

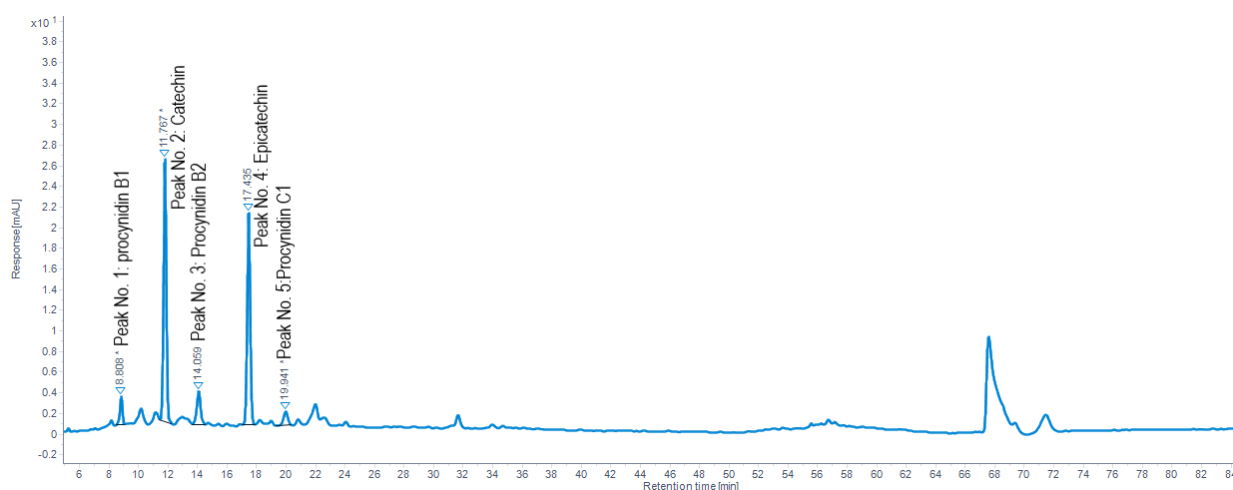
Assessment on using Vinomerix® Optima for Cardiovascular Health

Overview of the active compounds in Vinomerix® Optima:

Vinomerix® Optima is a grape seed-based extract that is standardized to not less than 96% polyphenols which contain not less than 80% OPC and not less than 16% monomers.

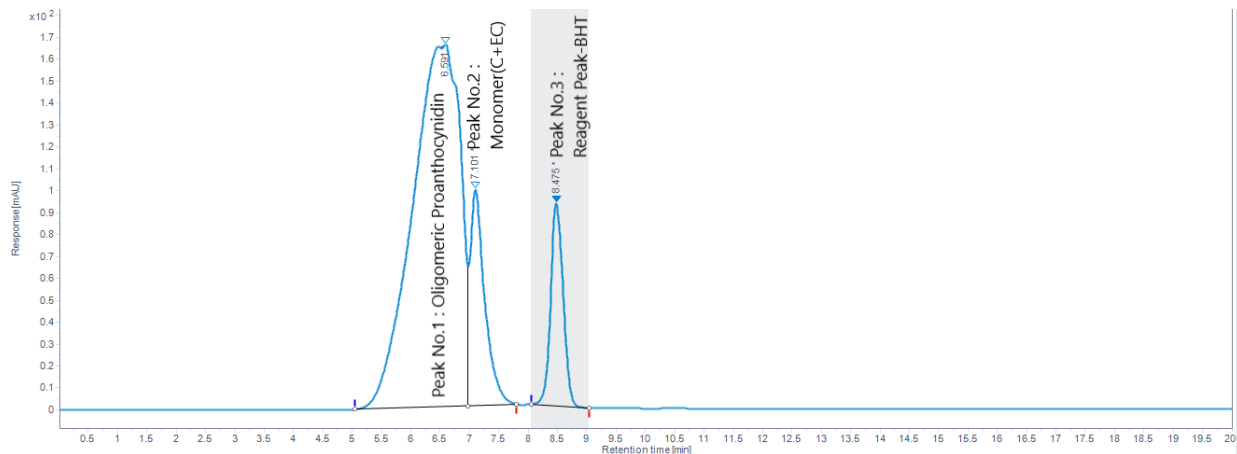
Overview of the Identification and measurement of the Active markers in Vinomerix® Optima:

Vinomerix optima is standardized using the cutting-edge High-Pressure liquid Chromatography which gives the accurate measurement of its active markers which are Oligomeric Proanthocyanidins (OPCs) and individual markers in monomeric form such as Catechins, Epicatechins. Standardization by HPLC not only quantifies the Active marker compounds but also gives HPLC Fingerprinting that can help to identify adulteration.



