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Summary Report:

Investigation of broad spectrum antioxidant activity of a proprietary phytonutrient nutraceutical against major free radicals found within the human body.

1. Introduction & Summary

Consumers of dietary supplements often seek products with antioxidants within their formulations due to the known ability of antioxidants to quench free radicals. However, there is often confusion regarding strength, spectrum of activity, absorption and bioavailability of antioxidant nutritional formulations. It is generally well accepted that ingested antioxidants from fruits, vegetables and other such substances are beneficial to human health, but the same cannot be said for antioxidant dietary supplement formulations. This is likely related to the research supported notion that broad spectrum antioxidant activity is present from multiple phytonutrients that are present within natural fruits and vegetables, not just single isolated nutrients. This mechanism is related to cofactor synergy which exists in natural foods, an explanation in which full mechanistic understanding is still developing. Due to the interest in the potential ability of ingested antioxidants to alter the harmful physiologic effects of free radicals, intense research has explored antioxidant activities of various nutritional substances and combinations. Research has also sought to identify compound combinations which have broad spectrum activity against multiple free radical agents, thus promoting broad spectrum antioxidant defense.

Antioxidants are substances which can interact with free radicals and render them harmless through electron donation, thus potentially protecting the body from oxidative harm. Free radicals are reactive oxygen species (oxidants), generated internally and externally, that can have adverse effects on normal physiological function of the human body when out of homeostatic balance and process. Free radicals are known agents of cellular damage at the membrane, mitochondrial and DNA levels. The adverse effect of free radicals on biologic systems is described generally as Oxidative Stress (OS).

This study undertook investigation of the anti-oxidant activity of a phytonutrient dietary supplement containing verified essential RDA nutrients and a proprietary blend of fruits, vegetables and, aloe vera gel concentrated components and also a high content green tea epigallocatechin gallate (EGCG) catechin complex (Eniva VIBE 2.0 Cardiac & Life™) against multiple free radical agents, including: peroxy (water and lipid soluble), hydroxyl, peroxynitrite, singlet oxygen and ferric free radicals. The investigational substance was sent for third party independent analysis (Brunswick Labs, MA). Antioxidant activity was measured by oxygen radical absorbance capacity (lipophilic and hydrophilic- ORAC total), hydroxyl radical averting capacity (HORAC), peroxynitrite radical averting capacity (NORAC), superoxide radical averting capacity (SORAC) and ferric reducing antioxidant capacity (FRAP) assays.

The results demonstrated broad spectrum antioxidant activity against all free radical species tested. This is likely related to the inclusion of multiple phytonutrient components within the formulation,

not solely a single isolated nutrient. While *in vitro* (laboratory) results do not always correlate to *in vivo* (in body) activity, the Eniva VIBE nutraceutical tested represents an over-the-counter (OTC) dietary supplement which has demonstrated *in vitro* antioxidant activity against a broad range of free radicals known to exist in the human body and stands as a consumer option for those seeking products verified for antioxidant activity.

2. Study Methodology

Methodology Synopsis:

The sealed and identified Eniva VIBE nutraceutical (Eniva VIBE 2.0 Cardiac & Life™) was sent for third-party *in vitro* analysis (Brunswick Labs, MA) for anti-oxidant capacity evaluation in relation to its ability to quench a variety of free radical species generated via various validated methodologies. The free radicals tested represent major radical species in the human body:

Test	Radical
ORAC-ROO (hydro)	Water-soluble peroxy radicals
ORAC-ROO (lipo)	Fat-soluble peroxy radicals
N-ORAC (ONOO-)	Peroxynitrite radical
H-ORAC (HO-)	Hydroxyl Radical
S-ORAC (O-)	Singlet oxygen (measured in SOD equivalence result)
*FRAP (FeIII)	Ferric Ion

* Although not a major endogenously produced free radical species, iron derivatives represent ingested pro-oxidants from the diet via an iron-catalyzed free radical--mediated oxidative stress mechanism.

1. Test Material:

This study undertook the investigation of the anti-oxidant activity of a dietary supplement formulation of verified essential nutrients and proprietary combinations of fruit, vegetable and aloe vera concentrated components also with a green tea epigallocatechin gallate (EGCG) catechin complex. This is identified as Eniva VIBE 2.0 Cardiac & Life™. Nutrient content and validated essential nutrients on file.

