

# A Sustainable analysis of Comparative Genomics to study of Antioxidant Compound

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**Abstract**— Neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease and Huntington's disease, present in a major health issue and the financial load for the health care systems and around the world. The impact of these the diseases will be further increase and decrease over the next decades caused by the increasing life expectancies of the health. No cure is currently available for the treatments of the these conditions; only drugs, which are merely alleviate the symptoms. Oxidative stress has been lengthy been associated with a neuro degeneration, whether as a cause or as part of the downstream and upstream results caused by the other factors and other effects. Thus, the use of the antioxidants to the counter cellulars and a oxidative stress and antioxidant within the nervous system has been suggested as a potential treatments option for the neurological disorders. Over the last decade, it has to be significant research has to be the found to be most and focused on the potential use of natural antioxidants to be target oxidative stress. However, clinical trial results have been lacked success for the treatment of the patients with neurological disorders. The knowledge that natural extracts shows other positive molecular activities in addition to the antioxidant activity, however, has led to further research of natural extracts for their potential use as well as prevention or treatment/management of the neurodegenerative diseases. The diversity of the wild mushrooms was a investigated from two protected forest areas in India and 232 mushroom specimens were morphologically identified. Among them, 77 isolates were screened for the antimicrobial potential against seven and bacterial and fungal pathogens. Out of the 77 isolates, 46 isolates which displayed significant antimicrobial activities were identified using ITS rRNA gene amplification and subsequently phylogenetically characterized using a random amplified polymorphic DNA (RAPD) and inter-simple sequences repeat (ISSR) markers. Sequencing of the ITS rRNA regions classified the isolates into 16 genera belonging to the 11 families. In total, 11 RAPD and 10 ISSR primers were selected to be the evaluate genetic diversity based on the their banding profile produced. In total 337 RAPD and 312 ISSR bands were to be detected, among which is percentage of the polymorphism ranges from 35.2% to 77.8% and 37.6% to 93.4% by using RAPD and ISSR primers respectively. The report has to be also demonstrated that both RAPD and ISSR could be efficiently differentiate wild mushrooms and it could thus be the considered as the efficient markers for the surveying genetic diversity. Additionally, selected six or more then six wild edible mushroom strains (*Schizophyllum commune* BPSM01, *Panusgiganteus* BPSM27, *Pleurotus* sp. BPSM34, *Lentinus* sp. BPSM37, *Pleurotus* sp. BPSM41 and *Lentinula* sp. BPSM45) were a analyzed for the their nutritional (proteins, carbohydrates, fat and ash content), antioxidant potential.

**Keywords**— antioxidants, natural products, in vitro, in vivo, clinical trials, plant extracts, photochemical, phenolics, *Ginkgo balboa*, secondary metabolites.

## I. INTRODUCTION

This review aims to give a refinement on the importance of the oxidative stress and its relevance in the neurodegenerative disease. One or more option for the counteracting of the oxidative stress in the application of the natural and artificial products get from plant extracts. And These have been thoroughly tested in vitro (chemical antioxidant activity and cell systems) and in vivo (animal disease models) and have been visible in promising results. However, results from clinical trials studies have been less than successful and other successful. Here, recent research on the natural and artificial extracts, and their a potential pitfalls in the clinical trials, are discussed in the review. Clinical trials have been demonstrated that a herb consumption is to be inversely related to the incidence of the cardiovascular diseases (Afshin, Michal, Khatibzadeh, & Mozaffarian, 2014; Belski et al., 2011; Hermsdorff, Zulet, Abate, & Martínez, 2010). These health benefits are partially attributed to the attenuation of the oxidative stress by the antioxidant components, and then the which exert an array of the cellular pursuit (Wang, Melnyk, Tsao, & Marcone, 2011). Oxidative stress has been established as the major factors in the development of a wide range of the cardiovascular diseases including hypertension (Siti, Kamisah, & Kamsiah, 2015). Dietary antioxidants are able to attenuate the oxidative stress and counteract to the onset and progression of the cardiovascular diseases. With a human and rat cardiomyocytes, we have proven that crude lentil phenol extract is able to attenuate angiotensin II-induced cardiomyocyte hypertrophy and to reduce intracellular reactive oxygen species (ROS) levels (Yao, Sun, & Chang, 2010). In animal studies, we have demonstrated that administration of the crude lentil phenol extract could substantially reduce aorta ROS level and increase and decrease total phenol content (TPC) and oxygen radical absorbance capacity (ORAC) in artery serums. Meanwhile, significant alleviation of the angiotensin II-induced hypertension, peripheral vascular remodeling and per vascular fibrosis have also been observed (Xuan et al., 2013; Yao, Sun, & Chang, 2012). Amenorrhea is a predominant factor in the development of various cardiovascular diseases and other diseases including atherosclerosis, heart attack and coronary disease and others. With a spontaneously hypertensive rats (SHRs), other researchers also have been proven that phenol extracts from the herb could reduce blood pressure and suppress inflammatory responses, such as intracellular ROS level, over expression of the proinflammatory enzymes including