HPLC Chromatogram of Vinomerix® Optima

Vinomerix® Optima has the 16-19 % Monomers (Catechin & Epicatechin) identified using HPLC. Various Literature says Monomers especially catechin & Epicatechin have better efficacy as they are more bioavailable. Vinomerix® Optima is also high in Dimeric Proanthocyanidins (Procyanidin B1 & B2) and Trimeric procyanidin C1. The unique combination of Monomers, Dimers and Trimers gives wholesome health benefits of grape seed extract.



Vinomerix® Optima Oligomeric Proanthocyanidins by GPC Method Chromatogram

Vinomerix® Optima is rich in bioavailable Oligomers Proanthocyanidins. It is standardized NLT 80% OPCs by USP compliant Gel Permeation Chromatography method.

Vinomerix® Optima is fully characterized using HPLC and GPC, ensuring a total polyphenol (Monomers + OPCs) content of no less than 96%. The whole profile characterization gives an added advantage to claim no adulteration, a common practice used by many other commercial grape seed extract sellers.

Overview of our rationale:

Vinomerix® Optima is a grape seed extract known for its high concentration of bioavailable monomers and oligomeric proanthocyanins (OPC). Grape Seed Extracts have been researched in detail over the years and it has been established that grape seed extracts, high in monomers (especially catechin and epicatechin) **have significant antioxidant and vasodilation properties**. Natrusolate's high monomer concentrated Vinomerix® Optima (more than 16% monomers) is an excellent source of radical scavenging bioactives that protect the heart as both an antioxidant as well as an eNOS activator (which promotes vasodilation). By this 2-way mechanism of action, **Vinomerix® Optima can help protect the heart as well as dilate endothelial cells lining the capillaries and arteries to sufficiently carry blood to the tissues.** For Vinomerix® Optima, we have relied on prior art to evidence the role of a standardized grape seed extract in cardiovascular health.

Please see below for detailed analysis of our rationale for Vinomerix® Optima.

Overview of our dosage assessment:

Effective Dosage Per Serving:

Vinomerix® Optima – 200 to 300 mg once per day

Rationale for Vinomerix® Optima in cardiovascular health:

Role of Grape Seed Extracts:

It is established that one of the main causes of cardio health issues is oxidative stress in the body. The oxidation of low-density lipoproteins (LDL) by free radicals is associated with the initiation of atherosclerosis and therefore, development of coronary artery disease.¹ Hence, it is critical to arrest oxidative stress caused by free radicals in the body. It has been shown that grape seed extracts, standardized to a high polyphenol content, can protect LDL from oxidation – thereby reducing the risk of heart disease.

It is also suggested that one of the main reasons for deteriorating cardio health is high blood pressure (hypertension). Hypertension is a major risk factor for fatal and non-fatal cardiovascular events. Studies have shown that blood pressure reductions lower the risk of myocardial infarction, stroke and other cardiovascular events.

We propose Vinomerix® Optima as a supplement that could enhance cardiac health by working in two ways:

- a. **Antioxidant:** As a powerful anti-oxidant which scavenges free radicals and prevents oxidation of LDL (one of the causes of cardiovascular decline).
- b. **Endothelial Health:** Activates eNOS, which enhances nitric oxide production which helps in vasodilation of the blood vessels

Some components of grapes, especially flavonoids, are endowed with beneficial cardiac effects. Flavonoids are plant products which act as potent free radical scavengers and could also aid in protecting the heart by preventing the oxidation of low-density lipoproteins (LDL). Additionally, there are studies that show the positive correlation between consuming grape seed extracts and lowering blood pressure. Hence, grape seed extracts rich in polyphenols and specifically monomeric compounds like catechin and epicatechin can have a positive impact on heart health.

Vinomerix® Optima has more than 15% monomers, primarily containing catechins and epicatechins, which can contribute to better heart health.

Mechanism of Action:

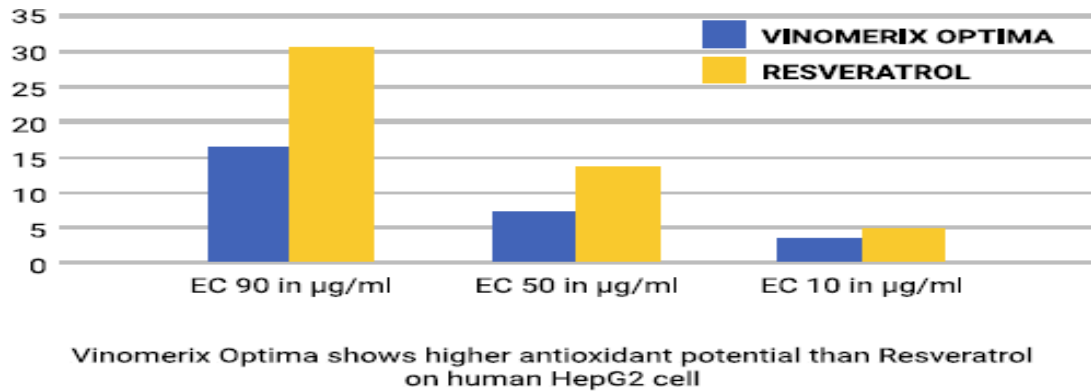
Vinomerix® Optima reduces risk of cardiovascular diseases by protecting the cardiovascular cells from oxidative stress and vasodilation (eNOS activation).²

