IN VITRO ANTIOXIDANT AND TOXICOLOGICAL EVALUATION OF HERBAL HEALTH SUPPLEMENTS, CLAIMED AS ANTIOXIDANT BOOSTERS, FROM LOCAL MARKETS OF LAHORE, PAKISTAN

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ABSTRACT: Our present work was aimed to evaluate in vitro ant-ioxidant potential and cytotoxicity of few locally synthesised herbal health supplements, claimed as antioxidant boosters. Six different samples were collected from famous traditional herbal medicine shops of Lahore, Pakistan. Antioxidant activities of all these synthesized Health supplements were determined by 2,2--diphenyl-1-picrylhydrazyl method (DPPH), TAC antioxidant assay, Hydrogen peroxide (H_2O_2) assay, and Anti-lipid peroxidation, LPO method. Toxicity study of the Health supplements was also evaluated by Brine Shrimps Toxicity Bioassay (BSLT). Although all the samples are claimed as "Antioxidant boosters" but percentage antioxidant index (%AI) of all the samples was not the same. Supplement Hs5 showed maximum antioxidant activity. The decreasing order of activity of the supplements was: Hs5 (72%) > Hs4 (63%) > Hs2 (42%) > Hs6 (33%) > Hs3 (25%) > Hs1 (21%). Further these supplements showed remarkable variations in their antioxidant potential when samples were analyzed by different assays. All these health supplements did not show significant toxicity at test concentration. Our results reveals that local health supplements are potential source of natural antioxidants and are safe to use at test concentration. This may also justify the use of these supplements for the cure of oxidative stress.

Key words: Health supplements, Antioxidants, Cytotoxicity, oxidative stress.

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INTRODUCTION

Oxidative stress and related diseases is a rising global problem (Sharifi-Rad *et al.,* 2020 and Sies, H. 2020). Reactive Oxygen Species included Oxygen (O_2), superoxide (O_2^-), hydroxide (OH^-), peroxide (H_2O_2)**,** Hydroperoxy radical (HOO^-), Nitric oxide (NO^-), Peroxynitrite ($ONOO^-$) and Lipid peroxide (ROO^-). They are continuously generated by oxidative products of biological reactions or exogenous factors (Jakubezyk *et al.,* 2020 and Jamshidi-Kia *et al.,* 2020). Under normal conditions there is a natural mechanism of endogenous antioxidants to neutralize these free radicals (Lobo *et al*., 2010). Some exogenous antioxidants namely vitamin E, Vitamin C and Vitamin A are not synthesised inside our body and thus are necessary to be supplied through diet and dietary supplements (Chung 2004). An imbalance between the generation and neutralization of free radicals by antioxidants results in Oxidative stress. Several chronic diseases such as cancer, diabetes, arthritis, Alzheimer, sperm mortality cardiovascular disease, , blindness premature aging and inflammation of bones are related to oxidative stress. (Garcia-Sanchez *et al*., 2020 and Luo *et al*., 2020 and Sarrafehi *et al.,* 2016 and Chandran *et al.,* 2020). We must take seasonal Fruits and fresh vegetables for these exogenous antioxidants. Several researches prove the effectiveness of antioxidant rich diets as a cure against these diseases (Chen *et al*., 2016 and Hrelia, *at al*., 2020). Worldwide, a large number of companies are now selling health supplements with a claim that their supplements serve as an alternative to natural foods (Jerome-Morais *et al*., 2011, Kasote *et al*., 2015). It is believed that these health supplements enhance dietry nutritional value. These supplements are formulated as a single component or a mixture of different components extracted from natural products. Mostly plants are the key source of these products. (Rahmatullah *et al*., 2012 and Hassan *et al*., 2017 and Hosseinzadeh *et al*., 2015). God,s pharmacy is amazing. Plants contain a lot of bioactive constituants such as, vitamins, amino acids, minerals, fibers, metabolites and fatty acids. Nature has made them a source of essential nutrients required to reduce disease risks (Jamous *et al*., 2018). They enhance the ability of the immune system, reduce inflammation, act directly against microorganisms, reduce aging process, prevent cardiovascular diseases and cance or and reduce the factors affecting the individual,s health (Krishnaiah *et al*., 2011 and Gulumian *et al*., 2018 and Paur *et al*., 2011). These health supplements are available in market as herbal teas, capsules, fluid extracts and tablets. Multivitamins are the most popular health supplements. The people, not taking a balanced diet may suffer . ***"IT IS DIFFICULT TO EAT AN APPLE BUT EASY TO TAKE A PILL"***. Presently, about five billion people are herbal products users around the world, especially in