iROS, COX-1, generation of  $O_2^-$ , as well as NADPH oxidizes (Mukai & Sato, 2009, 2011).

Lentil (*Lens culinaris*), black soybean (*Glycine max*), and black turtle bean (*Phaseolus vulgaris*) are dry legumes or moist legumes, and belong to the three different scientific genera, which are widely used to be cultured in the worlds, and preferred by the different groups of the consumers in different parts of the worlds. Numerous studies have been done proven that lentil, black soybean and black turtle bean have high concentrations of phenolics and potent antioxidants capacity to do (Tan, Chang, & Zhang, 2016; Wang et al., 2016; Xu, Yuan, & Chang, 2007; Zhang, Chang, & Liu, 2015). However, a direct and indirect comparison of the compositions and relative health promotion potential of these phenol rich legume varieties, and then the particularly when they are cooked, it is not available in the literature. Cooking (thermal treatment) is to be the essential for the human consumption since raw and legumes and other contain anti nutritional factors that it will cause illness without heating. In addition, dry legumes are not texturally palatable unless then they are to be soaked and cooked to the softness of the antioxidant.

In addition to the suppression of the oxidative stress, one or more commonly used in the therapeutic approach to the treat hypertension is the inhibition of the angiotensin-I converting enzyme (ACE), which mediates to the formation of the angiotensin II, a vasoconstrictor and the ROS initiator. Various plant extracts and pure phenolics possess ACE inhibitor activity and the ACE inhibition varies a great deal according to their chemical structures (Afonso, Passos, Coimbra, Silva, & Soares-da-Silva, 2013; Al Shukor et al., 2013; Guerrero et al., 2012; Ojeda et al., 2010). However, the ACE inhibition capability of the cooked legumes extract has not been received little study. (Xuan et al., 2013). In addition, phenolics compositions and antioxidant activity of the legumes are to be largely affected by the processing conditions (Haileslassie, Henry, & Tyler, 2016; Xu & Chang, 2009; Zhang & Chang, 2016). It is logical to assume that the processing-induced change in the phenol compositions might affect ACE inhibitor activity, but information regarding the effect of the thermal processing on the ACE inhibition is not available in the antioxidant. Our previous animal study. (Xuan et al., 2013) with rats revealed that the phenol extracts of the cooked lentil showed lower and higher effectiveness than the raw extracts in the attenuation of the angiotensin II-induced blood pressure elevation, peripheral vascular remodeling and per vascular fibrosis disease.

High phenolics content and compositions in the three legume varieties have been reported in the literature. However, results are to be inconsistent or even opposed due to the differences in the extraction methods and equipment employed for the analysis of the antioxidant. Therefore, the objectives of this study were to investigate and compare to

the effects of thermal treatments, purification, and fractionation on the phenolics substances, antioxidant activity, and ACE inhibitions of the three or more legume varieties, and to identify phenolics compounds using UV spectroscopy and LC-MS<sup>n</sup> analysis.

Fig.1: Summary of compounds identified in three legume varieties via LC-MS<sup>n</sup> analysis.

