Abstract:
Resveratrol is a natural polyphenolic compound belonging to the group called stilbene. It has medicinal functions which include anti-cancer, anti-oxidation, prevention of cardiovascular diseases. Red and green grapes (Vitis vinifera) berry skins and flesh were analyzed for trans-resveratrol using HPTLC and UV scanner. Both types of grapes’ skin showed small but significant amount of resveratrol. Different types of grape samples were measured and macerated with 99.9% methanol acidified with 0.1% HCl for 24hrs at 30°C in the dark. Sample application and HPTLC densitometric scanning were done using HPTLC machines on to 10cm x 10cm HPTLC plates. The wavelength for detection was evaluated from complete UV spectrum of resveratrol, 313nm. 25.7g of fresh green grape skin was observed that it contained approximately 0.363mg of trans-resveratrol. 20.0g of dried red grape skin contained approximately 0.802mg of trans-resveratrol. Fresh red and green grape flesh showed no presence of trans-resveratrol and also fresh red grape skin. The (retardation factor) Rf of resveratrol was obtained 0.34. Estimation of resveratrol in the samples was done by taking proportional comparison of area under the curve of the grape extract samples' peaks and resveratrol's peak at the Rf value of 0.34. However, all grape extract showed predominating peaks at Rf of 0.11. Green grape berry skin was considered to be containing much of the trans-resveratrol as compared to red grape berry skin and grape berry flesh of all the two types. Peaks at Rf value of 0.11 were probably of metabolites of trans-resveratrol or any other compound in the samples. Despite the observed small amount in some samples, it seems to be the metabolites of trans-resveratrol or other compound predominating in all the samples. Therefore, since the trans- isomer is affected much by UV and heat, the compound might have deteriorated during the sell or storage of the grapes.
Key words: Area under curve; grape skin; HPTLC; Rf values; Resveratrol.

Introduction

![Resveratrol (3,5,4′-trihydroxy-trans-stilbene)](image)

Figure 1: Resveratrol (3,5,4′-trihydroxy-trans-stilbene)

In as much as many researches are on going to combat the most troublesome disease in both developed and developing countries, Resveratrol has become one of the most studied natural polyphenolic compound belonging to the group called stilbene [1]. Resveratrol is a phytoalexin, which is a group of low molecular weight secondary metabolites of plants biosynthesized in response to different kinds of environmental stress and microbial attack [2]. Resveratrol has many medicinal functions which include anti-cancer, anti-oxidation, prevention and cure of cardiovascular diseases, prevention of Alzheimer’s disease, anti-platelets aggregation and anti-inflammation [3]. Moreover, it has been observed that it has the synergistic anti-HIV with Didanosine [4]. In addition, ethanol extract of black grape has shown to have significant activity against E.coli, S.aureus, P.aeruginosa and H.pylori [5].

The natural sources of resveratrol other than grapes include groundnuts, mulberries, blueberries, cranberries, bilberries, cassia, eucalyptus, polygonum and veratrum [6].

The oral absorption of resveratrol in humans is about 75% but extensive metabolism in the intestine and liver results in an oral bioavailability of less than 1% [7]. Three main metabolites found in human plasma include trans-resveratrol-3-O- sulphate, trans-resveratrol-3-O-glucuronide and trans-resveratrol-4′-O-glucuronide [4]. However, deconjugation enzymes such as β-glucuronidase and sulfatase, as well as specific tissue accumulation of resveratrol, may enhance efficacy at target site [7]. Through studies done on metabolite, resveratrol-3-O-sulphate was observed to have anti-estrogenic activity but showed poor cytotoxicity in human malignant and non malignant breast cell lines [8].

The researches which led to the discovery of resveratrol were based on the epidemiological data from the French people who had a low incidence of coronary heart diseases (CHD) despite the consumption of a diet rich in highly saturated fat. This is the so called “French paradox” [9].

The methods which have been used to analyze resveratrol in different matrices include wine, plant extract and serum involved gas chromatography-mass spectrometry (GC-MS) [10], liquid chromatography-mass spectrometry (LC-MS) [11], high performance liquid chromatography (HPLC) based on UV absorption, fluorimetry and electrochemical detection and the capillary electrophoresis (CE) [12].

Recently, high-performance thin-layer chromatography (HPTLC) has become a routine analytical technique due to its advantages of its reliability and cost effectiveness [1]. The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of mobile phase. In the present study, an accurate, specific and reproducible HPTLC method has been developed and validated [1], or determination of resveratrol in local fresh grape berry skin (Vitis vinifera).

Presence of resveratrol has not yet been acknowledged in Tanzania’s Vitis vinifera using the simple maceration method for extraction and HPTLC&UV/visible method for analysis, whereas, resveratrol is one of the most essential constituents of grape berry fruits and red wines. Therefore, the health benefits of taking grape berry fruits and locally brewed red wines are not acknowledged considering the usefulness of the compound resveratrol in health body maintenance.