developing countries. Most of people are poor and prefer them due to their availablility at affordable price. They believe in them as acceptable cure (Liang *et al*., 2020 and Unuofin *et al.,* 2020 and Tulewiez-Magulska *at al*., 2019). Many herbal products has not been scientifically studied. It was found later that some of them may cause toxicity (Jerome *et al*., 2011). For safty purposes there is always a requirement of clinical and scientific studies on these herbal products. Since a large number of third world population especially, from Africa and Asia, still believe and rely on their traditional medicine, therefore many local pharmaceutical companies tried to capture their buyers by selling the traditional health supplements with a label or slogan of Antioxidant Boosters.We carried out antioxidant as well as cytotoxicity analysis of some local health supplements which are claimed as antioxidant boosters. Literature proves the use of antioxidant rich diets as a cure of diseases caused by oxidative stress. (Batool *et al*., 2019, and Davalos *et al.,* 2003 and de Siqueira *at al.,* 2018 and Gulumian *et al*., 2018). Oxidation is not a simple process. (Hassan *et al.,* 2020 and Riaz *et al*., 2017 and Wakkumbura *et al.,* 2020). Thus, we can not correctly assess the total antioxidant capacity by using a single method (Moon *et al*., 2009). The selection of method depends on the type of function we want to evaluate. we carried out different antioxidant assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), TAC antioxidant assay, Hydrogen peroxide (H_2O_2) assay, and Anti-lipid peroxidation, LPO method. In vitro toxicity testing is commonly carried out using Brine shrimps (Artemia) (Lewan *et al*., 1992). Several toxic products such as fungal toxins, antifouling biocides, leachates and dental materials are usually tested by using Artemia species (Ayaz *et al.* 2016 and Couladis 2002 and Hamidi *et al*., 2014 and Milhem, *et al*., 2008 and Rahmatullah *et al*., 2010). These species are frequently practiced in laboratory testing due to many advantages such as commercial availability of eggs, low cost, easy to culture, no feeding required during the assay, lot of nauplii production within hours and short life cycle.

MATERIALS AND METHODS

Chemicals: Analytical grade (MERK / BDH) chemicals were used. Brine shrimps eggs were purchased from an aquarium store in Samanabad, Lahore. Solutions were made in double distilled (DD) water.

Collection and Sample preparation: Some famous traditional herbal medicine shops of Lahore, Pakistan were surveyed for six different commercially available health supplements (drinks, tablets, pills and capsules). Samples were labeled as Hs1, Hs2, Hs3, Hs4, Hs5 and Hs6. All samples were claimed to be effective against oxidative stress related diseases and are sold with the labels of : anti- cancer, anti- heart, anti- aging, protection of immune system, anti-inflammatory (effect of bones) and anti-fat. We grinded the tablets and pills into powder form. Double distilled water was used to make stock solution of each sample. Further dilutions were prepared from the stock solution to perform different Assays (Dávalos *et al*., 2003, Prior *et al.,* 2005).

A-Antioxidant Assays

i- 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay: We used Blois method (1958) with a little modifications. In a vial 0.5 ml solution of each sample was taken. Then 2.5 ml of the DPPH solution (0.1mM) was mixed. Mixture was incubated for 30 minutes at 37 ^oC. The color change was observed from violet to yellow. Measured the absorbance at 517nm with Spectrophotometer (UV-VIS). Distiled water is taken as blank refrence. %age antioxidant Index was calculated.

ii– Hydrogen peroxide (H_2O_2) scavenging activity assay: Ruch *et al* (1989) method was performed with little modifications. In a vial 2.5ml solution (2mg/ml) of each sample was taken. Then 0.5ml solution (2mM) of H_2O_2 (pH 7.4) was mixed. Mixture was incubated at 25^oC for 15 minutes. Measured the absorbance at 230nm with Spectrophotometer (UV-VIS). Phosphate buffer is taken as blank refrence. The %age of Hydrogen peroxide scavenged was calculated.

iii-Total antioxidant capacity (TAC) Assay: Prieto *et al* (1999) method was used with little modifications. In a vial 0.5ml of each sample (100µg/ml) and 4ml of the reagent [$(NH_4)_6Mo_7O_{24}$ (4Mm) + H_2SO_4 (0.6M) + K_3PO_4 (26mM)] were mixed. Mixture was incubated at 95°C for 90 minutes. Measured the absorbance at 734nm with Spectrophotometer (UV-VIS). Distilled water is taken as blank refrence Percentage antioxidant Index was calculated.

