

## Phytochemical Investigation, Antioxidant Capacity and Determination of Vitamin C content in Fruits from Local Three Wild Trees viz *Saba comorensis* (Rubber vine, Bungo), *Spondias cytheera* (Golden apple, Embe Sakua) and *Tamarinda indica* (Tamarinda, Ukwaju) found in Zanzibar

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### Abstract

Traditionally, the locals (Zanzibaris) prefer drinking juices prepared from fruits of the wild trees viz *Saba comorensis* (Rubber vine, Bungo), *Spondias cytheera* (Golden apple, Embe sakua) and *Tamarinda indica* (Tamarinda, Ukwaju) as fruit/food supplements singly or as a mixture to manage high body temperatures due to various ailments or infections and improving their body immune system. In the effort to establish the ethno botanical basis of the fruit juices, the three plants were phytochemically screened based on tests of coloration and precipitation. Vitamin C content was determined iodometrically. *Saba comorensis* and *Spondias cytheera* were found to contain reasonable amounts of vitamin C per 100 g of each raw fruit. *Saba comorensis* contained the highest amount of vitamin C (31 mg/100 g), while 100 g of *Spondias cytheera* was found to contain about 18 mg of vitamin C. Amount of vitamin C per 100 g found in *Tamarinda indica* was only 9.36 mg. The phytochemical screening of the plant extract undertaken by four solvents with different polarities; water, methanol, ethanol and diethyl ether indicated the presence of alkaloids, saponins, flavonoids, carotenoids, tannins and cardiac glycosides, reducing sugars and amino acids. Among the flavonoids, naringin was mostly found in Ukwaju confirming its sourness. The antioxidant capacity based on total phenolic content evaluated using DPPH was found to be highest in Bungo fruit at 58.4 mg/100 g. These results shows that these fruits have reasonable amounts of vitamin C, can be good sources of bioactive compounds, anti-inflammatory (analgesic) and antioxidants, thus supports both food nutrients/supplement and ethnopharmacological uses of these fruits.

**Keywords:** *Saba comorensis*; *Spondias cytheera*; *Tamarinda indica*, Ethnopharmacological; Phytochemical

### Introduction

Numerous medicinal studies indicate that an increase in the consumption of fruits rich in antioxidants and bioactive substances is associated with regeneration of some antioxidants within our body thus improving immunity system encompassed with enhanced performance of major body organs. Consumption of fruits also leads to a decrease in the incidence of various diseases like stroke, hypertension, cardio- and cerebro-vascular and cancer [1-3]. They scavenge free radicals and active oxygen species forming intracellular substances by inhibiting lipid peroxidation via inactivating lipoxigenase [4,5]. Moreover, fruit consumption was found to terminate free radicals which are responsible for the oxidative damage of lipids, proteins and nucleic acids [6,7].

Traditionally, the locals (Zanzibaris) prefer drinking the juices from the Bungo, Embe sakua and Ukwaju as a cocktail or separately, when they develop high body temperatures due to various ailments or infections, skin rashes and scurvy related complications. In addition, they take them to improve their immune system against common ailments, arthritis and anti-ageing problems. This is a strong indication that these fruits are composed of antioxidant constituents including vitamin C (L-ascorbic acid).

It has also been established that L-ascorbic acid, a water soluble vitamin, is very vital to human health [8]. To mention but a few, vitamin C is involved in protein metabolism which is vital to the production of collagen and certain neurotransmitters, protects the fat soluble vitamin A, vitamin E and fatty acids from oxidation [9]. A striking pathological change resulting from ascorbic acid deficiency is the weakening of the endothelial wall of the capillaries due to reduction in the amount of intercellular substances, therefore a clinical manifestation of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, joint pains and normal connective tissue metabolism [10]. Deficiency of L-ascorbic acid has been found to promote growth of cancer tissues, and other diseases in which oxidative stress plays a role [8]. Oxidative stress results when there is an insufficient capacity of the biological system to neutralize excess free radicals that have been produced. A recommendable dose of vitamin C varies from 90 mg to 150 mg for adults above 19 years and slightly lower dose for children [9]. Among the numerous naturally occurring antioxidants, ascorbic acid, carotenoids and phenolic compounds are found to be more effective in strengthening body immunity [11].