	Compounds	Monoisotopic mass	Lentil	Black soybean	Black turtle bean
Phenolic acids	Gallic acid	170.0215			X
	Protocatechuic acid	154.0266	x	x	
	<i>P</i> -Hydroxybenzoic acid	138.0316	x	x	
	<i>p</i> -Coumaric acid	164.0473	x	x	
	2-Hydroxycinnamic acid	164.0473	#	#	
	3-Hydroxycinnamic acid	164.0473	#	#	
	Vanillic acid	168.0422	x	x	
	Caffeic acid	180.0422	x	x	
	Ferulic acid	194.0579	x	x	X
	Syringaldehyde	182.0579	x		X
	Trans-cinnamic acid	148.0524			X
	Sinapic acid	224.0684	x	x	X
Flavonoids	Kaempferol	286.0477	x	*	X
	Epicatechin	290.0790	x	x	X
	Cyanidin	287.0550	*	*	
	Kaempferol 3-rhamnoside	432.1056	*	*	
	Kaempferol-3-O-rutinoside	594.1585	x	x	X
	Luteolin 7-glucoside	448.1006	*	*	*
	Kaempferol-3-O-glucoside	448.1005	x	x	X
	Kaempferol 3-(6-malonylglucoside)	534.1010	x	x	
	Quercetin-3-O-glucopyranoside	464.0950		x	X
	Myricetin	464.0950			X
	Apigenin	270.0528	x	x	
	Pelargonidin	270.0528		*	
	Genistein	270.0528		x	
	Glycitein	284.0685		x	
	Genistin	432.1057		x	
Condensed tannins	Procyanidin B <sub>1</sub>	578.1425			X
	Procyanidin B <sub>2</sub>	578.1425		x	
	Procyanidin C <sub>1</sub>	866.2058	x	x	

	Compounds	Monoisotopic mass	Lentil	Black soybean	Black turtle bean
Other compounds	Riboflavin	376.1383	x		
	Indole-3-acrylic acid	187.0633			X
	Carvone	150.1045		x	
	Indole-3-acrylic acid	187.0633		x	
	N,N'-Dicyclohexylurea	224.1886	x	x	
	Adenosine	267.0968		x	
	3-Nitro-2-6-dipiperidinopyridine	290.1743			X
	Dioctyl phthalate	390.2770	x		

X indicates confident identification via matching mass spectra of the analyses to the spectra of standards in the in-house, or on-line database (see text for details).

- Indicates compounds that they are identical via mass-spectra matching.
- Indicates tentative identification based only on of the matching of the monoisotopic masses.

Fig.2: Identification of antagonistic wild mushrooms based on ITS rRNA gene sequences.

Isolate No.	Accession number	Closest species with accession number	Similarity	Identification
BPSM01	KJ865831	<i>Schizophyllum</i> sp. (KR155096)	99%	<i>Schizophyllum commune</i>
BPSM02	KJ865832	<i>Trametes hirsuta</i> (KP216914)	99%	<i>Trametes hirsute</i>
BPSM03	KJ865833	<i>Marasmiellus palmivorus</i> (JQ653438)	99%	<i>Marasmiellus palmivorus</i>
BPSM04	KJ865834	<i>Trametes</i> sp. (KP686448)	99%	<i>Trametes elegans</i>
BPSM05	KJ865835	<i>Schizophyllum commune</i> (AB470852)	99%	<i>Schizophyllum commune</i>
BPSM06	KJ865836	<i>Trametes hirsuta</i> (JN048768)	99%	<i>Trametes hirsute</i>
BPSM07	KJ865837	<i>Trametes hirsuta</i> (KC461301)	99%	<i>Trametes hirsute</i>
BPSM08	KJ865838	<i>Trametes hirsuta</i> (KP216887)	99%	<i>Trametes hirsute</i>
BPSM09	KJ865839	<i>Pholiotalimonella</i> (KM496470)	98%	<i>Pholiotaadiposa</i>
BPSM10	KJ865840	<i>Pleurotus</i> sp. (KJ670292)	99%	<i>Pleurotus pulmonarius</i>
BPSM11	KJ865841	<i>Fomitopsis</i> sp. (KC595913)	99%	<i>Fomitopsis</i> sp.
BPSM13	KJ865843	<i>Marasmiellus palmivorus</i> (JQ653437)	99%	<i>Marasmiellus palmivorus</i>
BPSM14	KM985651	<i>Auriculariapolytricha</i> (FJ617294)	100%	<i>Auriculariapolytricha</i>
BPSM16	KM985653	<i>Bjerkandera</i> sp. (KM099498)	100%	<i>Bjerkanderaadusta</i>
BPSM17	KM985654	<i>Hymenopellischiangmaiae</i> (GU980131)	100%	<i>Hymenopellischiangmaiae</i>
BPSM18	KM985655	<i>Xylaria</i> sp. (JQ862668)	100%	<i>Xylaria</i> sp.
BPSM19	KM985656	<i>Bjerkanderaadusta</i> (KJ831843)	100%	<i>Bjerkanderaadusta</i>
BPSM20	KM985657	<i>Polyporus</i> sp. (AJ542518)	100%	<i>Polyporus</i> sp.
BPSM21	KM985658	<i>Xylaria</i> sp. (KP263113)	99%	<i>Xylaria</i> sp.
BPSM22	KM985659	<i>Auriculariapolytricha</i> (FJ617295)	99%	<i>Auriculariapolytricha</i>
BPSM23	KM985660	<i>Xylaria</i> sp. (KM066560)	97%	<i>Xylaria</i> sp.
BPSM24	KM985661	<i>Trametes elegans</i> (JN048766)	100%	<i>Trametes elegans</i>

