. stolonifer, Muccor mucedo* and *C. neoformans* caused soft rot of the fruits while *A. niger and A. flavus* caused dry rot of the fruits.

Table 2: Fungal flora of D. tripetala

Fungal flora of *D. tripetala*	**% incidence**
Sclerotium rolfsii	80 ± 0.005
Fusarium oxysporum	60 ± 0.002
Rhizopus stolonifer	100 ± 0.001
Cryptococcus neoformans	40 ± 0.003
Aspergillus niger	50 ± 0.002
Aspergillus flavus	60 ± 0.001
Muccor mucedo	80 ±0.003

Values are means of triplicate determinations ± standard error
Legend
± = Standard error

Pathogenicity study

Pathogenicity study revealed that all the fungal isolates caused rot on freshly harvested fruits of *D. tripetala.* Some of the fungi caused soft rot while others caused dry rot of the fruits. The fungi completely penetrated the fruits within seven days of incubation.

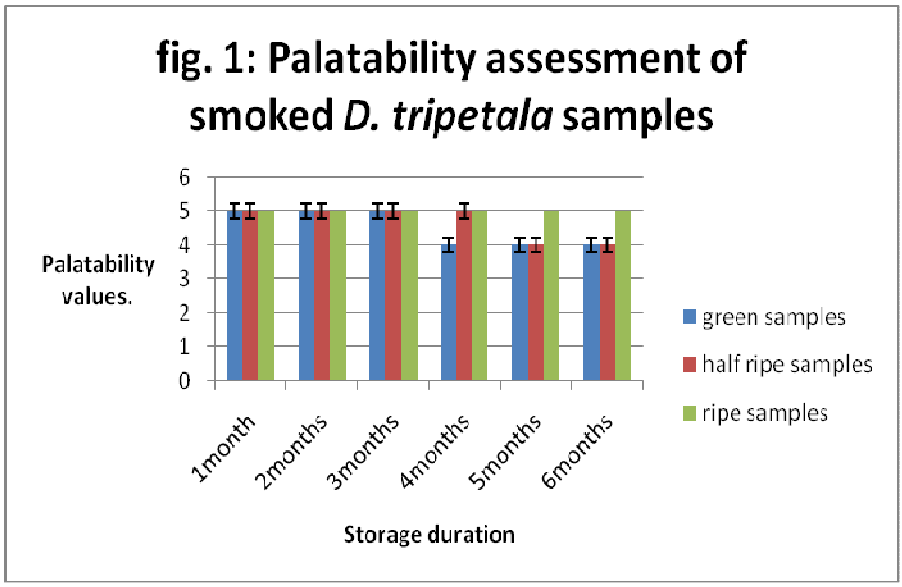
Shelf life preservation of D. Tripetala

Results of the shelf life preservation of smoked half ripen fruits, green mature fruits and ripe fruits of *D. tripetala* in relation to fungal growth are presented in Table 3. All the preserved samples did not support any of the fungal growth for a duration of six months indicating that the method of preservation by sun drying and smoking under low heat inhibited all the fungal isolates and therefore a good and cheap approach for the preservation of the fruits of *D. tripetala*.

Fungal isolates of *D. tripetala*	**Storage duration of *D. tripetala***					
	Smoked mature green fruits of *D. tripetala*					
	1 month	2 months	3 months	4 months	5 months	6 months
S. rolfsii	nil	nil	nil	nil	Nil	Nil
F. oxysporum	-	-	-	-	-	-
R. stolonifer	-	-	-	-	-	-
C. neoformans	-	-	-	-	-	-
A . niger	-	-	-	-	-	-
A .flavus	-	-	-	-	-	-
M. mucedo	-	-	-	-	-	-
	Smoked half ripe fruits of *D. tripetala*					
	1 month	2 months	3 months	4 months	5 months	6 months
S. rolfsii	nil	nil	nil	nil	Nil	Nil
F. oxysporum	-	-	-	-	-	-
R. stolonifer	-	-	-	-	-	-
C. neoformans	-	-	-	-	-	-
niger	-	-	-	-	-	-
flavus	-	-	-	-	-	-
M. mucedo	-	-	-	-	-	-
	Smoked ripe fruits of *D. tripetala*					
	1 month	2months	3 months	4 months	5 months	6 months
S. rolfsii	nil	nil	nil	nil	Nil	Nil
F.oxysporum	-	-	-	-	-	-
R. stolonifer	-	-	-	-	-	-
neoformans	-	-	-	-	-	-
niger	-	-	-	-	-	-
flavus	-	-	-	-	-	-
M. mucedo	-	-	-	-	-	-

Legend
= not found/nil

Fig. 1: Palability assessment of smoked D. tripetala samples.



Discussion

Phytochemical analysis showed that *D. tripetala* is rich in essential nutrient elements necessary for growth and development. The proximate and mineral compositions of *D. tripetala* portray it as an important fruit that can be used as substitute for most conventional fruits in the tropics. The values recorded for anti-nutrient elements were also appreciable as these elements also protect and deter predators from infesting and infecting the plants. The high nutritional qualities of most tropical fruits and vegetables are well documented (Chuku and Chuku, 2014, Ajala *et al*, 2014, Ogbuji *et al*, 2014, Elochukwu *et al*, 2014, Achinewhu, 1996). One outstanding quality of most fruits and vegetables is their low carbohydrate and high fibre and vitamins contents thus making their consumption very important for weight control (Ihekoronye and Ngoddy, 1985). Fungi have been implicated as the most serious spoilage organisms of most agricultural products both in the field and store (Onuegbu, 2002, Chuku and Anunobi, 2010, Chuku, 2011, 2012 and 2013). These organisms are capable of surviving in several places even where other organisms cannot. This wide range of adaptation by fungi makes them highly infectious amongst most plants and animals. Fungi secrete enzymes that cause tissue maceration; cell leakage and eventual cell rot (Onuegbu, 1999, Chuku *et al*, 2005). Fungi through their numerous types of infection have caused serious losses in quality and quantity of harvested

crops. Observation during the pathogenicity test revealed profuse mycelia growth from the inoculated sites which spread over the entire fruit surfaces which indicated that they were associated with the fruit rot. The high pathogenicity of the fungal organisms which are often associated with soil, air and contaminated environment have been reported (Onion, *et al*, 1981, Omokaro and Isoboye, 2014). Research on shelf life preservation indicated that sun drying and smoking the fruits of *D. tripetala* under low heat greatly stabilized the fruit quality in terms of mould growth and palatability. Smoking must have greatly reduced the moisture content of the fruits and thus concentrated the nutrients. The low moisture level of the smoked fruits also caused negative effects on the fungal isolates which could not penetrate the fruits and as such could not establish on the fruits (Chuku, 2014). Palatability test also indicated that the fruits remained acceptable for about six months (Okaka, 1997).

Limitation of the study

The major limitation of this study was the seasonal nature of the fruits of *D. tripetala* which restricted the sampling period to the flush season during which the fruits were in abundance. Another limitation encountered in course of the study was sporadic rainfall which was monitored to avoid soaking the samples being sun dried.

Conclusion

From the results of the present study, it can be concluded that *D. tripetala* is rich in phytochemicals and as such should be consumed in large quantity. However, the fruit is prone to attack by several fungal organisms that are capable of reducing its quality. A combination of sun drying and smoking under low heat preserved the shelf life of the fruits as evidenced by inhibition of fungi and palatability assessment.

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