There are two known isomers of resveratrol which are trans and cis- and they have maximum absorption in the UV/visible spectrum at 306nm and 280nm respectively. The HPTLC analysis performed on 20cmx10cm aluminium plates coated with 200µm layer of silica gel 60F254, prewashed with methanol and activated at 110 °C for 5min and developed with 30ml chloroform-ethylacetate-formic acid (2.5 :1:0.1) showed Rf value of 0.34. The validated method used for analyzing resveratrol in Arachis hypogaea [1].

The optimum extraction conditions to obtain a good yield of trans-resveratrol from the grape berry skin when freeze dried were methanol 80%, temperature 60-80°C and time of 30 minutes,[ Romero-Perez, I. et al].
However, acidifying the methanol with 0.1% HLC also aids in quantitative extraction of resveratrol from grape skin [13].

Infection on the grape with powdery mildew relatively increased the concentration of resveratrol and its glycosides (piceid) in the skin of the grape berry. Therefore, since the compounds are produced by plants to attack pathogens such as bacteria or fungi, the concentration of the compounds increases when grapes are infected [10]. It is suggested that conditions leading to infection by Botrytis cinerea enhance resveratrol production, but that extensive grey mold development may destroy the induced phytoalexin [14].

Resveratrol exists in two isomeric forms, trans- and cis- and as the glycoside piceid is also called polydatin. The trans isomers is the more common and is believed to be photo- and thermal stable and biologically active. In addition to the cis- isomer a product, diphenyl-acetylene derivative of trans-resveratrol resulting from oxidation of the central double bond to a triple bond occurs in continuous expose of trans-isomer to UV in methanol at 365nm [15]. Therefore, during collection and preparation for extraction, the samples were protected from the light, to avoid light-induced isomerization. Since there is transformation of trans-resveratrol into the cis form after subjecting the trans form to UV light, Lopez-Hernandez.

Resveratrol is best absorbed through the buccal route as wine formulation than being formulated as tablet to be absorbed in the gastrointestinal tract [16]. Resveratrol was found to be better absorbed from natural grape products than from tablets from a small research done of 7 volunteers [9].

Researchers have shown that there is an increase in the mean levels and prevalence of selected cardiovascular risk factor in Tanzania [17]. Therefore since the risk factors are increasing everyday it is of great importance to study and know possible prophylactic measures in the simple food staffs and other preventive methods possible. Similarly, cancers, infections and many other diseases can be prevented or cured with no much effort of using medicines by just having a good dietary intake considering the presence of compounds like resveratrol which have activity against most of the common diseases and is present in some simple foods like grapes and groundnuts.

So it was of great importance to verify the presences of resveratrol in the local grapes to ascertain the claims about the health benefits of taking grape berries or using products from them like red wines.

### Methods and Materials

**Materials used:**

Plant materials vitis vinifera (grapes) were purchased from the local market from different sources at different places around Dar es Salam.

All the chemicals used in the experiments were of analytical grade. The reference standard resveratrol 98% pure of molecular weight 228.24 was purchased from Temperament herbal extract Pvt.Ltd,(India). Grass HPTLC plates of size 10cmx10cm coated with 200µm layer of silica gel(Switzerland) were used. Camag (Switzerland) linomat 5 sample applicator filled with a 100µl syringe was used. Camag TLC Scanner 3 and the former were controlled with Camag winCATS planar Chromatrography manager software version 1.4.3.6336. Camag developing chambers were used.

**Method used:**

**Extraction method used:**

20g of ground grape dried skin were macerated with 99.9% methanol acidified with 0.1% HCl for 24hrs at 30 °C. Similarly, fresh grape skin and flesh of red and white grapes were used for the extraction as follows:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of sample used</th>
<th>Amount of methanol used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried red grape skin</td>
<td>20.0g</td>
<td>100ml</td>
</tr>
<tr>
<td>Fresh red grape skin</td>
<td>36.4g</td>
<td>80ml</td>
</tr>
<tr>
<td>Green grape skin</td>
<td>25.7g</td>
<td>70ml</td>
</tr>
<tr>
<td>Red grape flesh</td>
<td>55.1g</td>
<td>110ml</td>
</tr>
<tr>
<td>Green grape flesh</td>
<td>50.3g</td>
<td>110ml</td>
</tr>
</tbody>
</table>

The grape skins were dried at 35 °C for six days. The extracts were placed in 5ml volumetric flasks after filtration using the filter paper and stored in the dark at temperature range of -0.7 to -1.5 °C.
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**HPTLC analysis**

**Stock solution preparation.**

Stock solution of resveratrol was prepared by dissolving 10.8mg of resveratrol in a volumetric flask with 25ml of methanol. The stock solution was stored in the dark at temperature range (-0.7 to -1.5 ºC). Validation was done by plotting increase of amount of resveratrol per spot and the peak area response. Therefore, by means of simple dilution, solutions of 100%, 80%, 70%, 50%, 25% were prepared and each solution was applied on three different brands on a single 10cmx20cm HPTLC glass plate and developed with chloroform-ethyl acetate-acetic acid (2.5:1:0.1) after a saturation time of 20 minutes.

**Sample application and development**

Both test and standard samples (5µl each) were applied onto the HPTLC plates as 10mm wide brands and 15mm wide apart from middle of brands by spray on technique along with air for simultaneous drying of brands by means of Camag linomate V sample applicator filled with a 100ul syringe.

