

## BIOPRESERVATION POTENTIALS OF NON-FOOD PLANTS ON SOME EDIBLE FRUITS IN DELTA STATE, NIGERIA.

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### ABSTRACT

The study was carried out to investigate the biopreservation potentials of some non-food plants on some edible fruits in Delta State, Nigeria. Non-food plants such as *Emilia sonchifolia*, *Tridax procumbens* and *Jatropha curcas* were sourced from different locations in Delta State, Nigeria and the plant materials were extracted. Edible fruits purchased from different locations in Delta State, Nigeria were subjected to preservation using extracts from non-food plant materials. The visual observation using sign of change in colouration, spoilage and rot showed that visibility of rot was observed in the control (0.0) nine days after storage with total rot occurring at day fifteen. 25% concentration treated fruits showed sign of rot at day twelve with complete rot at day fifteen while both 50% and 75% concentrations recorded rot signs 15 days after storage with complete rot at day 18. The extracts coated with 100% treatment concentration recorded rot and spoilage at day 18. One hundred (100%) treatment was more effective compared to other treatment concentrations in the preservation of shelf-life of *I. gabonensis*. With extract of active components, some refinement, *Jatropha curcas* may be considered, as a source of medicament in the control of fungal diseases in edible fruits.

**Keywords:** Biopreservative, non-food plants, fruits, post-harvest

### INTRODUCTION

The use of various substances including plant extracts, chemicals and waxing material during postharvest storage of fruits is becoming popular among growers in recent years to enhance the shelf life of fruits (Anisa *et al.*, 2015). However, the use of these substances has their own limitations, as some of them are believed to be ecologically unsafe and economically unviable, besides leaving their residue on the fruit surface, which may have adverse effect on human health. Considerable postharvest losses of fruit are brought about

by decay caused by fungal plant pathogens. Inhibiting the growth of fungi causing rot of fruit has great significance fruit preservation for commercial and home use. However, in recent years, the use of synthetic fungicides has increased consumers concern and their use is becoming more restrictive due to carcinogenic effects (Rial-Otero *et al.*, 2005), residual toxicity problems, environmental pollution, occurrence of microbial resistance, and high inputs. Use of plant extracts having fungicidal and insecticidal properties is one of the

alternative methods. Coating of plant leaf/flower extract forms a thin film around each fruit, which can act as a semi-permeable membrane to regulate the diffusion of oxygen and carbon dioxide into and out of the fruit, thereby reducing the rate of metabolism and also preventing water loss (Anisa *et al.*, 2015).

Natural products of plant origin play an important role in pharmaceutical industries, chemicals which are naturally present in plants are converted traditionally and medicinally into substances that regulate human fertility (Gayanthri, 2012). Some secondary compounds produced by plants could be very effective against parasites and pathogens such plants include pawpaw, mango, citrus as well as *Emilia sonchifolia* (Brianna *et al.*, 2011). *Emilia sonchifolia* (composite) is a herbaceous plant which grows up to about 10 – 40cm in height. Develops ripe fruits between August to October, the flowers are hermaphrodite and are pollinated; the plant is pantropic and probably originated from South Asia (Edu *et al.*, 2017).

Deterioration and spoilage of fruits have been a major challenge facing storage of agricultural products. According to Islam *et al.* (2016), the most important problems regarding fruits production in tropical and subtropical regions of the world are post-

harvest losses and deterioration of nutritional quality of fruits (Shrestha *et al.*, 2018). In developed countries the post-harvest losses in fresh fruit is estimated to be about 5-25% while that in developing countries it is about 20-50% (Islam *et al.*, 2016). During post-harvest operations like natural ripening, physical handling and storage, approximately 30-50% fruits go wasted. This high wastage of fruits is due to highly perishable nature and climacteric pattern of respiration (Islam *et al.*, 2013). In addition to natural deterioration various post-harvest disease infections also play a major role in post-harvest losses of fruits. Considerable postharvest losses of fruit are brought about by decay caused by fungal plant pathogens (Malik *et al.*, 2015).

Postharvest losses of perishable fruits, vegetables and other horticultural crops due to their high moisture, high sugars (in fruits) and low pH that fosters fungal growth leading to fruit deterioration and decay. All these reduce the quality and sometimes cause completely unmarketable produces that fail to meet the standards for exports. Because of this, producers and traders sold their produce in less demanding local markets at lower prices; resulting in an economic loss (Sanders and Korsten, 2013). Besides, fungal pathogens can attribute to quality loss and health hazards through the production of toxins.