iv-Anti-lipid peroxidation (ALP) Assay: Halliwell and Guttridge (1989) method was used with little modifications. In a vial 0.5ml of the sample (5mg/ml) was mixed with 0.5ml of of $FeCl_3$ (6 mM). Added 1ml of egg yolk (20%) and 1 ml of KCl reagent (1.15%). Using a water bath, mixture was incubated at 37°C for 90 minutes. Same procedure was performed for blank simultaneously. Then added 2.0 ml ice cold reagent [0.25N HCl + ((15%) TCA+ (0.38%) TBA + (0.5%) BHT)] to the mixture. 2.0 ml of this reagent, without TBA was added in blank. Heated again using water bath for 60 minutes at 80°C. Cooled and centrifuged at 3000rpm for 20 minutes. Pink colored product is formed. Supernatan was separated. Measured the absorbance at 532nm with Spectrophotometer (UV-VIS). Same experiment was repeated the with 10mg/ml of sample solution. Percentage lipid peroxidation Index was calculated.

B-Cytotoxicity testing against Brine shrimps lethality Assay (BSLT): A 1000ppm stock solution was prepared for each sample by dissolving 2mg of the sample in 1ml of double distilled water. Different concentrations of 900ppm, 800ppm, 700ppm, 600ppm, 500ppm, 400ppm, 300ppm, 200ppm were prepared from stock solution. In each vial took 2m lof each concentration. Then 10 shrimps with 2 ml of sea water were added. For negative control took 2 mL of artificial seawater and 10 shrimp larvae in DMSO. For positive control only distilled water is used with shrimps and sea water. Left these vials for overnight. After 12 hrs. 24 hrs. and 48 hrs with the help of magnifying glass counted the number of shrimps deaths in each vial. Calculated half the Lethal concentration (LC50).

RESULTS

Table 1, shows the composition (in mg/l) of components of each Health supplement as printed on their labels. These components include different parts of local medicinal plants (seeds, flowers, bark, leaves and roots) along with micronutrients. Literature shows antioxidant potential and related benefits of these plants as they have natural bioactive components such as Essential oils, phenolic compounds, flavonoids, flavones, fatty acids and polyacetylenes. **Fig 2,** shows the results for the comparison of different assays (DPPH, TAC, H_2O_2 scavenging activity, LPO) performed, to identify the percentage antioxidant potentials. These are economic, valid, easy and accurate methods. Although all the samples are antioxidant rich but free radical scavenging capacity showed results that did not agreed with the company claims. Supplement Hs5 gives highest anti-oxidant potential in DPPH protocol. It has % age AI potential of 72 %. The results of other supplements are lower to it. The order of %age AI is : Hs5 (72%) > Hs4 (63%) > Hs2 (42%) > Hs6 (33%) > Hs3 (25%) > Hs1 (21%). **Fig 3,** shows similar trends in IC50 values. Sample Hs5 shows this value at very low concentration. The IC50 value represents the amount of substance required to inhibit the proceeding assay (DPPH) by half. Surpisingly many variations can be seen in our results. Each supplement, when performed using different assays, does not show the same antioxidant potential. Supplement Hs1 shows the minimum antioxidant potential in DPPH assay and maximum in TAC. Reactivity order of Hs1 for performed assays is, TAC (55%) > H_2O_2 (38%) > LPO (27%) > DPPH (21%). Supplement Hs2 has minimum antioxidant potential in LPO assay and maximum in TAC. Reactivity order of Hs2 for performed assays is, TAC (60%) > DPPH (44%) > H_2O_2 (41%) > LPO (26%). Supplement Hs3 has minimum antioxidant potential in DPPH assay and maximum in TAC. Reactivity order of Hs3 for performed assays is, TAC(69%) > H_2O_2 (42%) > LPO (28%) > DPPH (22%). Supplement Hs4 has minimum antioxidant potential in LPO assay and maximum in DPPH. Reactivity order of Hs4 for performed assays is, DPPH (62%) > H_2O_2 (43%) >TAC (37%) > LPO (23%)**.** Supplement Hs5 has minimum antioxidant potential in

Table 1. Medicinal Composition of Health supplements.

