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Bio-Preservative Potential of Calotropisprocera extract on Tomato (Lycopersicumesculentummill.) Fruits Sold in Ago-Iwoye Market, Southern Nigeria and its Microbial Spoilage

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ABSTRACT

Calotropisprocera extract has been established to possess antimicrobial properties against microorganisms of medical importance. This study investigated the effect of the exudates on post-harvest spoilage of tomato fruits and also studied the microorganisms associated with its spoilage. Fresh and wholesome tomato fruits were purchased from Ago-Iwoye metropolitan market, Southern Nigeria. One hundred (100) pieces of epicarp intact tomato fruits were sorted and distributed into nine (9) small baskets each and treated with 10%, 15%, 20% and 25% C. procera extract with leaves underlain covering and without leaf respectively including a control set up. They were monitored every other day for eight weeks and spoilt fruit samples were enumerated and microbiological analysis was conducted. Samples without leaf significantly enhanced the shelf life as level of spoilage set in at 11th, 13th, 21st and 15th day with 4%, 5%, 5% and 4% spoilage respectively. However, less than 50% of the 20% concentration maintained wholesome freshness till 35th day whereas control started on the 3rd day and by 15th day all got spoilt. Total of nine species of microorganisms were identified as Escherichia coli, Micrococcus sp., Staphylococcus aureus, Corynebacterium sp., Mucorsp., Aspergillusniger, A. fumigatus, Alternaria sp. and Saccharomyces cerevisiae. Mucorsp. had the highest rate of occurrence 43 (51.2%) and the lowest was Alternaria sp. 2 (2.4%). The results therefore established a good support for the application of this bio-preservative metabolite in food processing industries.

Keywords: *Tomato fruits; Calotropisproceraextract; bio-preservative, microorganisms, spoilage, Ago-Iwoye;*

INTRODUCTION

Tomato is a red berry-type edible fruit, which belongs to the family *Solanaceae*. The botanical classification of tomatoes was first identified as *Solanumlycopersicum.* However, this designation was changed to '*Lycopersicumesculentum'.* The word 'tomato' was being derived from the Latin name '*Lycopersiconesculentum* Mill.' Syn. *Solanumlycopersicum* (Cutler, 1998). It is botanically classified as a fruit, although, some persons think of it as a vegetable (Ajayi, 2013; United States Department of Agriculture, 2013). A widely grown vegetable fruit, rich in minerals and vitamins and is one of the highly nutritious food ingredient used in savouring and flavouringof cooked food and can be used in many dishes, salads, sauces and drinks (Effiuwevwere, 2000; Canene-Adams *et al*., 2005; Ogunbanwo *et al.*, 2014). Lycopene, a form of B-carotenoid pigment have been responsible for deep red colouration of ripened tomato. A powerful antioxidant that help to prevent prostate cancer, reduce the cardiovascular risk associated with type 2 diabetes and improve the ability of the skin to protect itself against the harmful ultra violet rays, decrease the risk of breast, lung, stomach, bladder, uterine, head and neck cancers (Amusa, 2002; Freedman, 2008; Zhang *et al.*, 2009; Shidfar *et al.*, 2010; Zdenka *et al.*, 2010).

Calotropisprocera is a shrub or small tree up to 2.5 m high, stem usually simple, rarely branched, woody at base and covered with a fissured, corky bark and branches somewhat succulent (Orwa*et al.*, 2009). All parts of the plant exude white latex when cut or broken which acts as a defense strategy against insects, viruses and fungi (Deepak, 1995). *Calotropisprocera* belongs to family Asclepiadaceae and sub family of the