Several post-harvest treatment methods and technologies like cold (refrigeration) storage, CA storage, MAP, treatment with ethylene inhibitors like 1-MCP, PGR treatment, wax treatment, etc. have been developed over the years for lengthening shelf life and maintaining post-harvest qualities of fruits (Pandey *et al.*, 2017). But the accessibility and affordability of poor farmers to these advanced technologies is a matter of concern in most developing and under developed countries. Most of the fruit growers in those countries suffer heavy post-harvest losses of fruits as a result of natural deterioration and severity of diseases. Postharvest diseases cause serious loss of both quality and quantity of fruits every year (Shrestha *et al.*, 2018). The study is therefore conducted to investigate the biopreservation potentials of some non-food plants on some edible fruits in Delta State, Nigeria.

## MATERIALS AND METHODS

Fresh fruits of bush mango (*Irvingia gabonensis* Baill), healthy and diseased fruits of pawpaw (*Carica papaya* L.), healthy and rotted fruits of Avocado pear (*Persea americana* Mill) used for the study were purchased from the Main market at Abraka, Ethiope East Local Government Area, Delta State, Nigeria. The fruits were selected on the basis of colour and absence

of external injuries. Fresh leaves of *Emilia sonchifolia*, *Tridax procumbens* and *Jatropha curcas* used for the study were collected from different locations within Abraka community.

## Preparation of Plant Materials

The leaves were air dried under the laboratory condition at room temperature for 15days. The dried leaves samples were ground well in to a fine powder with the help of mixer grinder (Thilagavi *et al.*, 2015). A 10gm air dry plant was soaked into 50ml methanol and chloroform for 24hrs in an orbital shaker at normal temperature. The extracts were filter through the Whatman No: 1 filter paper. The extract was allowed to dry using rotary evaporator. The condensed extracts were stored in airtight container at 4<sup>0</sup>C for further investigation.

## The isolation and inhibitory activity of fungal isolates from leaf extracts

The inhibitory activity of fungal isolates from diseased *Carica papaya* fruits was carried out using the method adopted from Ilondu and Bosah (2015). Sections, 4 mm long, excised from the margins of diseased spot with sterile razor blade were surface-sterilized for 2 min in 2% aqueous solution of commercial bleach (sodium hypochlorite solution), rinsed in two changes of sterile distilled water. The disinfected tissue

pieces were blotted between sterile Whatman No. 1 filter paper and aseptically plated on potato dextrose agar (PDA) plates (3 pieces per plate). The plates were then incubated at room temperature ( $32 \pm 2^\circ\text{C}$ ) for five days. Any observed mycelial growth was repeatedly transferred to fresh PDA plates until pure cultures of isolates were obtained. The antifungal efficacy of leaf extracts was determined by the agar-well diffusion method (Juan *et al.*, 2006). A 5% (w=v) test solution of the extract was prepared in dimethylsulfoxide. Sabouraud dextrose agar was used for filamentous fungi. Each extract solution (0.0625, 0.125, 0.25, 0.5 and 1.0%) and controls were dropped in a 6-mm-diameter well. Plates were incubated for 24 h at  $37^\circ\text{C}$  for

filamentous fungi; all cultures were kept under aerobic conditions. The diameter of the inhibition zone around each well was measured and recorded. Antimicrobial activity was expressed as the ratio of the average of inhibition zones produced by the extract under test and the average of inhibition zones caused by the positive controls. Each test was performed in triplicate. Antifungal activity was recorded in terms of inhibition of mycelial growth (%) and calculated using the formula.

$$\text{Inhibition of mycelial growth (\%)} = (C - T/C) \times 100$$

Where 'C' is average diameter of fungal colony in control plates and 'T' is average diameter of fungal colony in poisoned plates.



Plate 1. *Aspergillus niger* fungi species isolated from diseased *Persea americana* fruit



**a. *Aspergillus niger***

**b. *Rhizopus stolonifer***

Plate 2. Fungi species isolated from diseased *Carica papaya* fruit

### Data Analysis

Data obtained from the study were recorded and subjected to statistical analysis using Microsoft Excel and the values expressed as Duncan's multiple range tests with  $P < 0.05$  were employed to analyze the results and to compare control and treatment fruits.