Sr. No.	Health Supplement	Medicinal Composition	mg/L
1	**Hs1**	Felon herb	54.14
		Common fumitory	43.23
		Common clubmoss	47.77
		Chinaberry	24.62
		Asteraceae	34.14
		Chiretta	33.66
		Wild indigo	28.94
		Chebulic myrobalan	45.78
		Heart-leaved moonseed	56.25
2	**Hs2**	Sweet Fennel	37.62
		P- ajowan	52.22
		Black cumin	16.69
		Common Rue	37,17
		Mineral water	-
3	**Hs3**	Coriandrum-S	31.56
		Sandalwood	32.52
		S- bezoar	31.56
		Agate-Kushta (processed)	23.76
		Calcium- Coral	42.17
		(processed)	16.78
		Calcium- Oyster Shell	21.78
		(processed)	52.23
		Elaichi-seeds	
		Cardamom	
4	**Hs4**	Chaff-flower	31.28
		Mormon-tea	12.65
		Opium Poppy	10.11
		Licorice-root	12.85
		Malabar nut	37.25
		Wax tree-Leaves	22.75
		Mint,-Exract.	44.85
		Preservatives	07.06
		Wild sugarcane	12.24
5	**Hs5**	Kiker-pods	72.46
		Vegetable rennet	66.43
		Nux vomica	12.06
		Calcium-Coral	28.15
		(processed)	
6	**Hs6**	Sweet fennel	39.62
		P- ajowan	33.52
		Cumin seeds	39.67
		Common rue	37.62
		Silk serum-Laksha	23.45
		Indian Bay leef	18.23
		Excepients	09.41

LPO and maximum in DPPH. Reactivity order of Hs5 for performed assays is, DPPH (71%) > TAC (43%) > H_2O_2 (34%) > LPO (23%). Sample Hs6 shows minimum antioxidant activity in H_2O_2 and maximum in TAC assay. Reactivity order of Hs6 for performed assays is, TAC (52%) > DPPH (31%) > LPO (29%) > H_2O_2 (22%). Higher concentrations of bioactive compounds is always toxic (1000ppm to 10,000ppm). **Fig 4,** shows LC50 value of the brine shrimps lethality bioassay (BSLT). Percentage mortality of shrimps is only 3% in positive control i.e distilled water. While 96% of the brine shrimps are died in DMSO i.e a negative control. Literature proved the toxic effects of DMSO (Mclaughlin *et al.,* 1998).

DISCUSSION

Our results does not show the significant antioxidant potential for two tested health supplements i.e Hs1 and Hs3. Antagonism may be one of the reason of their lower activity (Caesar *et al*., 2019 and Izzo *et al*., 2020). This is a phenomen which decreases the rate of oxidation of food components when formulated in combinations. So antioxidant activity of these health supplements may not agree with their company claims. Mostly, health supplements are sold in the form of mixture and not as a single component. These components may reduce the antioxidant potential of Health supplement, if interfere with each other. (Liu *et al*., 2003). On the other hand Sample Hs2, Hs4, Hs5 and Hs6 show good antioxidant activity even, they are combinations of different ingredients too. Synergism may be one of the reason of this effect. This is a phenomen which enhances the rate of oxidation of food components when formulated in combinations (Wang *et al*., 2011 and Yarnell *et al*., 2015 and Mundy *et al*., 2016). So components in these health supplements may not interfere with each other (Zhao *et al*., 2020 and Bhuyan *et al*., 2020). The brine shrimp lethality bioassay (BSLT) is crried out for cytotoxic evaluation of the Health supplements. This frequently used protocol evaluate the toxicity in biomaterials, organic compounds and dental materials (Milhem *et al*., 2008 and Ullah *et al*., 2013). Results of our performed protocols were found according to literature (Yadav *et al.,* 2020). At high concentration all the Health supplements were toxic. The lethality effect was almost nil at lower concentration. To best of our knowledge, for comparing the results of our present analysis no earlier reports are available. So this may be the first report to show the radical scavenging activity of these Health supplements. Based on the possible relationship between Health supplements bioactivity and brine shrimp lethality, we suggested that our contribution may serve for further ethnobotanical and phytochemical research.