2. Test Methodologies:

Antioxidant activity was evaluated by validated testing methodologies for quantifying the ORAC-ROO (hydro+lipo), H-ORAC, N-ORAC, FRAP and SOD scores. Assays performed via standard methodologies at Brunswick Labs, MA.

- a. **Peroxy Radicals** (water and lipid soluble): Quantification through oxygen radical absorbance capacity (ORAC). Reported in micromole Trolox equivalents per liter.
- b. **Hydroxyl Radicals**: Quantification through hydroxyl radical averting capacity (HORAC). Reported in micromole Trolox equivalents per liter.
- c. **Peroxynitrite radicals**: Quantification through peroxynitrite radical averting capacity (NORAC). Reported in micromole Trolox equivalents per liter.

- d. **Iron ferric radical:** Ferric reducing antioxidant capacity (FRAP). Reported in micromole Trolox equivalents per liter.
- e. **Superoxide radical:** Superoxide radical averting capacity (SORAC). Reported in kilounits Superoxide Dismutase(SOD) equivalence per liter.

3. Results:

1. Antioxidant activity: Eniva VIBE 2.0 Cardiac & Life™

The antioxidant activity of the test material was evaluated against 6 major free radical known to exist in the human body. Please find the results summarized in Table 1.

Table 1:

Test Sample	ORAC-ROO hydro (umolTE/L)	ORAC-ROO lipo (umolTE/L)	H-ORAC (HO-) (umolTE/L)	N-ORAC (ONOO-) (umolTE/L)	F-RAP (FeIII) (umolTE/L)	SOD (kunits SODEq/L)
VIBE 2.0 Cardiac & Life™	150,734	3,012	15,944	15,880	76,794	14,878

* Actual result documents attached at end of report.

Figure 1: Peroxyl radical antioxidant capacity of test material

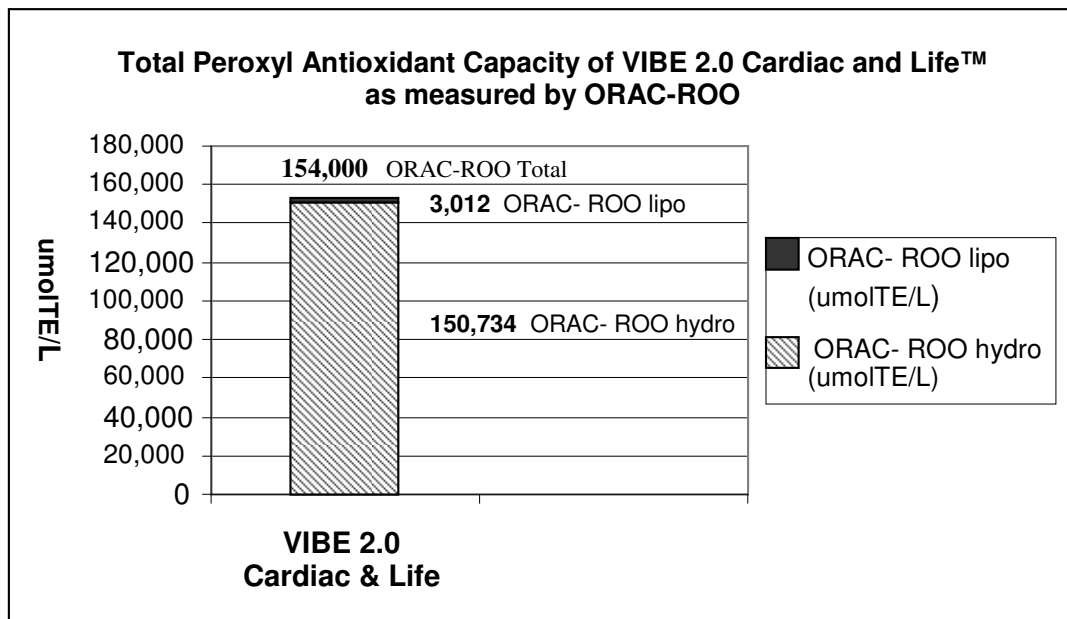


Figure 2: Hydroxyl, peroxyntirite, ferric radical antioxidant capacity of test material.