Antioxidant Activity of Vinomerix® *in vitro* study:

At Natrusolate®, we conducted an *in vitro* study to evaluate the antioxidant potential of Vinomerix® Optima by LUCS (Light-up cell system) technology on a human liver cell model. In this model, it was found that Vinomerix® Optima was 50% more potent than resveratrol in its antioxidant efficacy.³ Due to the high antioxidant potential, Vinomerix® Optima can scavenge the free radicals in the body and prevent decline in heart health.

1 Nuttall, Kendall, Bombardelli, Morazzoni. An evaluation of the antioxidant activity of a standardized grape seed extract, Leucoselect Journal of Clinical Pharmacy and Therapeutics (1998) 23, 385-389

2.Feng Z, Wei RB, Hong Q, Cui SY, Chen XM. Grape seed extract enhances eNOS expression and NO production through regulating calcium-mediated AKT phosphorylation in H2O2-treated endothelium. Cell biology international. 2010 Oct;34(10):1055-61. Refer to report on LUCS Study, Indus Biotech attached in email



Vasodilation by eNOS activation:

We conducted an invitro study to evaluate effect of VINOMERIX OPTIMA on potential to activate eNOS (endothelial nitric oxide levels in HUVEC (Human Umbilical Vein Endothelial Cell) Model. In this study, VINOMERIX optima at 0.5% concentration shown 122% increase in eNOS with respect to vehicle control. Up regulation of increased endothelial nitric oxide synthase (eNOS) can increase endothelial nitric oxide (NO) and endothelial dependent vasodilation. By enhancing NO production, grape seed extract promotes vasodilation, which helps to lower blood pressure.

Human Clinical Data:

Anti-oxidant potential:

Prevention of oxidation of LDL:⁴

In a study conducted on 20 subjects, it was found that a standardized extract of grape seed, Leucoselect®, increased serum anti-oxidant activity. This study showed that 300 mg dose of polyphenols produced a measurable increase in serum anti-oxidant activity using a chemiluminescent assay.

Similarly, Vinomerix® Optima, if used at 300 mg per day, can have a similar efficacy based on the LUCS *in-vitro* study.

Anti-oxidant potential:⁵

In a study conducted by *Joe A. et al*, the antioxidant efficacy of grape seed extract standardized to contain Monomers (19.8%) and Oligomers (69.8%) was compared to grapes, grape juice, red wine, and other grape seed extracts using an oxidation-reduction colorimetric assay. The study also evaluated the in-vitro model of heart diseases and measured plasma antioxidant levels using the RANDOX assay.

4 Nuttall, Kendall, Bombardelli, Morazzoni. An evaluation of the antioxidant activity of a standardized grape seed extract, Leucoselect Journal of Clinical Pharmacy and Therapeutics (1998) 23, 385-389

5 Vinson JA, Proch J, Bose P. MegaNatural® gold grapeseed extract: in vitro antioxidant and in vivo human supplementation studies. Journal of medicinal food. 2001 Mar 1;4(1):17-26.

The outcomes of the study revealed the following results for the grape seed extract standardized to Monomers (19.8%) and Oligomers (69.8%):

- It exhibited a superior antioxidant index when compared to the other tested samples.
- There was a dose-dependent inhibition of low-density lipoprotein oxidation.
- The RANDOX assay showed a significant increase of 12% in plasma antioxidant activity after 1 and 2 hours.

These findings highlight the potent antioxidant properties of the grape seed extract, particularly when standardized to contain Monomers and Oligomers, demonstrating its potential benefits in combating oxidative stress-related conditions, such as heart diseases.

Similarly, Vinomerix® Optima is standardized to NLT 16% Monomers, which is higher concentration of monomers than the grape seed extract tested in the Joe A et al study – hence, we can use this study to show the benefits of a high monomer concentration and anti-oxidant potential in the human body.

Anti-oxidant potential in smokers:⁶

The objective of this study was to assess the impact of a formulation of grape seed extract standardized to Monomers (15%) and Oligomers (80%) on the susceptibility of low-density lipoprotein (LDL) to oxidation in a group of heavy smokers.