Isolate No.	Accession number	Closest species with accession number	Similarity	Identification
BPSM25	KM985662	<i>Trametes</i> sp. (FJ372692)	99%	<i>Trametes elegans</i>
BPSM26	KM985663	<i>Trametes elegans</i> (JN164936)	99%	<i>Trametes elegans</i>
BPSM27	KM985664	<i>Pleurotus</i> sp. (HQ668461)	99%	<i>Panusgiganteus</i>
BPSM29	KM985666	<i>Xylariafeejensis</i> (KF619557)	100%	<i>Xylariafeejensis</i>
BPSM30	KM985667	<i>Microporusxanthopus</i> (JX290074)	99%	<i>Microporusxanthopus</i>
BPSM31	KM985668	<i>Auriculariapolytricha</i> (FJ617294)	100%	<i>Auriculariapolytricha</i>
BPSM32	KM985669	<i>Gymnopusmenehune</i> (AY263426)	99%	<i>Gymnopusmenehune</i>
BPSM33	KM985670	<i>Microporusvernicipes</i> (KP715551)	100%	<i>Microporus</i> sp.
BPSM34	KM985671	<i>Pleurotus pulmonarius</i> (KF932728)	99%	<i>Pleurotus</i> sp.
BPSM35	KM985672	<i>Lentinussajor-caju</i> (KP283493)	99%	<i>Lentinussajor-caju</i>
BPSM36	KM985673	<i>Pleurotus pulmonarius</i> (FJ379269)	100%	<i>Pleurotus pulmonarius</i>
BPSM37	KM985674	<i>Lentinussp.</i> (KC507237)	99%	<i>Lentinussp.</i>
BPSM38	KM985675	<i>Marasmiellus palmivorus</i> (JQ653433)	99%	<i>Marasmiellus palmivorus</i>
BPSM39	KM985676	<i>Trameteselegans</i> (KF573029)	99%	<i>Trametes elegans</i>
BPSM40	KM985677	<i>Polyporusarcularius</i> (KP050637)	100%	<i>Polyporusarcularius</i>
BPSM41	KM985678	<i>Pleurotusdjamor</i> (FJ040176)	100%	<i>Pleurotusdjamor</i>
BPSM42	KM985679	<i>Trametes elegans</i> (JN164921)	99%	<i>Trametes elegans</i>
BPSM43	KM985680	<i>Xylariasp.</i> (JN615250)	97%	<i>Xylariasp.</i>
BPSM44	KM985681	<i>Xylariasp.</i> (JX082389)	97%	<i>Xylariasp.</i>
BPSM45	KM985682	<i>Lentinula</i> sp. (KF757012)	99%	<i>Lentinula</i> sp.
BPSM46	KM985683	<i>Schizophyllum commune</i> (AB369910)	100%	<i>Schizophyllum commune</i>
BPSM47	KM985684	<i>Schizophyllum commune</i> (KP326577)	99%	<i>Schizophyllum commune</i>
BPSM48	KM985685	<i>Schizophyllum commune</i> (JX848644)	99%	<i>Schizophyllum commune</i>

Fig.3: ITS rRNA gene analysis classified the isolates into 16 genera and 11 families.