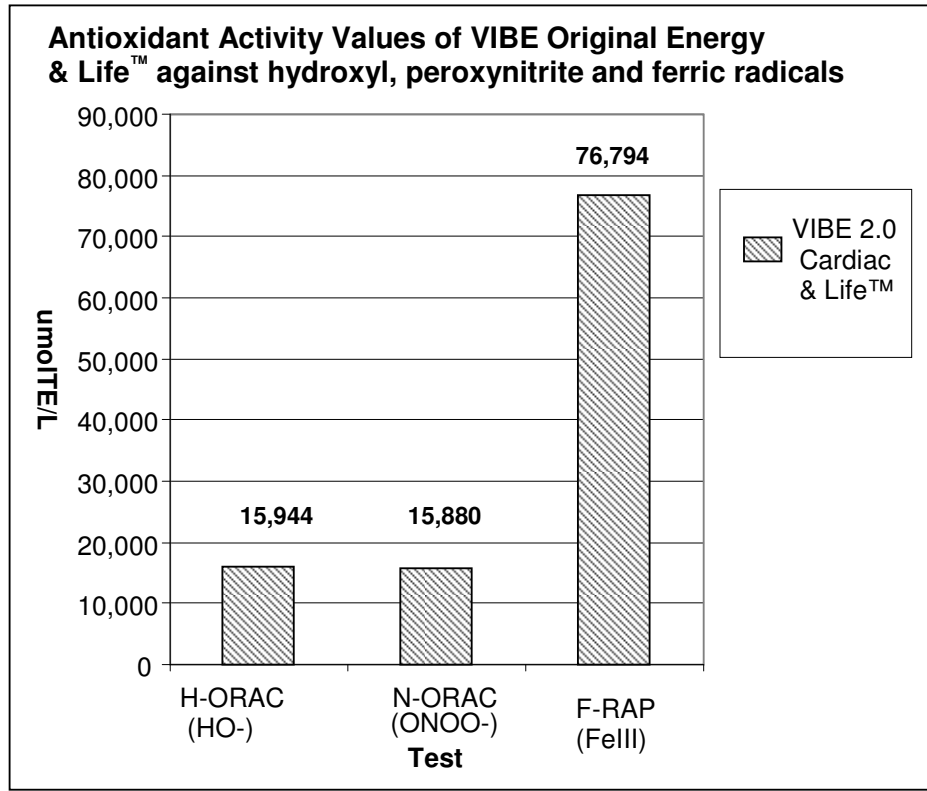
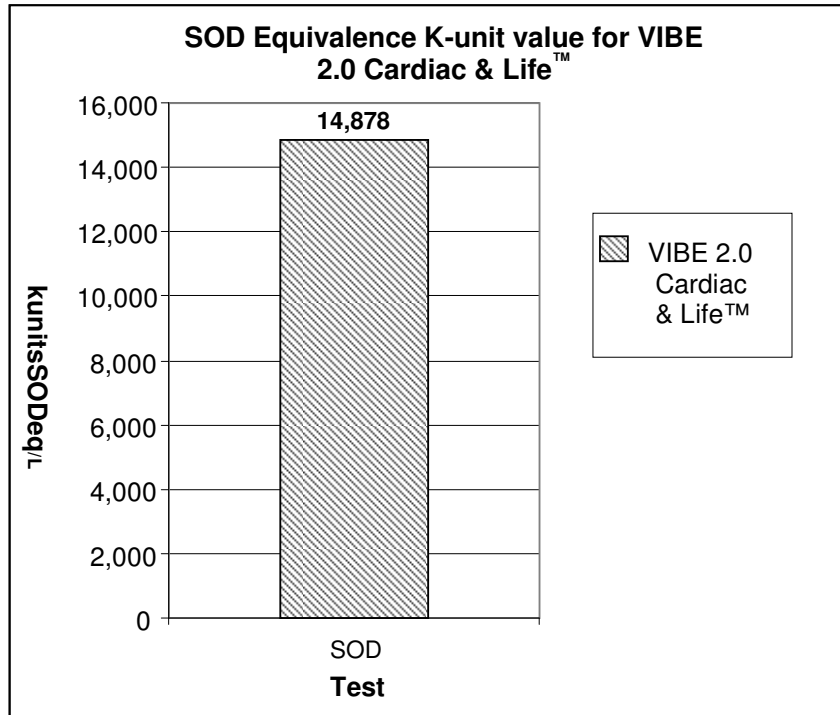


Figure 3: SOD equivalence value for test material



4. Conclusions: Eniva VIBE Cardiac & Life™

1. The test material demonstrated significant broad *in vitro* antioxidant activity against all free radicals generated.
2. The *in vitro* free radical species tested against represent major free radicals within the human body known to play a role in aging and several degenerative conditions.
3. The broad antioxidant activity is likely related to the multi-ingredient formulation of essential nutrients and additional phytonutrients, versus a single nutrient alone.