Apocynaceae which includes over four hundred genera and almost five thousand species distributed throughout the tropics with a very wide range of ecological amplitude. It is a salt tolerant, drought resistant weed found along degraded roadside, and in overgrazed pastures commonly known as calotrope, calotropis, Dead Sea fruit, desert wick, giant milkweed, swallow-wort, mudarfibre, rubber bush, rubber tree or sodom apple in English; pomme de Sodome in French; tumfafia in Hausa; and ewe bomubomu among Yorubas. It could serves as food, fodder, fuel fibre, timber, tannin or dyestuff, or latex which contains 11-23% of rubber. The liquid latex of *C. procera* can be used as a renewable source of hydrocarbons and intermediate energy resources (Arora, 1982) and also effective in inhibiting the activity of the tobacco mosaic virus. The latex is cardiotoxic with the active ingredient being calotropin (FAO, 1986; Orwa *et al.,* 2009). Belewu and Aina, (2000) reported extracts from the stem and succulent leaves of *C. procera* has been reported to contain rennet enzymes called calotropin that coagulates milk. Traditionally, the extracts from the Calotropis plant has been used as the sole coagulant of milk in African countries for the production of a soft cheese called '*wagashie'* (Ashaye *et al*., 2006).Intensive research has been undertaken to comprehend the measure as it affect some radical control and tomato's disease occurrence has been a major global concern (Butler, 1992). Wokomi (2008) has reported on microorganisms associated with spoilage of tomato fruits in Nigeria. Eni *et al.*,2010; Etebu *et al.*, 2013; Oyemaechi*et al.*, 2014; Wogu and Ofuase, 2014; Onuorah and Orji, 2015).

Biological and physical factors are often responsible for tomatoes spoilage that results into adverse changes (taste, smell, appearance or texture) in its quality. However, Ghosh (2009) revealed the main microorganism responsible for spoilage in tomato is fungi.

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Numerous reports on tomato's spoilage organisms in the developed countries are available but there is dearth of information on the spoilage organisms of tomatoes in a developing city such as Ago-Iwoye, Southern Nigeria.

Spoilage of tomatoes is those adverse changes in the quality of tomatoes that are brought about by the action of predominantly biological and physical factors. These may be changes in taste, smell, appearance or texture of the fruits. Ghosh (2009) revealed that fungi were the source of spoilage of most of the tomato samples accessed than bacteria.

Numerous reports on tomato's spoilage organisms in the developed countries are available but there is dearth of information on the spoilage organisms of tomatoes in a developing city such as Ago-Iwoye, Southern Nigeria.

However, due to the seasonal variations in the availability of *Calotropisprocera*, and its scarcity in urban cities, there is the need to find suitable forms of processing the plant material for easy storage and transport. Therefore, this study sought to determine the preservative potential of extract from fresh Sodom apple (*Calotropisprocera*) succulent leaves and stems on tomato fruits with a view of elongating the life span of the fruits for consumption and industrial purposes. Isolation of microorganisms responsible for its spoilage under storage was also investigated.

MATERIALS AND METHODS

Description of Study Area

The present study on *Calotropisprocera* extract to preserve tomatoes was done in the Department of Microbiology, OlabisiOnabanjo University, Ago-Iwoye, Ijebu North Local Government Area, Southern, Nigeria.

Sample Collection and Size

One big basket of strong ripened tomato was purchased from Ago-Iwoyemetropolitan market while *Calotropisprocera* extract was collected from different locations in Ijebu-Igbo, Ijebu North Local Government Area, Southern, Nigeria. The extract was collected in a sterile bottle, labeled and brought into the laboratory, and was prepared into different concentrations (10%, 15%, 20%, and 25%). The tomatoes fruit samples were kept free from dust and insects at ambient temperature.

Media

Plate Count Agar (PCA), Potato Dextrose Agar (PDA). They were prepared according to the manufacturers' specification.

Processing of Tomato Sample

Strong, ripened and uninfected fruits were sorted and then distributed into nine (9) smaller baskets containing one hundred pieces each and treated as follows: control, 10%, 15%, 20% and 25% extract with leaves underlain covering and without leaf respectively.

The tomatoes were stored up to eight weeks and monitored every other day to observe and record the effect of the extract on its storage. Spoilt samples from the control and experimental samples were collected in a sterile bottle and were taken into the laboratory for microbiological analysis.