The plates were developed to a distance of 70mm with 30ml chloroform-ethyl acetate-glacial acetic acid (2.5:1:0.1) for resveratrol as the mobile phase. The chamber was saturated for 20mins at room temperature (30ºC).

**HPTLC scanning**

The wavelength for detection was evaluated from complete UV spectrum of resveratrol. Densitometric scan was performed with a Camag TLC scanner at the wavelength of 313nm. The Rf of resveratrol was obtained at 0.34.

**Results and Discussions**

**Validation results**

The densitogram, table and graph of the representation of the relationship of amount of resveratrol per spot and the area under the peak are shown in Tables 2 and Figures 2 and 3 below. There was a linear relationship between the increase in amount of resveratrol per spot and the average area under the peak as observed in the figures and table.

![Figure 2: The validation densitogram](image-url)
Table 2: Examining the variation in peak area and amount of sample at a spot

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Area under the Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>19 514.4</td>
</tr>
<tr>
<td>80</td>
<td>15 782.5</td>
</tr>
<tr>
<td>70</td>
<td>13 814.4</td>
</tr>
<tr>
<td>50</td>
<td>9 712.5</td>
</tr>
<tr>
<td>25</td>
<td>4 853.7</td>
</tr>
</tbody>
</table>

Figure 3: Graph of concentration of resveratrol against average peak area from Densitometric scan by Camag TLC scanner

Extract analysis results
The densitograms of the samples (extracts) showed absence of trans-resveratrol and the other with significantly small amount of the compound, especially in the skin extracts as expected. The densitogram for each sample were observed as in the following figures 4-10.
At Rf of 0.11 a peak of area 1 181.2 was obtained, representing presence of metabolites or isomer of trans-resveratrol in the standard. The peak at Rf of 0.34 with an area of 19 774.6 was of trans-resveratrol which is the standard prepared at a concentration of 0.432mg/ml.

The peak area of the standard at Rf of 0.34 was then used to determine the concentration of trans-resveratrol in the extracts.
The peak at R<sub>f</sub> of 0.11 had an area of 5585.5, while the peak of trans-resveratrol at R<sub>f</sub> of 0.34 showed a significantly small peak area of 242.2. This showed the presence of trans-resveratrol in the fresh green grape skin, though in very little amount.

\[ 242.2 \times (10.8\text{mg} \times 98\%) = 0.005185\text{mg/ml} \]
\[ 19774.6 \times 25\text{ml} \]

\[ 0.005185\text{mg/ml} \times 70\text{ml} = 0.363\text{mg} \]

Therefore 25.7g of fresh green grape skin contained approximately 0.363mg.
The peak at Rf of 0.11 showed a peak area of 3 323.4 and there was no peak obtained at the Rf of 0.34 which showed the absence of trans-resveratrol from the fresh green grape flesh.

The peak at Rf of 0.11 gave an area of 3 218.8 and similarly, there was no peak at the Rf of 0.34 of the flesh extract of fresh red grape showing absence of trans-resveratrol in the fresh red grape flesh.
The peak at R_f of 0.11 gave an area of 4,882.1, though with an additional small peak at 0.15 but there was no peak at R_f of 0.34. Regardless of the expected presence of trans-resveratrol in the skin of red grape the densitogram showed it absent.

The peak at R_f of 0.11 gave a relatively larger area 16,553.6 showing a higher concentration of metabolite or isomers of trans-resveratrol since the extraction was done using dried sample. Similar to the extract above a peak 0.17 was obtained and the peak at R_f of 0.34 gave an area of 374.7.

\[
374.7 \times (10.8 \text{mg} \times 98\%) = 0.00802 \text{mg/ml}
\]
Therefore **20.0g** of dried red grape skin contained approximately **0.802mg**. (0.004%). Implying that fresh red grape skin should have contained (0.00074%).

Basically, there was no trans-resveratrol in the flesh of both the green and red grape berries, whereas, it was present though in little amounts in both the green and red berries’ skin.

In comparison between the green and red grape berry skin, trans-resveratrol was relatively higher in the green grape berry skin with (0.0014%) for fresh (wet) sample compared to the expected (0.00074%) of the red grape berry skin fresh (wet) sample.

**Conclusion**

Despite the observed small amount in grape skin, it seems to be the metabolites of trans-resveratrol, the isomer of trans-resveratol or other compounds predominating in all the samples. Therefore, since the trans-isomer is affected much by UV and heat, the compound might have deteriorated during the sell or storage of the grapes in the local market. However, a relatively good concentration might have been present initially.

**Recommendations**

We recommend other similar researches to be done in three phases of sample collection that is, direct from the farm, from the local market and other samples exposed to extreme conditions.

Validation methods for HPTLC analysis maybe developed using other safer, cheaper and environmentally friendly reagents for example ethyl acetate and acetic acid (29:1).

We also recommend analytical researches of trans-resveratrol to be done in the locally produced and imported grape juices existing in Tanzanian markets. Other analytical researches can be done in food like groundnuts which are commonly consumed in our settings.

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**Source of Conflict:** Nil

**References**

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