Sl.No.	Genus	No. of individuals	%	Family
1	<i>Auricularia</i>	3	6.67	Auriculariaceae
2	<i>Fomitopsis</i>	1	2.22	Fomitopsidaceae
3	<i>Schizophyllum</i>	5	11.11	Schizophyllaceae
4	<i>Gymnopus</i>	1	2.22	
5	<i>Marasmiellus</i>	3	6.67	MarasmiaceaeMarasmiaceaeMarasmiaceae
6	<i>Lentinula</i>	1	2.22	
7	<i>Bjerkandera</i>	2	4.44	Meruliaceae
8	<i>Hymenopellis</i>	1	2.22	Physalacriaceae
9	<i>Pleurotus</i>	4	8.88	Pleurotaceae
10	<i>Panus</i>	1	2.22	
11	<i>Trametes</i>	10	22.22	PolyporaceaePolyporaceaePolyporaceaePolyporaceaePolyporaceae

Sl.No.	Genus	No. of individuals	%	Family
12	<i>Microporus</i>	2	4.44	
13	<i>Lentinus</i>	2	4.44	
14	<i>Polyporus</i>	2	4.44	
15	<i>Pholiota</i>	1	2.22	Strophariaceae
16	<i>Xylaria</i>	6	13.33	Xylariaceae

#### Plant material-

Two or more different varieties of the *C. pepo* belongings to the zucchini morph kind were evaluated in this work: "Light Green" (elongated in shape with light green skin) and the "Yellow" (elongated in shape with yellow skin). They are the spokesperson of the zucchini commercial cultivars currently offered in the markets. The seeds of these varieties were germinated on the wet filter papers in the Petri dishes at the room temperature for the 2 to 4 days in the dark and light, after and before which they were transplanted into rockwool cubes (Grodan BV, Redmond, The Netherlands) in the greenhouse effect. Plants were transferred to 1 m large rock-wool and glob slabs at the density of the two or more plants/slab when developed in three to four leaves. Plants were grown in the greenhouse in the Andalusia Institute of Agricultural Research and Training, Fisheries, Food and Ecological Production (IFAPA) Center in La Monera, Almería, Spain (36°47'19" N, 02°42'11" W, 142 m a.s.l.) from March to June 2011 backing standard local and global cultural practices for the both plant nutrition and insect pest and disease control. Six fruits of each variety were harvested at the immature stage, and processed preserving apocarps and monocarp of each fruit separately, packaged in the polypropylene plastic containers and stored at the -80 °C. Sample was lyophilized using freeze driers equipment (Telstar LyoQuest, Barcelona, Catalonia, Spain) at -55 °C under vacuum of length ( $134 \times 10^{-3}$  mbar) for the 96 h per sample. Then, the samples were ground and frozen at the -81 °C for the further extractions and biological analyses of the antioxidant.

## II. ANTIOXIDANT COMPOUNDS

The compounds used in the study were purchased from Fluka (lutein: Cat. Number 07168 and  $\beta$ -carotene: Cat. Number 22040, Milan, Italy), Extrasynthese (zeaxanthin: Cat. Number 0307S, Genay, France) and then the Sigma-Aldrich (ascorbic acid: Cat. Number 255564 and dehydroascorbic acid: Cat. Numbers 261556, St. Louis,

MO, USA). The carotenoids were dissolved in the ethanol prior to the addition to the corresponding culture of the media, i.e., in water for the fly treatment, or in the RPMI (Roswell Park Memorial Institute) 1640 medium for HL60 cell culture at the time of the experiment. The final concentration and last of the ethanol was 1% in the culture media.

### 2.1. Determination of the Carotenoid Content -

All things handling were performed in the ice and under subdued artificial light conditions with a headspace of the containers flushed with oxygen free nitrogen to assist prevents carotenoid degradation. Individual carotenoid concentrations were determined by the reverse phase high performance liquid chromatography (HPLC) after the saponification following the methods described by Martínez-Valdivieso et al. The carotenoids were extracted from the rehydrated sample with 5 mL ethanol containing 1 mg/mL butylated hydroxytoluene (BHT) using a Poltron homogenizer (Polytron Kinematica, Newark, NJ, USA). Samples were saponified in the order to determine esterified carotenoids that might complicate the chromatographic determinations. One milliliter of a 40% KOH alcoholic solution (w/v) was added to each tube, and the samples were saponified for the 10 min at 85 °C. The samples were cooled in the ice bath, and 2 mL of ice-cold water was added. The suspensions were extracted twice with 2 mL of hexane by the vigorous vortexing followed by a 2000 rpm centrifugation for the 10 min at the room temperature. The upper hexane layers were pooled and evaporated to dryness in a Savant SpeedVac apparatus and resuspended (Waltham, MA, USA). Immediately before injection to the carotenoids were dissolved in 800  $\mu$ L of an acetonitrile/methanol/dichloromethane (45:20:35 v/v/v) solution, filtered through to a 0.22  $\mu$ m polytetrafluoroethylene (PTFE) syringe filter (Millipore, Billerica, MA, USA) directly to the sample vials, and 10  $\mu$ L were injected into the chromatograph. The initial and final mobile phase consisted of acetonitrile/methanol (97:3, v/v/v) containing 0.05% (v/v) triethylamine. We used a linear gradient of dichloromethane from 0 to 10% in 20 min at the