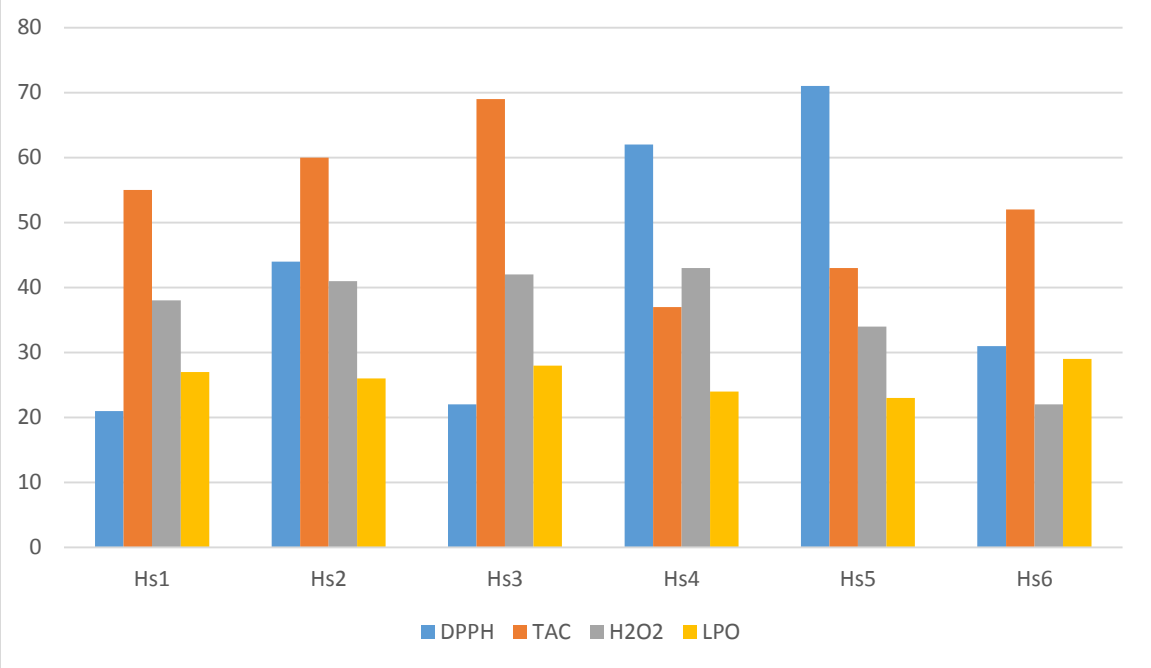

Figure 2- Comparison of %age AI of health supplements in performed antioxidant assays (DPPH, TAC, H_2O_2, LPO)