The traditional uses of these seasonal fruits in Zanzibar motivated our investigation on the amount of vitamin C content, phytochemical

composition, and anti-oxidant potentials to ascertain their health benefits and ethnopharmacological use of these locally available fruits which grow in the wild.

## Materials and Methods

### Plant material

Fruits used in this study were purchased from local markets in Zanzibar and were authenticated at Botany Department, Pwani University. The fruits were rinsed thoroughly with distilled water, peeled off the outer skin and were cut into smaller pieces and stored at 5°C before use.

### Preparation of fruit juices and powder

The juices were extracted from the individual plant fruits using a juice blender (Sing sung model BL-50). The juices were lyophilized using a freeze dryer (Toption-Topt-10A) and the concentrates obtained stored in airtight containers refrigerated at 5°C until subsequent use.

### Quantitative test for vitamin C

The amount of vitamin C in each of the fruit juices was determined by titrating with standardized iodine solution [12].

**Preparation of iodine solution:** 5.00 g of potassium iodide and 0.268 g of potassium iodate were dissolved in 200 ml of distilled water in a 500 ml volumetric flask followed by addition of 30 ml of 3 M sulfuric acid. The mixture was mixed thoroughly and diluted to the mark with distilled water.

**Preparation of vitamin C standard solution:** 0.250 g of pure vitamin C tablets was powdered, dissolved in 100 ml of distilled water and made to 250 ml by making use of a volumetric flask.

**Standardization of the iodine solution with the standard vitamin C solution:** 25.00 ml of the vitamin C solution and 10ml of 1% starch solutions were added into a 125 ml Erlenmeyer flask. The resulting solution was titrated against the iodine solution. The end point was determined by the appearance of blue color which remained after at least 20 seconds of swirling. The titration process was repeated thrice to obtain mean titre value.

**Titration of *Tamarinda indica*, *Saba comorensis* and *Spondia cytheera* juices:** 25.0 ml of blended individual juices obtained by blending 56.86 g of the plant fruits were added into a 125 ml Erlenmeyer flask and titrated against iodine solution. The titration processes for the individual extracts were repeated to obtain three concordant readings and mean titre values for the individual juices taken.

**Quantification of vitamin C content:** The presence of vitamin C in the fruit extracts was first confirmed and the amount of vitamin C in each fruit juice determined by stoichiometric calculation in relation to that of standard solution.

### Determination of antioxidant activity of the fruit juices

There are various methods available in the assessment of total antioxidant capacity [13-17]. In this study the antioxidant activity was evaluated by making use of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (from Sigma, USA) through free radical assay [17].

The juice samples were reacted with the stable DPPH radical in ethanol solution. The reaction mixture constituted 0.5 ml of fruit juice, 3 ml of absolute ethanol and 0.3 ml of DPPH radical solution of 0.5 mM in ethanol. The absorbance of resulting solution after 95 to 105 min was determined using UV-VIS spectrophotometer (Shimadzu double-beam spectrophotometer) at 518 nm. The mixture of ethanol 3.3 ml and fruit sample 0.5 ml served as a blank while the control solution was prepared by mixing 3.5 ml of ethanol and 0.3 ml of DPPH solution. The scavenging activity percentage is determined as per equation below [18]:

$$AA\% = 100 - \left[ \frac{(Abs_{sam} - Abs_{blank}) \times 100}{Abs_{control}} \right]$$

The experiment was done in triplicate for each substance and statistical significant test done at 95% confidence level using student t-test to ascertain the accepted value.

### Phytochemical screening of the fruit juices

The phytochemical studies were done to ascertain the chemical components which may be responsible for the medicinal values and health benefits. The screening of the concentrates was qualitatively done according to the established protocols [19-21]. Stock solution of each concentrate 10 mg extract/ml of distilled water was also prepared, besides the powder, and used for the qualitative identification of the phytochemical constituents.

