



ESTIMATION OF RESVERATROL IN FEW NATIVE FRUITS OF NORTH-EAST INDIA

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ABSTRACT

Resveratrol- a naturally derived stilbene, is gaining extreme popularity because of its highly appreciated multi-beneficial properties as anticancerous, antioxidant and anti-inflammatory molecule. Major dietary sources of resveratrol include grapes, berries, peanut, legumes and red wine. N.E India, rich for its wild heritage possesses many indigenous plants that have the potential to be rich source of the stilbene. *Emblica officinalis*, *Averrhoa carambola*, *Elaeagnus pyriformis* from native N.E India were screened for resveratrol content and HPLC based result confirm the dietary potential of resveratrol. In *Phyllanthus emblica* resveratrol is amounted to 43.5µg/g while in *Elaeagnus pyriformis* and *Averrhoa carambola* it is 4µg/g and 1.6µg/g respectively which is higher than many available sources such as grape and peanut. The fruits are used as alternative source of resveratrol and as neutraceuticals for many diseases by natives of India, and are easily available and low cost.

KEYWORD: Bioavailability, Resveratrol, HPLC, *Emblica officinalis*, *Averrhoa carambola*, *Elaeagnus pyriformis*

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INTRODUCTION

Resveratrol (3,5,4'-trihydroxystilbene or 3,4',5-stilbenetriol), is a member of a family of polyphenols called viniferins secreted by certain plants in response to external stimuli such as fungal attack or UV radiation or pathological attack, and has been demonstrated to possess varied biochemical and physiological benefits^{1,2}. In nature, molecular formula of resveratrol is C₁₄H₁₂O₃, and molecular weight is 228.25 Daltons, and available as two structural isomers, *cis* and *trans*, the *trans* form being more common and possessing greater biological activity. Resveratrol was first identified in grapevines (*Vitis vinifera*) in 1976³. Since then, its presence in varying concentrations has been discovered in many other plants specially berries such as strawberry, blueberry, mulberry, grapes etc, and a wide variety of flowers and leaves including *Gnetum*, white hellebore, corn lily, butterfly orchid tree, eucalyptus etc⁴. The health benefits of resveratrol ranges from antioxidant property, cardio-protection capacity, anticancer activity, anti-inflammatory effects, estrogenic/anti-estrogenic properties to modulate cellular signal transduction pathways⁵⁻¹³, inhibition of oxidative apoptosis¹⁴⁻¹⁷ and to lower lipid peroxidation in blood platelets¹⁸. Resveratrol is the lead molecule accounted for French Paradox, a phenomenon of less coronary diseases and high longevity of French people despite a high fat diet and consumption of red wine. The potentials of naturally available nutraceutical resources having anti-cancer property and other life style related diseases/disorders prevention property has always been a huge research area in Biological Sciences. Till date, resveratrol is the most studied secondary metabolite of stilbene family and researchers has put ample effort to find out varied naturally available sources of resveratrol for better benefit of human race. North Eastern (NE) part of India, comprising of seven states, is rich in biodiversity. The wild heritage of this geographical area bears varieties of fruits which may harbour nutraceutical values which need screening. In this study an effort is made to identify and quantify the wonder compound

'resveratrol' in few locally available fruits from NE India.

METHODOLOGY

i) Short listing of plants

The selection of the fruits was based on traditional knowledge of the ethnical tribes and residents of NE Indian states, and existing scientific literature as follows.

- a) *Phyllanthus emblica* (common name Indian gooseberry). The deciduous annual plant belongs to family Phyllanthaceae and is mainly found in the sub-tropical parts of India, China and Indonesia. It is a very rich source of Vitamin C, and has been reported to contain several phenolic compounds, tannins, phyllembelic acid, phyllembin, rutin, curcuminoides and emblicol¹⁹.
- b) *Averrhoa carambola* (common name carambola) belongs to family Oxalidaceae. This evergreen perennial tree is native to Southeast Asia and the Indian Subcontinents. It is a very good source of Vitamin C, B-complex vitamins such as folates, boflavin and flavonoids such as quercetin, epicatechin, and gallic acid.
- c) *Elaeagnus pyriformis* (common name silverberry) belongs to Elaeagnaceae family. The fruit is mainly available in Manipur, Mizoram and other sub tropical places of India and rich in vitamins, minerals, flavonoids and few essential fatty acids²⁰.