### RESULTS AND DISCUSSION

The quantitative analysis showed that saponin and alkaloid were quantitatively higher in methanolic extracts of *E. sonchifolia* while tannin and flavonoid were also higher in chloroform extracts of *E. sonchifolia*. It was also observed that the presence of several phytochemical constituents at various concentrations. Methanolic extract revealed the presence of high concentrations of flavonoid, saponin

and alkaloid, and moderate concentration of tannin and steroid with low concentrations of glycoside and anthraquinone. Chloroform extract revealed moderate concentration of tannin and low concentration of other phytochemicals in *T. procumbens*. The quantification showed that saponin was higher in chloroform extract than methanol extract while flavonoid concentration was observed to be the same in both extracts in *T. procumbens*. The result also showed that different methanolic extract of *J. curcas* revealed the presence of high concentrations of flavonoid, tannin, saponin and terpenoid with moderate concentration of steroid and alkaloid while glycoside, anthraquinone and reducing sugar were present in low concentrations. Chloroform extract revealed high concentration of saponin and

low concentration of flavonoid, tannin, steroid, glycoside, alkaloid, anthraquinone, terpenoids and reducing sugar . The quantification showed that alkaloid was

higher in methanol while saponin was higher in chloroform extract (Tables 1, 2 and 3).

Table 1. Quantitative phytochemical screening of *Emilia sonchifolia* leaf extracts

S/N	Phytochemicals	Methanol	Chloroform
1	Tannin	2.2	3.1
2	Saponin	3.2	1.2
3	Alkaloid	3.1	3.2
4	Flavonoid	2.3	2.2

Table 2. Quantitative phytochemical screening of *Tridax procumbens*

S/N	Phytochemicals	Methanol	Chloroform
1	Tannin	2.1	2.1
2	Flavonoid	3.1	1.3
3	Saponin	2.6	3.3
4	Alkaloids	1.7	1.8

Table 3. Quantitative phytochemical screening of *Jatropha curcas* leave extracts

S/N	Phytochemicals	Methanol	Chloroform
1	Tannin	1.6	1.6
2	Flavonoid	2.1	0.8
3	Saponin	2.2	2.5
4	Alkaloids	2.4	1.7

### Fungal Isolation and Antifungal Activities

Two fungal species including *Aspergillus niger* and *Rhizopus* sp. were isolated from diseased fruit of *Carica papaya*. The study showed that as the concentration of the extract increased, the effect of the extract was significantly higher on the organisms (Table 4 and 5).

In the course of the research *Aspergillus niger* was isolated and identified as the fungi associated with rot of *P. americana*. The study showed that as the concentration of the extract increased, the effect of the extract was significantly higher on the organisms (Table 6 and 7).

Table 4. Zones of mycelial growth (cm) of fungi isolated from *Carica papaya* fruit exposed to different concentrations of leaf extract

Extract Concentration (mg/ml)	Methanol		Chloroform	
	<i>Aspergillus niger</i>	<i>Rhizopus</i> sp.	<i>Aspergillus niger</i>	<i>Rhizopus</i> sp.
0.0	4.30	4.30	4.30	4.30
0.0625	0.0	0.3	0.0	0.2
0.125	0.0	0.2	0.0	0.2
0.25	0.0	0.3	0.0	0.3
0.5	0.2	0.3	0.0	0.4
1.0	0.2	0.4	0.0	0.4

Table 5. Percentage zones of growth inhibition of fungi isolated from *Carica papaya* fruit exposed to different concentrations of leaf extract

Extract Concentration (mg/ml)	Methanol		Chloroform	
	<i>Aspergillus niger</i>	<i>Rhizopus</i> sp.	<i>Aspergillus niger</i>	<i>Rhizopus</i> sp.
0.0	4.30	4.30	4.30	4.30
0.0625	100.0	93.0	100.0	95.0

0.125	100.0	95.0	100.0	95.0
0.25	100.0	93.0	100.0	93.0
0.5	95.0	93.0	100.0	90.0
1.0	95.0	90.0	100.0	90.0

Table 6. Zone of mycelial growth (cm) of fungi isolated from *Persea americana* fruit exposed to different concentrations of leaf extract