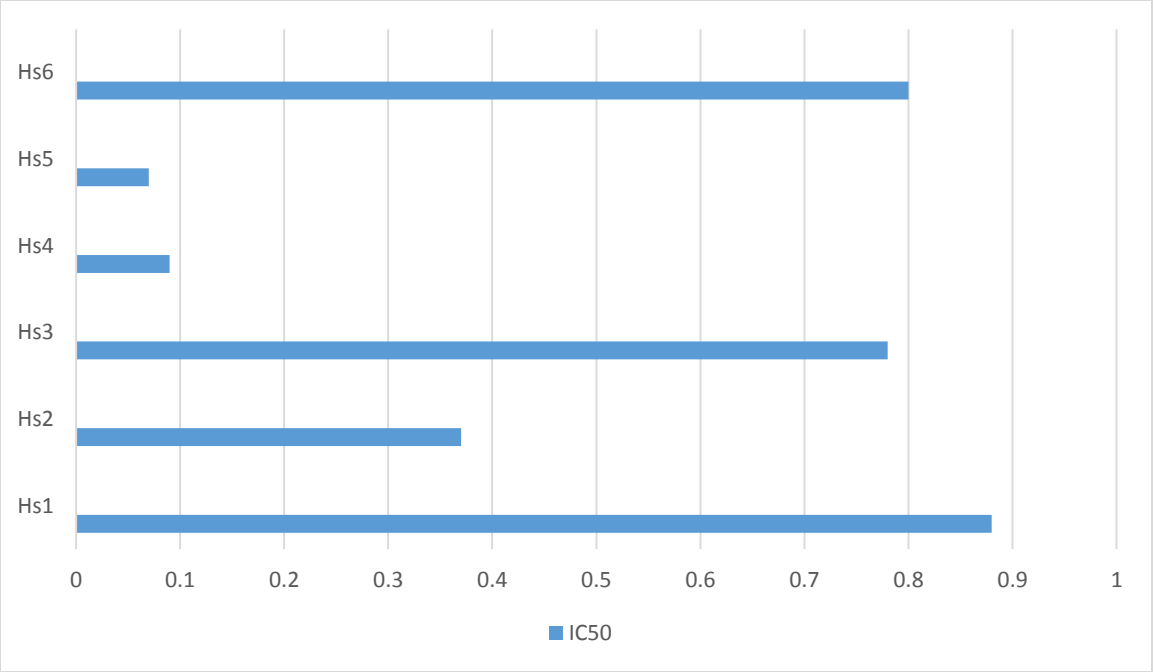

Figure 3- Comparison of IC 50 values of health supplements using DPPH assay

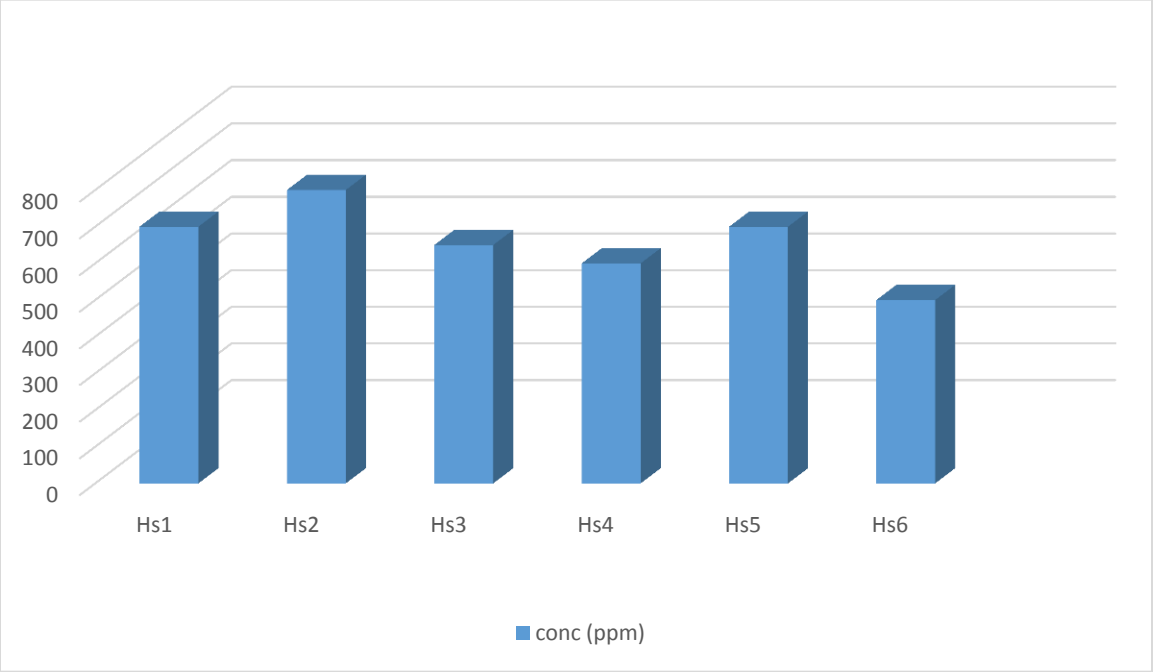

Figure 4- Comparison of LC 50 values of health supplements in Brine Shrimps Lethaliy bioassay (BSLT)

Conclusion: It is concluded in the end that our selected Health supplements showed good antioxidant potential when analysed with DPPH, H_2O_2, TAC and LPO protocols. Further, toxicity testing of these products reveals that these health supplements are save to take at test concentration.

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REFERENCES

Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Subhan, F., Khan, M. A., & Ahmad, S. (2016). Molecularly characterized solvent extracts and saponins from Polygonum hydropiper L. show high anti-angiogenic, anti-tumor, brine shrimp, and fibroblast NIH/3T3 cell line cytotoxicity. *Frontiers in pharmacology*, *7*, 74.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200.

Bhuyan, D. J., Perera, S., Kaur, K., Alsherbiny, M. A., Low, M., Seto, S. W., & Zhou, X. (2020). Synergistic Effects of Chinese Herbal Medicine and Biological Networks. In *Approaching Complex Diseases* (pp. 393-436). Springer, Cham.

Batool, R., Khan, M. R., Sajid, M., Ali, S., & Zahra, Z. (2019). Estimation of phytochemical constituents and in vitro antioxidant potencies of Brachychiton populneus (Schott & Endl.) R. Br. *BMC chemistry*, *13*(1), 32.

Chen, W., Jia, Z., Pan, M. H., & Anandh Babu, P. V. (2016). Natural products for the prevention of oxidative stress-related diseases: Mechanisms and strategies.

Couladis, M., Badisa, R. B., Baziou, P., Chaudhuri, S. K., Pilarinou, E., Verykokidou, E., & Harvala, C. (2002). Antioxidant and cytotoxic activities of Hypericum sp. on brine shrimps and human cancer cell lines. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *16*(8), 719-722.

Caesar, L. K., & Cech, N. B. (2019). Synergy and antagonism in natural product extracts: when 1+ 1 does not equal 2. *Natural product reports*, *36*(6), 869-888.

Chung, M. K. (2004). Vitamins, supplements, herbal medicines, and arrhythmias. *Cardiology in Review*, *12*(2), 73-84.

Chandran, R., & Abrahamse, H. (2020). Identifying plant-based natural medicine against oxidative stress and neurodegenerative disorders. *Oxidative Medicine and Cellular Longevity*, *2020*.