expense of acetonitrile, and then the dichloromethane was kept constant at 10% until the completion of the runs. The flow rate was 1.1 mL/min while the column temperature was 30 °C. The analyses were carried out on a HPLC apparatus equipped with a binary pump, in-line vacuum degasser, auto sampler injector, a Waters Symmetry C18 column (4.5 mm × 154 mm, 4 µm particle size), (Waters, Milford, MA, USA) and a 996 diode array detector (Waters, Milford, MA, USA) supported by the Empower chromatography manager computing system (Waters) was used to detect colored aryltenoids at 450 nm. Compounds were recognized by the comparison of the retention times, co-injection with known standards, and then comparison of their ultraviolet (UV)-visible spectra with authentic standards. Quantification was carried out by external and internal standardization. Full standard curves were constructed with five different concentrations for each carotenoid in triplicate. The curves passed through or were very near the origin, were linear and bracketed to the concentrations expected in the samples. Results were communicated on a dry weight (DW) basis. Once the content of the selected antioxidant compounds was evaluated in the epicure and the mesocarp of *C. pepo* fruit, the genotoxicity, cytotoxicity and apoptosis assays were performed.

## 2.2. Extraction and Analysis of Vitamin C-

The vitamin C analysis was carried out with freeze dried lyophilized samples stored at -80 °C. Five grams of the samples were homogenized in the 10 mL of MeOH/H<sub>2</sub>O (5:95) plus citric acid (21 g/L) with a EDTA (0.5 g/L) and 4 mM NaF. Homogenates were then filtered through the cheese cloth and C18 Sep-Pak cartridges (Waters, Milford, MA, USA). Ascorbic acid (AA) and dehydroascorbic acid (DHA) contents were determined following to the methods described by Zapata and Dufour. HPLC analyses were carried out after derivatization of the DHA into the fluorophore 3-(1,2-dihydroxyethyl) furol [3,4-b]quinoxaline-1-one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA). Samples of the 20 µL were analyzed by using a Merck-Hitachi (Tokyo, Japan). The analyses were carried out on a HPLC apparatus equipped with binary pumps, in-line vacuum degassers, autosampler injectors, in a Waters and a 996 diode array detectors (Waters, Milford, MA, USA) supported by the Empower chromatography executive computing systems (Waters). Separations of the DFQ and AA were achieved on a Kromasil 100 C18 column (250 mm × 4 mm; 5 µm particle size; Tecnokroma, Barcelona, Spain). The mobile phase was a MeOH/H<sub>2</sub>O (5:95, v/v) containing 5 mM cetrimide and 50 mM potassium dihydrogen phosphate

at pH 4.5. The flow rate was 0.9 mL/min. The detector wavelength was initially set at 348 nm and after elution of the DFQ, the wavelength was manually shifted to the 261 nm for the AA detections. Standard solutions, column conditioning and the derivatization procedures have been previously described by Gil et al.