Extract Concentration (mg/ml)	<i>Aspergillus niger</i>	
	Methanol	Chloroform
0.0	4.30	4.30
0.0625	0.0	0.0
0.125	0.0	0.0
0.25	0.1	0.0
0.5	0.3	0.0
1.0	0.3	0.2

Table 7. Percentage zone of growth inhibition of fungi isolated from *Persea Americana* fruit exposed to different concentrations of leaf extract

Extract Concentration (mg/ml)	<i>Aspergillus niger</i>	
	Methanol	Chloroform
0.0	4.30	4.30
0.0625	100.0	100.0
0.125	100.0	100.0
0.25	97.0	100.0
0.5	93.0	100.0
1.0	93.0	95.0



**Shelf-Life Extension and Physiological Weight Loss (PWL)**

The result of shelf-life extension of *I. gabonensis* fruit using different crude extract concentration of *E. sonchifolia* showed varying degree of biopreservative potentials (Tables 8 and 9). The visual observation using sign of change in colouration, spoilage and rot showed that visibility of rot was observed in the control (0.0) nine days after storage with total rot occurring at day fifteen. 25% concentration treated fruits showed sign of rot at day twelve with complete rot at day fifteen while both 50% and 75% concentrations recorded rot signs 15 days after storage with complete rot at day 18. The extracts coated

with 100% treatment concentration recorded rot and spoilage at day 18. One hundred (100%) treatment was more effective compared to other treatment concentrations in the preservation of shelf-life of *I. gabonensis*.

The study recorded a significant difference among treatments at various days of storage with respect to the physiological loss in weight. With increasing period of storage, the physiological loss in weight also increased in all the treatments. There was significant difference in the physiological loss of weight across the different concentrations and the control at day 18 .

Table 8. Shelf-life of *I. gabonensis* fruits within 18 days of treatment with *E. sonchifolia* extracts

Treatment Concentrations	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
<b>Leaf Extracts</b>						
0	-	-	+	++	+++	+++
25	-	-	-	+	+	+++
50	-	-	-	-	+	+++
75	-	-	-	-	+	+++
100	-	-	-	-	-	+++

**Key:** - = No sign of rot and no colour change      ++ = Become soft and rotten gradually  
 + = No sign of rot but had slightly change in colour      +++ = Fruit has rotten completely

Table 9. Physiological weight loss of *I. gabonensis* fruits within 18 days of treatment with *E. sonchifolia* extracts

Treatment Concentrations	Weight Loss (kg)						
	Initial Weigh	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
0	0.112	0.103	0.093	0.084	0.076	0.068	0.060 <sup>d</sup>
25	0.126	0.117	0.110	0.104	0.099	0.093	0.087 <sup>b</sup>
50	0.132	0.124	0.118	0.112	0.106	0.100	0.094 <sup>a</sup>
75	0.101	0.091	0.082	0.075	0.069	0.062	0.050 <sup>e</sup>
100	0.121	0.102	0.096	0.090	0.084	0.079	0.074 <sup>c</sup>

Plate 3. Leaf extract of *E. sonchifolia* on *I. gabonensis* (Bush mango)

The isolation of *Aspergillus niger* and *Rhizopus* sp. from *Carica papaya* revealed and supported the work of Chuku *et al.* (2008), who reported that *Fusarium* sp. and *Rhizopus stolonifer* are responsible for the soft rot of tomato. Colonization of fruits and vegetable by the invading microorganism is a critical phase in the microbial spoilage of produce. Also, the prevalence of fungi as the spoilage

organisms of fruits and vegetables is due to a wide range of factors which are encountered at each stage of handling from pre-harvest to consumption and is related to the physiological and physical condition of the produce as well as the extrinsic parameters to which they are exposed. The study showed that the plant extracts had effects on the mycelial growth of *Aspergillus niger*. This organism is

primarily an environmental fungus and may, like most other members of the genus, show variable susceptibility to antifungal agents. Our result is in agreement with the study of Ogbekor *et al.* (2007) who reported that the *J. curcas* leaves extract inhibited the mycelium growth of *several fungal organisms*. *J. curcas* extracts caused complete inhibition of the mycelium growth of fungal species, which is responsible for anthracnose, dieback, root rot, leaf spot, and blossom rot and seedling blight of tropical fruit (Saetae and Suntornsuk, 2010).