**Test for alkaloids:** 1 g of powdered sample of each specimen was separately boiled with water and 10 ml of HCl on a water bath and filtered to obtain a clear filtrate. 6 drops of Mayor's reagents was then added to 2 ml of filtrate. A creamish or pale yellow precipitate indicates the presence of alkaloids.

**Test for amino acids:** 1 g of each specimen sample extracted with 10 ml of distilled water, 1 ml of the extract taken, added few drops of ninhydrin reagent, appearance of purple color confirms the presence of amino acids.

**Test for cardiac glycosides:** 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

**Test for carotenoids:** 1 g of each specimen sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added. A blue coloration at the interface confirms the presence of carotenoids.

**Test for flavonoids:** 1 g of the powdered fruit was boiled with 10 ml of distilled water for 5 minutes and filtered when hot then added few drops of 20% NaOH solution to 1 ml of the filtrate. A change to yellow coloration which on addition of acid changed to colourless solution depicted the presence of flavonoids.

**Test for naringin:** The Paul Davis method via Soxhlet extraction was performed in the entire fruit specimen using ethanol, appearance of yellow color on addition of 0.1 M NaOH in the presence of diethylene glycol confirms the presence of naringin.

**Test for reducing sugars:** Approximately 1 g of each specimen placed in a test tube added 10 ml distilled water and boiled for 5 minutes. The mixture was filtered while hot and the cooled filtrate made alkaline to litmus paper with 20% NaOH solution. The resulting solution was boiled with equal volume of Benedict's qualitative solution on a water bath. The formation of a brick red precipitate depicts the presence of reducing compounds.

**Test for saponins:** 1 g of each dried powder was separately boiled with 10 ml of distilled water in a bottle bath for 10 minutes. The mixture was filtered while hot then allowed cooling. 2.5 ml of the filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 minutes, frothing indicates the presence of saponins in the filtrate.

**Test for tannins:** 1 ml of the extract was treated with few drops of 1% ferric chloride and observed for brownish green or a blue-black coloration.

## Results and Discussion

Experimental results for the determination of vitamin C content revealed that Bungo juice had exemplary content of vitamin C at  $31.0 \pm 1.05$  mg/100 g. The fruit juice obtained from Embe sakua had moderate amounts while that of Ukwaju was quite minimal with the values being  $18.0 \pm 0.51$  mg/100 g and  $9.36 \pm 0.22$  mg/100 g, respectively (Table 1). The statistical analysis was done using t-test at 95% confidence level for three concordant readings.

In comparison with common fruits found around the world, vitamin C in Bungo (*Saba comorensis*) is less than that of citrus fruits, such as orange (53 mg/100 g), but is comparable to that of mango fruit (28 mg/100 g). Vitamin C content established in Embe sakua (*Spondias cytheera*) is of very good ranking and is comparable to that reported for tomatoes (*Hycopersicon* sp.) and custard apple (*Annona reticulata*) at 19 mg/100 g. Ukwaju (*Tamarinda indica*) fruit can be quantitatively compared in terms of vitamin C content with banana (*Musa paradisiaca*) and water melon (*Citrullus lanatus*) reported at 9 mg/100 g and 10 mg/100 g, respectively (IMFND, 2000).

The antioxidant test revealed that Bungo (*Saba comorensis*) has the highest antioxidant activity with scavenging activity of 58.4 mg/100 g while that of Embe sakua (*Spondias cytheera*) and Ukwaju (*Tamarinda indica*) were relatively low at 32.3 mg/100 g and 16.8 mg/100 g, respectively (Table 2). The highest antioxidant activity of Bungo may be associated with the high vitamin C content.

We cannot rule out the interference in absorbance measurements due to presence of polyphenols in the fruit juices [22] and naringin in case of Ukwaju. Studies done on medicinal plants, fruits and vegetables strongly support the idea that the constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [16,23]. The qualitative survey of these fruit juices reveals the presence of alkaloids, flavonoids, saponins, cardiac glycosides, tannins, reducing sugars, amino acids, carotenoids and naringin which were found only in Ukwaju extract (Table 3).