Dávalos, A., Gómez-Cordovés, C., & Bartolomé, B. (2003). Commercial dietary antioxidant supplements assayed for their antioxidant activity by different methodologies. *Journal of agricultural and food chemistry*, *51*(9), 2512-2519.

de Siqueira Leite, K. C., Garcia, L. F., Lobón, G. S., Thomaz, D. V., Moreno, E. K. G., de Carvalho, M. F., ... & de Souza Gil, E. (2018). Antioxidant activity evaluation of dried herbal extracts: an electroanalytical approach. *Revista Brasileira de Farmacognosia*, *28*(3), 325-332.

de Menezes Epifanio, N. M., Cavalcanti, L. R. I., dos Santos, K. F., Duarte, P. S. C., Kachlicki, P., Ożarowski, M., ... & de Almeida Chaves, D. S. (2020). Chemical characterization and in vivo antioxidant activity of parsley (Petroselinum crispum) aqueous extract. *Food & Function*.

Gulumian, M., Yahaya, E. S., & Steenkamp, V. (2018). African herbal remedies with antioxidant activity: A potential resource base for wound treatment. *Evidence-Based Complementary and Alternative Medicine*, *2018*.

García-Sánchez, A., Miranda-Díaz, A. G., & Cardona-Muñoz, E. G. (2020). The role of oxidative stress in physiopathology and pharmacological treatment with pro-and antioxidant properties in chronic diseases. *Oxidative Medicine and Cellular Longevity*, *2020*.

Halliwell, B. (1989). Protection against oxidants in biological systems. The superoxide theory of oxygen toxicity. *Free radicals in biology and medicine*.

Hassan, A., Akmal, Z., & Khan, N. (2020). The Phytochemical Screening and Antioxidants Potential of Schoenoplectus triqueter L. Palla. *Journal of Chemistry*, *2020*.

Hrelia, S., & Angeloni, C. (2020). New Mechanisms of Action of Natural Antioxidants in Health and Disease.344

Hassan, W., Noreen, H., Rehman, S., Gul, S., Amjad Kamal, M., Paul Kamdem, J., & BT da Rocha, J. (2017). Oxidative stress and antioxidant potential of one hundred medicinal plants. *Current topics in medicinal chemistry*, *17*(12), 1336-1370.

Hosseinzadeh, S., Jafarikukhdan, A., Hosseini, A., & Armand, R. (2015). The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris. *International Journal of Clinical Medicine*, *6*(09), 635.

Hamidi, M. R., Jovanova, B., & Panovska, T. K. (2014). Toxicological evaluation of the plant products using Brine Shrimp (Artemia salina L.) model. *Maced pharm bull*, *60*(1), 9-18.

Izzo, A. A., Di Carlo, G., Borrelli, F., & Ernst, E. (2005). Cardiovascular pharmacotherapy and herbal medicines: the risk of drug interaction. *International journal of cardiology*, *98*(1), 1-14.

Jakubczyk, K., Dec, K., Kałduńska, J., Kawczuga, D., Kochman, J., & Janda, K. (2020). Reactive oxygen species-sources, functions, oxidative damage. *Polski merkuriusz lekarski: organ Polskiego Towarzystwa Lekarskiego*, *48*(284), 124.

Jerome-Morais, A., Diamond, A. M., & Wright, M. E. (2011). Dietary supplements and human health: for better or for worse?. *Molecular nutrition & food research*, *55*(1), 122-135.

Jamous, R. M., Abu-Zaitoun, S. Y., Akkawi, R. J., & Ali-Shtayeh, M. S. (2018). Antiobesity and antioxidant potentials of selected palestinian

medicinal plants. *Evidence-Based Complementary and Alternative Medicine*, *2018*.

Jamshidi-Kia, F., Wibowo, J. P., Elachouri, M., Masumi, R., Salehifard-Jouneghani, A., Abolhasanzadeh, Z., & Lorigooini, Z. (2020). Battle between plants as antioxidants with free radicals in human body. *Journal of Herbmed Pharmacology*, *9*(3), 191-199.

Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International journal of biological sciences*, *11*(8), 982.

Krishnaiah, D., Sarbatly, R., & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and bioproducts processing*, *89*(3), 217-233.

Luo, J., Mills, K., le Cessie, S., Noordam, R., & van Heemst, D. (2020). Ageing, age-related diseases and oxidative stress: What to do next?. *Ageing research reviews*, *57*, 100982.

Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews*, *4*(8), 118.

Liang, B., Zhou, Y., Fu, L., & Liao, H. L. (2020). Antiarrhythmic Mechanisms of Chinese Herbal Medicine Dingji Fumai Decoction. *Evidence-Based Complementary and Alternative Medicine*, *2020*.

Lewan, L., Andersson, M., & Morales-Gomez, P. (1992). The use of Artemia salina in toxicity testing. *Alternatives to Laboratory Animals*, *20*(2), 297-301.

Moon, J. K., & Shibamoto, T. (2009). Antioxidant assays for plant and food components. *Journal of agricultural and food chemistry*, *57*(5), 1655-1666.