Isolation of Microorganisms

One milliliter of the serially-diluted sample (10^{-5}) was dispensed into a conical flask containing sterile Potato Dextrose Agar (PDA) and two percent chloramphenicol to inhibit bacterial for fungi growth and also into Plate Count Agar (PCA) for bacteria growth. The contents were properly mixed and dispensed aseptically into sterile petri-dishes. They were incubated at 28 ºC for 72 h and 37 ºC for 24 h for fungi and bacteria respectively. The colonies that developed were sub-cultured to obtain pure cultures and enumerated as colony forming unit (cfu/g) after multiplying with the dilution factor. They were later stored on slants for identification.

Characterization and Identification of the Isolates

The distinct colonies that developed in the pure culture plates were observed for the morphological and cultural characteristics including the nature of margin, elevation, shape, colour and transparency (Macroscopic examination). The bacteria isolates were further characterized and identified by Gram staining and biochemical test as described by Holt *et al*., (1994). These include catalase, coagulase, indole, fermentation and oxidase tests. Confirmation of the identification was based on the use of Bergey's manual of systems bacteriology (Sneath *et al.*, 1986).

Results and Discussion

The microorganisms present in samples of spoilt tomato fruits were identified based on their cultural, morphological and biochemical characteristics (Table 1). The bacterial isolates were *Micrococcus sp.*, *Escherichia coli*, *Corynebacterium sp.* and *Staphylococcus aureus*. Contamination of foods with human flora *S. aureus*and *Micrococcus sp*. could be via food handlers/producers and equipment as toxigenic strains of *S. aureus*has been confirmed in foodborne illness. It could also be as a result of storage conditions, distribution, marketing practices and transportation (Yadav*et al.*, 2011). Jushi and Patel (2008) confirmed the presence of *S. aureus*and *Escherichia coli* in their findings. Fungi isolates were identified morphologically (microscopy and macroscopy) as *Mucor*sp., *Aspergillusniger*, *A. fumigatus*, *Saccharomyces cerevisiae*and *Alternaria sp.* (Table 2).

Table 3 revealed among fungi that *Aspergillusniger* had the highest rate of occurrence 43 (51.2%) in the spoilt fruits observed, followed by *Mucor*sp. 22 (26.2%), *A. fumigatus*11 (13.1%), *Saccharomyces cerevisiae*6 (7.1%) and *Alternaria sp.* 2 (2.4%).Whereas bacterial isolates *Micrococcus sp.* had the highest rate of occurrence 34 (48.6%), *Staphylococcus aureus*23 (32.9%), *Escherichia coli* 8 (11.4%) and *Corynebacterium sp.* 5 (7.1%). The studies of Akinmusire (2011), Ibrahim (2011) and Onuorah and Orji (2015) revealed that fungus may be the major microorganism responsible for the spoilage of tomato fruits as *Aspergillusniger*had the highest percentage occurrence among all the isolates. The occurrence of fungal spoilage of tomato fruits is a source of potential health hazard to man as a result of the production of naturally occurring toxic chemicals (aflatoxins) which are capable of inducing mycotoxicoses in man.

Figure 1 showed the tomato fruits with and without leaves (experimental and control samples) during storage. Potential effect of *Calotropisprocera* extract (without leaves) on tomato fruits as investigated was shown in Table 4. In the experimented samples (10%, 15%, 20% and 25% extract concentration), the level of spoilage in tomato fruits set in at 11th, 13th, 21st and 15th day with 4%, 5%, 5% and 4% spoilage respectively. However, less than 50% of 20% concentration maintained freshness and wholesome fruits till 35th day as compared with 25% concentration that maintained same till 27th day. For control samples, spoilage started on the 3rd day and by 15th day all has gotten spoilt.

Table 5 showed the preservative potential of *Calotropisprocera* extract (with leaves) on tomato fruits at different concentrations. The results revealed that the fruits with 20% concentration treatment also had less than 50% freshness till 35th day as compared with others treatments.