*J. curcas* fruits and seeds could be used as antifungal compound to control major postharvest diseases of fresh horticultural produce *in vitro*. Seeds and leaves extract of *J. curcas*, have shown molluscidal and insecticidal properties. They showed that methanol extracts of *J. curcas* had the highest activity against vector snails of the human parasites *Schistosoma* spp (Rahman *et al.*, 2011). The result of shelf-life extension of *I.*

*gabonensis* fruit using different crude extract concentration of *E. sonchifolia* showed varying degree of biopreservative potentials with 25% concentration treated fruits showing sign of rot at day twelve with complete rot at day fifteen while both 50% and 75% concentrations recorded rot signs 15 days after storage with complete rot at day 18. The extracts coated with 100% treatment concentration recorded rot and spoilage at day 18. This is similar to the study of Okigbo *et al.* (2010) and Verghese (2000) reported the efficacy of botanical extracts of neem, *mahua* (*Madhuca* sp.), and mint (*Mentha* sp.) leaves on pomegranate (*Punica granatum*) fruit and reported that these extracts were effective in retaining marketable quality even after 22 days of storage.

Similarly, Kulkarni *et al.* (2008) reported that shelf life was maximum and number of rotten fruits was least in azadirachtin (1% at 3 ml/L) + *Trichoderma harzianum* (5 ml/L) treated grapes. Anisa *et al.* (2015) has reported the potentials of

neem (*Azadirachta indica*), Chinaberry (*Melia azedarach*) and marigold (*Tagetes erecta*) as biopreservative on the post-harvest loss of guava. The interaction between treatment and storage was non-significant. The effect of treatments over entire storage period was statistically significant having lowest mean value of 18.06% spoilage in neem 20%, and the highest value of 55.56% in control. No spoilage was observed on 4<sup>th</sup> day of storage.

The study recorded a significant difference among treatments at various days of storage with respect to the physiological loss in weight. With increasing period of storage, the physiological loss in weight also increased in all the treatments. Similar result has been reported by Ashwini and Nikhita (2018) who stated that the physiological loss of weight in mango fruits showed an increasing trend in all the treatments. Weight loss of fruit is primarily due to transpiration and respiration. Water is lost by transpiration due to differences in

vapour pressure of water in atmosphere and tomato surface (Tonna *et al.*, 2012).

The phytochemical screening of *Jatropha curcas* leaf revealed the presence of tannins, saponins, flavonoids, alkaloids and other secondary metabolites. These phytochemicals are biologically active and can be responsible for the antifungal activity of the plant. The mechanisms through which these secondary metabolites exert their antifungal activities differ. The presence of these phytochemicals has also been reported by Oyama *et al.* (2016). The reported that tannins, saponins, flavonoids, alkaloids, oxalates and cyanogenic glycosides are essential metabolites on *J. curcas*.

Tannins have been reported to form irreversible complexes with proline rich protein (Shimada, 2006) thereby inhibiting cell protein synthesis. Another secondary metabolite observed in the leave extract of *J. curcas* was alkaloid. Alkaloids have analgesic effects and have been clinically used. Alkaloids have been acclaimed for

their antimicrobial activities, especially against gram negative bacteria (Cushnie *et al.*, 2014). Other secondary metabolites present in *J. curcas* whose antimicrobial activities have been documented are flavonoids and saponins.

## CONCLUSION

The study revealed that the plant *Emilia sonchifolia* acted as a potential source of essential secondary metabolites. The phytochemical screening revealed the presence of flavonoid, tannin, saponin, protein, alkaloids, terpenoid, anthraquinone and reducing sugar. Methanolic and chloroform extract of *E. sonchifolia* exhibited remarkable biopreservative activity against pathogenic fungi causing rot of *Irvingia gabonensis* fruit. The biopreservative potential of the extract suggests that they can be used for preservation of fruits. It also evaluated the antifungal activities of *Tridax procumbens* whose result showed that *T. procumbens* possess several essential secondary metabolites and antifungal potentials

against *Aspergillus niger* and *Rhizopus stolonifera* causing fruit rot of *Carica papaya*. The study also revealed the methanol and chloroform extracts of *Jatropha curcas* connoting that they possess phytochemicals which exerted antifungal activities on *Aspergillus niger* causing fruit rot of *Persea americana*. With extract of active components, some refinement, *Jatropha curcas* may be considered, as a source of medicament in the control of fungal diseases in edible fruits.

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