ii) Collection of plant material

Phyllanthus emblica and *Averrhoa carambola* were collected from local market of Assam and *Elaeagnus pyriformis* was collected from farms of Manipur. Frozen fruits (100 in number) were taken and skin was removed manually by hand. Skin was then lyophilized to dry and was finely ground. 10 g of finely ground skin powder was macerated in 100 ml of methanol with 0.1% HCl. The solution was centrifuged at 1000 rpm for 15 min. Supernatant was collected and equal volume of water was mixed prior to evaporation followed by liquid-liquid extraction

with equal volume of ethyl acetate. Organic layer was evaporated to dryness and was recovered by 2-5 ml of 50% ethanol. All the procedures were performed in dark room at an ambient temperature of 25° C.

iii) HPLC

A modular Shimadzu UFLC system comprising LC-20AD solvent delivery module, 25CTO-20AC column oven, SPD-20A/20AV UV/VIS detector, CBM-20A/20Alite interface and LC Solution 1.25 work station was utilized. The separation was performed on a reverse phase C18 Column of size 250 X 4.60 mm (5 micron) with methanol and water in a ratio of 65:35 as a mobile phase in isocratic mode at a flow rate of 1ml/min. 20µl of sample and standard was injected in the instrument for analysis. Detection was performed at 286 nm. The run time was 15 min. Samples and standard were filtered in 0.2 micron syringe filter before injecting into column. Quantitative analysis of resveratrol in plant material was performed by the external standard method using pure resveratrol (Sigma R5010 >99% pure) as standard. A stock solution of 100ppm was prepared in ethanol and stored at -20° C in dark. Stock was diluted to 10, 20, 30, 40, 50,

60, 70, 80ppm and utilized as reference standard. Each determination was carried out in triplicate. The concentration of resveratrol was calculated from peak areas using the graphical method. A standard curve for trans resveratrol in 10% ethanol was generated for the quantification of resveratrol in the fruit extracts.

RESULTS AND DISCUSSION

Resveratrol, mainly in its trans-isoform has gained huge popularity in research community as the lead molecule behind beneficial effect of wine. Many commercial resveratrol supplements are available in the market, however, the best benefits of the compound can be achieved with its natural resources. In this study, the standard peak for resveratrol was observed at retention time 6.2 – 6.5 minutes. Standard curve was prepared by taking standard concentrations in X-axis and Area of peak in Y axis. Concentration of resveratrol in the test samples were determined by plotting area against respective spots in X axis. The Fig 1 shows the chromatogram of standard resveratrol solution.

Figure 1
Representative HPLC curve for Resveratrol standard

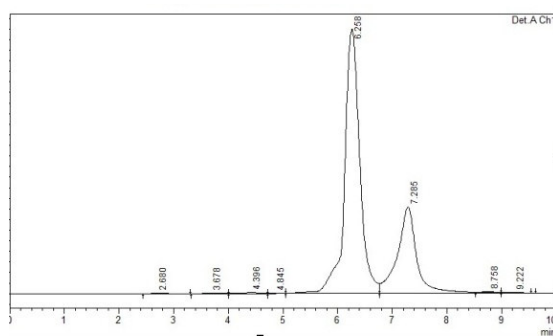


Fig 1: Reversed phase HPLC analysis of trans-resveratrol standard analyzed on a C18 Column 250 X 4.60 mm (5 micron) with methanol and water in a ratio of 65:35 as a mobile phase at flow rate of 1ml/min at 286nm

When 20 µl of the test samples was eluted in HPLC, peaks were observed at retention time of 6.2 to 6.5 minutes. The peak area of different samples was different based on the content of resveratrol in the respective samples. After standard curve preparation, the concentration of resveratrol in the three samples was

calculated. The concentration varied in each of the three test samples and significant level of resveratrol were observed. Concentration of resveratrol in *Phyllanthus emblica* was found to be highest among the three samples while *Averrhoa carambola* recorded the least amount. In *Phyllanthus emblica* resveratrol was

amounted 43.5 $\mu\text{g/g}$ while in *Elaeagnus pyriformis* and *Averrhoa carambola* it was 4

$\mu\text{g/g}$ and 1.6 $\mu\text{g/g}$ respectively. Fig2 shows the chromatogram for test samples.