Post-harvest decay of fruits result in substantial economic losses around the world (Hongyin*et al*, 2011) and many great alternative strategies have been employed to control postharvest disease and losses which include: biological control, bioactive compounds, heat treatment, (Sharma, 2009; Mari, *et al.*, 2007). The result of this study clearly reflects that the preservative potential of Sodom apple (*Calotropisprocera*) leaves and extract has inherent ability to control the spoilage of tomatoes fruits. This is in agreement with Shittu, *et al.*, (2004) that reported that *C. procera* leaves has the ability to reduce total viable count. The result obtained revealed more on the effect of Sodom apple on tomato fruits. It can be concluded that enzymes - polygalacturonases and xylanases were responsible for the spoilage of tomato fruits (Dennis and Davis, 1980). Compounds derived from the plant have been found to have digitalic and emeto-cathartic properties. Other compounds have been found to have bactericidal and vermicidal properties. The latex contains a proteolytic enzyme called caloptropaine (Orwa*et al.*, 2009). Preservative effect of *Calotropisprocera* extract (without leaves) on tomato fruits at different concentrations seems to be more effective than the one covered with leaves. This could serve as source of contamination to the fruits.

Figure1. *Tomato fruits with and without leaves (experimental and control samples) during storage*

Table1. *Morphological, Biochemical Tests and Identification of Bacteria Isolates*

No of Isolates	Morphology	Gram Reaction	Biochemical Test				Probable Organisms
			Co	I	Ca	O	
1	White, small and irregular Shaped colony with smooth margin. Cocci in singles	Gram +	-	+	+	+	*Micrococcus sp.*
2.	Whitish yellow, small and Irregular colony with Smooth margin. Cocci in cluster	Gram +	+	-	+	-	*Staphylococcus aureus*
3	Rose-pink with entire Margin. Rod in single	Gram –	-	+	-	+	*Escherichia coli*
4	White, club shaped colony. Rod with entire in singles	Gram +	+	-	+	-	*Corynebacterium sp.*

"+" Positve; "-'' Negative; Co-Coagulase; I-Indole; Ca-Catalase; O-Oxidase

Table2. Morphological Characterization of Fungi isolates

Macroscopy	Microscopy	Probable Organism
White becoming gray-brown on surface	Hyphae broad, non septate, rhizoids and stolon present	*Rhizopusstolonifer*
Dark brown to black colouration with entire margin	Branched septate hyphae with globoseconodiophore vesicles	A*spergillusniger*
Rapid growth of creamy, flat, smooth edge colonies	presence of pseudohyphae	*Saccharomyces cerevisiae*
Entire margin, rough circular shape moderately raised mycelium and bark blackish in colour	Multi celled, septate and irregularly branched. Conidiophores arised singly or in clusters	*Alternaria sp.*
Dark green – black colour With umbonate elevation	Branched septate hyphae with dome shaped conidiophores vesicles and roughened conidia	*Aspergillusfumigatus*

Table3. *Prevalence frequency occurrence of microbial isolates in tomato fruit*

Bacterial isolates	Number	Percentage Occurrence
Micrococcus sp.	34	48.6
Staphylococcus aureus	23	32.9
Escherichia coli	8	11.4
Corynebacterium sp.	5	7.1
Fungi isolates		
Mucor sp.	22	26.2
A. fumigates	11	13.1
Saccharomyces cerevisiae	6	7.1
Alternaria sp.	2	2.4
Aspergillusniger	43	51.2

CONCLUSION

Within this context is the utilization of natural plant extracts which are sources of antimicrobial substances, regarded as safe and degraded by natural soil microbes; they do not pose any health residual or environmental problems at any concentration in which they are used. Nevertheless, it revealed the various pathogenic organisms that injurious to human health were isolated from the spoilt samples. Therefore further studies are necessary to provide information concerning spoilage of tomatoes fruits by revealing the nature of contamination. The use of substances with effective antimicrobial effects against spoilage microorganisms is necessary during storage.

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