The study employed a randomized, double-blind, crossover design involving 24 healthy male heavy smokers aged over 50 years. Participants were administered 2 capsules twice daily for a duration of 4 weeks in the first phase. Each capsule contained 75 mg of standardized grape seed extract and soy-phosphatidylcholine, while the placebo consisted of 75 mg lactose and soy-phosphatidylcholine. Following a washout period of 3 weeks, the participants underwent another 4-week phase with the opposite treatment. Blood samples were collected at the beginning and end of each phase and analyzed for plasma lipids and LDL susceptibility to oxidation.

The study outcomes revealed significant reductions in oxidative indices, specifically the concentration of thiobarbituric acid reactive substances (TBARS), in subjects taking the grape seed extract formulation ($-14.7\% \pm 21.1\%$ vs. $-5.0\% \pm 18.1\%$, $P < .01$) compared to the placebo and baseline values. Additionally, the lag phase, which represents the time until the initiation of LDL oxidation, was prolonged in the group taking the grape seed extract formulation ($-15.4\% \pm 24.4\%$ vs. $-0.1\% \pm 16.0\%$, $P < .05$) compared to the placebo and baseline values.

These findings indicate that the grape seed extract formulation effectively reduced oxidative stress and improved LDL resistance to oxidation in heavy smokers.

⁶ Vigna GB, Costantini F, Aldini G, Carini M, Catapano A, Schena F, Tangerini A, Zanca R, Bombardelli E, Morazzoni P, Mezzetti A. Effect of a standardized grape seed extract on low-density lipoprotein susceptibility to oxidation in heavy smokers. *Metabolism*. 2003 Oct 1;52(10):1250-7

Lowering Blood Pressure in Hypertension:⁷

The objective of this study was to investigate the potential of grape seed extract (GSE) as a functional ingredient for lowering blood pressure (BP) in individuals with pre-hypertension.

The study employed a single-centre, randomized, two-arm, double-blinded, placebo-controlled design, lasting for a duration of 12 weeks. The participants consisted of middle-aged adults with pre-hypertension. A total of thirty-six subjects were randomly assigned in a 1:1 ratio to either the Placebo group (n=18) or the GSE group (n=18). Twenty-nine participants completed all the specified procedures outlined in the study protocol, with seventeen in the Placebo group and twelve in the GSE group. Throughout the study, the participants consumed a juice containing either 0mg (Placebo) or 300 mg/day of GSE (150 mg per dose) twice daily for 6 weeks. This intervention period was preceded by a 2-week Placebo run-in period and followed by a 4-week no-beverage follow-up. Compliance with the juice consumption was closely monitored. Blood pressure measurements were taken at screening, 0, 6, and 10 weeks of the intervention, and blood samples were collected at 0, 3, 6, and 10 weeks of the intervention.

The study outcomes demonstrated that GSE had a significant impact on reducing systolic blood pressure (SBP) by 5.6% (P=0.012) and diastolic blood pressure (DBP) by 4.7% (P=0.049) after 6 weeks of intervention. These reductions were significantly different from the effects observed in the Placebo group for SBP (P=0.03) and showed a tendency towards significance for DBP (P=0.08). These findings indicate that GSE supplementation effectively decreased both systolic and diastolic blood pressure in individuals with pre-hypertension during the 6-week intervention period.

Lowering blood pressure: ⁸

The objective of this study was to investigate whether grape seed extracts (GSE), which contain potent vasodilator phenolic compounds, can effectively lower blood pressure in individuals with the metabolic syndrome.

The study design involved randomizing the subjects into three groups: (a) placebo, (b) 150 mg GSE per day, and (c) 300 mg GSE per day. The participants received their assigned treatment for a period of 4 weeks. At the beginning and end of the study, serum lipids and blood glucose levels were measured. Blood pressure was assessed using an ambulatory monitoring device at the start of the treatment period and again at the end.

The study outcomes revealed that both systolic and diastolic blood pressures were significantly reduced after treatment with GSE, as compared to the placebo group. This indicates that GSE supplementation effectively lowered blood pressure in subjects with metabolic syndrome.

⁷ Park E, Edirisinghe I, Choy YY, Waterhouse A, Burton-Freeman B. Effects of grape seed extract beverage on blood pressure and metabolic indices in individuals with pre-hypertension: a randomised, double-blinded, two-arm, parallel, placebo-controlled trial. *British Journal of Nutrition*. 2016 Jan;115(2):226-38.

⁸ Sivaprakasapillai B, Edirisinghe I, Randolph J, Steinberg F, Kappagoda T. Effect of grape seed extract on blood pressure in subjects with the metabolic syndrome. *Metabolism*. 2009 Dec 1;58(12):1743-6.