**Table 1:** Vitamin C content in the three wild fruit juices

Fruit local name	Scientific name	Vitamin C mg/100 g	Ranking
BUNGO	<i>Saba comorensis</i>	$31.00 \pm 1.05$	EXCELLENT
EMBE SAKUA	<i>Spondias cytheera</i>	$18.00 \pm 0.51$	VERY GOOD
UKWAJU	<i>Tamarinda indica</i>	$9.36 \pm 0.22$	GOOD

**Table 2:** Antioxidant Capacity of the juices from the three wild fruits

Plant juice	Sample absorbance	Blank absorbance	Control absorbance	Scavenging capacity (mg/100 g)
Bungo	$0.7034 \pm 0.034$	0.5587	0.3478	$58.4 \pm 0.034$
Embe Sakua	$0.6783 \pm 0.051$	0.4428	0.3478	$32.3 \pm 0.051$
Ukwaju	$0.8952 \pm 0.092$	0.6058	0.3478	$16.8 \pm 0.092$

**Table 3:** Phytochemical constituents of the wild fruit juices

	Bungo	Embe Sakua	Ukwaju
<b>Alkaloids</b>	++	+	++
<b>Amino acids</b>	++	++	++
<b>Cardiac glycosides</b>	+	+	++
<b>Carotenoids</b>	++	++	+
<b>Flavonoids</b>	++	+	++
<b>Naringin</b>	-	-	++
<b>Reducing sugar</b>	+	+	++
<b>Saponins</b>	++	++	+
<b>Tannins</b>	++	++	+

**Key:** + Moderate amount, ++ Good amount, - Absent

Bungo and Ukwaju fruit extracts shows a generally good amount of the evaluated phytochemical constituents. The presence of naringin in Ukwaju can therefore be associated with its fruit juice sourness [24-26]. The presence of carotenoids, which acts as precursors of vitamin A synthesis, has antioxidant and anti-inflammatory activities [27-29] which account for the analgesic property of these fruits extracts thus their use in ethnomedicine. The analgesic activity of the fruit juices may also be associated with the presence of alkaloids and flavonoids which are well known for their ability to inhibit pain perception [30,31]. The traditional use of Ukwaju juice as antiobesity agent can therefore be associated with the dietary phytochemicals identified in the fruit concentrate. These findings corroborate those reported in literature on dietary phytochemicals and their potential effect on obesity [27].

The use of the fruit juices in management of common diseases associated with bacterial infections can thus be accounted for by the presence of alkaloids, saponins and tannins which have been reported as constituents of medicinal plants used in management of common pathogenic strains [32].

The existence of cardiac glycosides in the extracts authenticate the use of the wild fruit juices in management of heart related diseases as such compounds have since been reported to have a participatory role in inhibiting congestive heart failure [33-35]. The presence of flavonoids and tannins and phenolic which have been reported as anti-cancer agents [36-39] may account for the use of these wild plant juices as potential anti-cancer agents.

## Conclusion and Recommendations

The study reveals that these fruits extract have moderate to significant amount of the antioxidant vitamin C. In this regard juice obtained from a 200 g of *Saba comorensis*, or 330 g of *Spondias cytheera* may be sufficient for daily body requirement of vitamin C. However, high amounts of about 600 g of *Tamarinda indica* will be required to meet the required dietary amounts.

Further studies are recommended for the determination of daily intake amounts of these fruits as sources of vitamin C. Other antioxidant like vitamin E may also be present in the fruit juices and its presence needs to be established since it is responsible for healthy skin, dentin and enamel.

The medicinal values of these extracts lie in bioactive phytochemical constituents combined with their nutrients. However, a comparative study needs to be done on anti-inflammatory activity of the wild juice extracts against standard analgesic drugs.

A mixture of these fruit extracts in water is a museum of phytochemical constituent thus confirming the ethnopharmacological value of these wild fruit juice cocktails. Thus, these wild fruits should be considered in world fruit compositional databases and more studies to be done as recommended in this study.

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