Figure 2
Representative HPLC curve for test sample

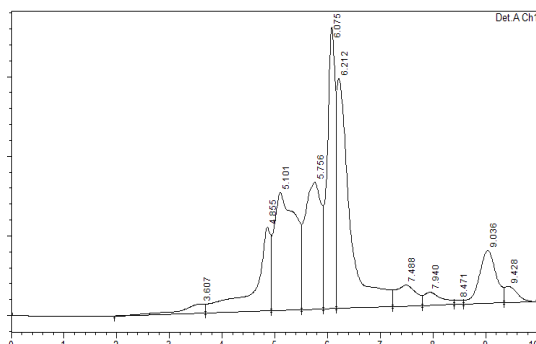


Fig 2: Chromatogram showing the peak at different retention time for experimental sample. The peak at retention time 6.21 min, represents resveratrol.

The amounts of resveratrol found in these fruits are higher when compared to renowned sources of resveratrol. Different variety of peanut has 0.022-1.792 $\mu\text{g/g}$ of resveratrol²¹, whereas in *Palomino fino* Grapes resveratrol concentration varies from 1.45-2.06 $\mu\text{g/g}$ depending on vintage and fungal infection²². Different researchers across the globe have conducted many studies to quantify resveratrol content in different berries. Among them a study states that *V. arboreum* Marshall (sparkleberry) has 0.519 $\mu\text{g/g}$ of resveratrol, *V. ashei* Reade (rabbiteye blueberry) has the highest of 0.586 $\mu\text{g/g}$, *V. corymbosum* L. (highbush blueberry) has maximum 0.853 $\mu\text{g/g}$ and *V. vinifera* L. (grapes) is found to have 2.475-6.356 $\mu\text{g/g}$ of resveratrol concentration²³. However, Grape variety Thompson seedless, Cabernet Sauvignon and Sauvignon blanc under healthy condition is found to have 0.63 $\mu\text{g/kg}$, 1.21 $\mu\text{g/kg}$ and 0.62 $\mu\text{g/kg}$ resveratrol respectively²⁴. Compared to the data presented above, it can be observed that the fruits of N.E India have a rich source of resveratrol. *Elaeagnus pyriformis* and *Phyllanthus emblica* contains almost 2 times and 7 times more resveratrol than other conventional source of resveratrol. This data also provides us with the information that the experimental fruits can be used as a nutraceutical because of the high amount of resveratrol present in it. Resveratrol is produced naturally by plants; however, the

amount of resveratrol varies in each case depending on the geographical locations and physiological conditions. By estimating the concentration of resveratrol in different food materials, a suitable dose can be predicted which may improve human health and may act as a nutraceutical. It is already known from previous reports that presence of resveratrol in red wine is associated with 'French paradox' of longer life span; and more focus has been given on resveratrol based research because of its cardio-protective and clinically important role and functions. Since culturally the consumption of red wine is not customary in Indian and NE Indian societies, and moreover since berries have been reported as rich reservoirs of resveratrol, we focused on the availability of berry-like fruits in NE India. In our study, we found a rich bioavailability of resveratrol containing fruits in NE India, and if consumed in proper amount may definitely confer health benefits as a hepato-protective nutraceutical as well as additional medicinal benefits like cardio protection, anti-aging etc. The fruits may serve as a dietary supplement as a source for anti oxidant component. In addition, owing to its varied health benefits, resveratrol supplements under various manufactures are mushrooming in the market. Most of these commercially available resveratrol supplements of U.S market contain extracts from the Japanese and Chinese knotweed plant *Polygonum cuspidatum* while others use red

wine or red grape extract as a source. The fruits of our experiment can serve as an alternative source for resveratrol supplements.

CONCLUSION

Our study elucidates that the three fruits are most potential sources for resveratrol production. This goes against the popular notion that resveratrol is produced only from grape and a very limited number of other edible species. Resveratrol rich food *Phyllanthus emblica*, *Averrhoa carambola*, *Elaeagnus pyriformis* can be used as nutraceutical fruits owing to its anti aging, cardio protective, anti-oxidant properties.

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ABBREVIATIONS

µg- microgram, g- gram, ppm-parts per billion

CONFLICT OF INTEREST

Conflict of interest declared none.

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