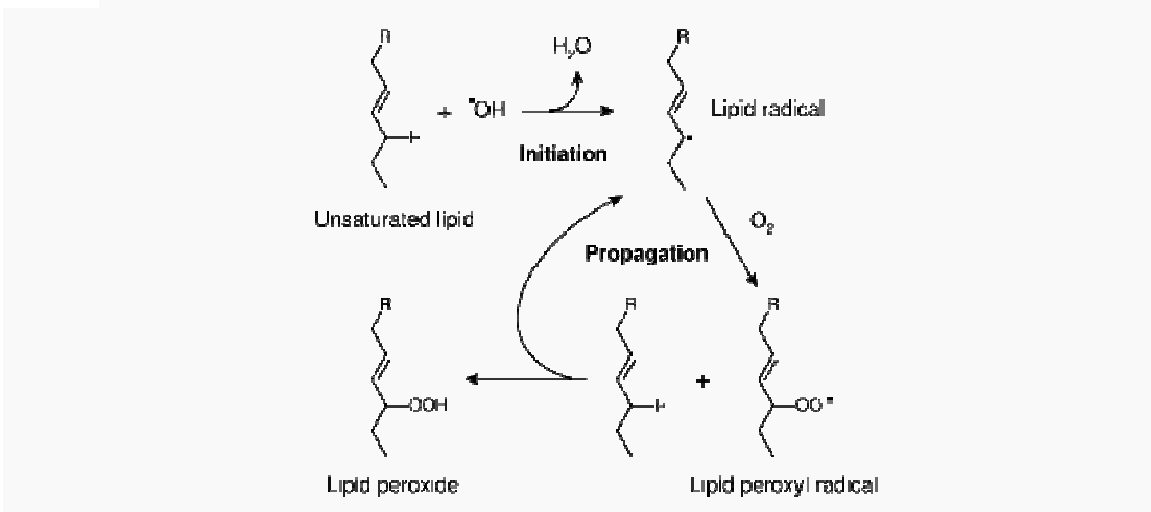
5. Discussion:

With the attention paid to antioxidants in recent research reports and the common press, consumers are seeking products which contain antioxidant substances. Unfortunately, many of these products do not offer any form of validated testing as to the strength of the antioxidants present, nor to their activity against various forms of free radicals which are found within the human body. While *in vitro* testing does not always equate to *in vivo* activity, it is argued here that manufacturers of dietary supplements should, at a minimum, be testing their products for *in vitro* antioxidant activity and making this data readily accessible to consumers. However, this is currently not the standard of practice for the nutritional marketplace industry.

The data presented here suggests broad antioxidant capacity against a variety of free radical species, including the peroxy (water and lipid soluble), hydroxyl, peroxynitrite, singlet oxygen and ferric free radicals. This broad spectrum of activity is likely related to the multi-nutrient formulation of essential nutrients, fruit, vegetable and aloe vera gel concentrates, as well as the high content green tea EGCG catechin complex.

It is well known that within the biologic system of the human body there exists the presence of various forms of ROS free radicals. The majority of these are generated via metabolic mechanisms. In examining the results, the highest antioxidant activity was seen against the peroxy radical, a key ROS that is well known to propagate cellular membrane and lipid damage (see Pictoral 1).

Pictoral 1: Initiation and propagation of lipid peroxidation



As well, ingested substances can also contribute to the generation of free radicals. One such substance is iron, which in its electrically unbalanced forms is a known pro-oxidant and contributor to the atherosclerotic process through an iron-catalyzed free-radical-mediated oxidative stress mechanism. Of interest in these test results are the test formulation's demonstrated activity against this ferric ion. Additionally, the formulation showed activity against singlet oxygen. This would imply superoxide dismutase enzyme like activity, a key human antioxidant defense enzyme. These two specific results are intriguing because of the relation to specific human health issues. It should also be noted the formulation tested does not possess mega-dosing of any RDA essential nutrient and the Vitamin E and Vitamin C content of both formulations were at 30 International Units and 120 milligrams, respectively..

While sole *in vitro* ORAC and similar antioxidant activity testing values cannot be extrapolated to bioavailability and absorption, they do provide verification of antioxidant activity- a likely first step in the process. In an era where there is often much hype and advertising around antioxidant supplement products, steps must be taken to provide the consumer with data that, at a minimum, offers some degree of validation to the claim of "antioxidant" formulation.

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