Milhem, M. M., Al-Hiyasat, A. S., & Darmani, H. (2008). Toxicity testing of restorative dental materials using brine shrimp larvae (Artemia salina). *Journal of Applied Oral Science*, *16*(4), 297-301.

Mclaughlin, J. L., Rogers, L. L., & Anderson, J. E. (1998). The use of biological assays to evaluate botanicals. *Drug information journal*, *32*(2), 513-524..

Mundy, L., Pendry, B., & Rahman, M. (2016). Antimicrobial resistance and synergy in herbal medicine. *Journal of Herbal Medicine*, *6*(2), 53

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry*, *53*(10), 4290-4302.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, *269*(2), 337-341.

Paur, I., Carlsen, M. H., Halvorsen, B. L., & Blomhoff, R. (2011). Antioxidants in herbs and spices. In *Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition*. CRC Press/Taylor & Francis.

Rahmatullah, M., Azam, N. K., Khatun, Z., Seraj, S., Islam, F., Rahman, A., ... & Aziz, S. (2012). Medicinal plants used for treatment of diabetes by the Marakh sect of the Garo tribe living in Mymensingh district, Bangladesh. *African Journal of Traditional, Complementary and Alternative Medicines*, *9*(3), 380-385.

Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, *10*(6), 1003-1008.

Riaz, M., Shahid, M., Jamil, A., & Saqib, M. (2017). In vitro antioxidant potential of selected aphrodisiac medicinal plants. *Journal of biological regulators and homeostatic agents*, *31*(2), 419.

Rahmatullah, M., Sadeak, S. M. I., Bachar, S. C., Hossain, M. T., Abdullah-al-Mamun, M., Jahan, N., ... & Rahman, S. (2010). Brine shrimp toxicity study of different Bangladeshi medicinal plants. *Advances in Natural and Applied Sciences*, *4*(2), 163-173.

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. *Frontiers in physiology*, *11*, 694.

Sies, H. (2020). Oxidative stress: Concept and some practical aspects. *Antioxidants*, *9*(9), 852.

Sarrafchi, A., Bahmani, M., Shirzad, H., & Rafieian-Kopaei, M. (2016). Oxidative stress and Parkinson's disease: new hopes in treatment with herbal antioxidants. *Current pharmaceutical design*, *22*(2), 238-246.

Unuofin, J. O., & Lebelo, S. L. (2020). Antioxidant Effects and Mechanisms of Medicinal Plants and Their Bioactive Compounds for the Prevention and Treatment of Type 2 Diabetes: An Updated Review. *Oxidative Medicine and Cellular Longevity*, *2020*.

Ulewicz-Magulska, B., & Wesolowski, M. (2019). Total phenolic contents and antioxidant potential of

herbs used for medical and culinary purposes. *Plant Foods for Human Nutrition*, *74*(1), 61-67.

Ullah, M. O., Haque, M., Urmi, K. F., Zulfiker, A. H. M., Anita, E. S., Begum, M., & Hamid, K. (2013). Anti–bacterial activity and brine shrimp lethality bioassay of methanolic extracts of fourteen different edible vegetables from Bangladesh. *Asian Pacific journal of tropical biomedicine*, *3*(1), 1-7.

Wang, S., Meckling, K. A., Marcone, M. F., Kakuda, Y., & Tsao, R. (2011). Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities. *Journal of agricultural and food chemistry*, *59*(3), 960-968.

Wakkumbura, H. P., Wickramaarachchi, W. M. D., Arawwawala, L. D. A. M., Liyanage, J. A., & Rajapakse, R. P. V. J. (2020). Assessment of the Quality and Evaluation of the Antioxidant Potential of a Novel Sri Lankan Ayurvedic Polyherbal Formulation. *Evidence-Based Complementary and Alternative Medicine*, *2020*.

Williamson, E. M. (2001). Synergy and other interactions in phytomedicines. *Phytomedicine*, *8*(5), 401-409.-58.

Yarnell, E. (2015). Synergy in Herbal Medicines. *Journal of Restorative Medicine*, *4*(1), 60-73.

Yadav, A. R., & Mohite, S. K. (2020). Toxicological Evaluation of Psidium guajava Leaf Extracts using Brine Shrimp (Artemia salina L.) Model. *Research Journal of Pharmaceutical Dosage Forms and Technology*, *12*(4), 258-260.

Zhao, Q., Luan, X., Zheng, M., Tian, X. H., Zhao, J., Zhang, W. D., & Ma, B. L. (2020). Synergistic Mechanisms of Constituents in Herbal Extracts during Intestinal Absorption: Focus on Natural Occurring Nanoparticles. *Pharmaceutics*, *12*(2), 128.