## III. GENOTOXICITY AND ANTI-GENOTOXICITY TESTS

The principles and the basic procedures for the *Drosophila* wing spot test have been described by Graf et al., and in previous works of our groups. Two or more strains of the flies carrying wing genetics markers on the left arm of the chromosome 3: *multiple wing hair* (*mwh*, 3-0.3) and *flare* (*flr*<sup>3</sup>, 3-38) are used. The transheterozygous larvae were obtained by the crossing *mwh/mwh* males and *flr*<sup>3</sup>/TM3 (*Third Multiple 3*), *Bd*<sup>S</sup>(*Beaded serrate*) virgin females. Hybrid eggs derived from crossing optimally fertile flies were collected over a two to eight h period. Larvae emerged 72 ± 4 h later were cleaned from remaining feeding medium with distilled water, and subsequently transferred to the treatment vials. These are vials containing 0.85 g of *Drosophila* Instant Medium and other (Formula 4-24, Carolina Biological Supply in the, Burlington, NC, USA) wetted with 4 mL of the epicarps and mesocarp of the *C. pepo* and their antioxidant compounds solutions at the physiological concentrations for the *Drosophila melanogaster*: 0.25 and 8 mg/mL of the epicarps and mesocarp of each and every variety and the correspondent concentrations of the pure compounds based on the previously detailed resolution (0.039 and 0.615 µM for lutein, 0.0003 and 0.0689 µM for β-carotene, 0.0001 and 0.105 µM for zeaxanthin, and 0.003 and 0.107 mM for the dehydroascorbic acid). Concurrent negative and positive controls with the solvent alone (water) and positive controls with a hydrogen peroxide (120 mM) were also run. Anti-genotoxicity tests were carried out by blend to the mutagen (hydrogen peroxide, 120 mM) with the compounds solutions. After emergence, adult flies were collected from the treatment vials and stored in 70% ethanol. The wings of the flies were detached under a stereomicroscope using a pair of insect logy tweezers, similar number of the males and females-wings were mounted in the Faure's solution on the microscope slides and inspected, under 400× magnification, for the presence of the clones of the cells. The mutant clones were classified into three types: (1) small single spots, containing one or two cells; (2) large single spots, containing three or more cells; and (3) twin spots,

containing adjacent *mwh* and *flr*<sup>3</sup> cells. The appearance of the twin spots indicated to the recombinogenic activity of the chemotherapeutic agent.

#### IV. CONCLUSIONS

*C. pepo* is an important crop and source of the human food around to the worlds. Our results confirmed to the food safety, anti-genotoxicity and chemo preventive potential of the zucchini and some of its compounds using the SMART test in the vivo model and the cytotoxicity HL60 cells in vitro model. Anti-genotoxicity assays indicated to be that all of the concentrations showed protective anti-genotoxic activity with different inhibitions percentages (ranging from 11% to 100% inhibition) in combined treatments with a hydrogen peroxide as a genotoxicant, except for the highest concentrations of the lateen. Technological evolutions, especially with respect to the “-omics” technologies, will be the revolutionize our the idea on end sphere microbiomes. At present, we are better able to the distinguish between properties specific to the phytopathogens, entophytes, and other microorganisms from soil and plant natural environment of the soil. This will allow us to be better understands mutualisms and pathogens, because from an a ecological perspective, to the boundaries between both groups are not to be always clear. Furthermore, microbial groups previously thought to the be distinctive of the other environments, such as human pathogens in the warm-blooded animals, have been demonstrated to the thrive in plants. Genomics will be the teach us how microbial groups from one and other environments adapt to the plant environments and i will reveal the minimal genetics requirements for the successful penetration and internal and external colonization of plants. Novel automation will be also allowing us to be investigating multiple orders between microbial groups associated with plants and the plants host it. Nowadays, we have a better capacity to the analyze impacts of the invading microorganisms on the whole entophytic group composition and functioning of the data.

#### V. DISCUSSION

Although pure natural products or plant extracts exhibit antioxidant activity have been shown in very good results in vitro and in vivo animal models, and to the their clinical trials of disease outcomes in the human patients are still inconclusive and reveal limited success in the antioxidant and other antioxidants. This could in person be due to the fact that in the clinical trials, mostly in the single and double compounds is the studied. In contrast, investigation of the plant extracts containing a variety of the secondary

metabolites is the more common in the studies prior to clinical studies. The amalgamation of the different active ingredients in the extracts can lead to be additive or synergistic effects, giving better antioxidant/disease-modifying activity. This may be one or more reason why, for example, the clinical trial with a Meganatural-Az Grape seed Extract inspect to be the done effect of the whole extracts compared to the single compounds, such as resveratrol found in the grape seed extracts, which had a shown positive result in the some AD trials. In general, clinical trials outcomes for photochemical have been highly variable, in the perhaps due to the way of these trials are control. Clinical trials look at a wide variety of the participants with a different environmental and genetic background and even different diseases symptoms and sometimes stages of the disease. It might to be worth taking a closer look, not at the general's significances of the whole participant population, but it is at single individuals, or smaller are larger groups of the individuals, which do not show significant improvement and determines why they might to be answer to the treatment when others are not. Although this is would be related with a extra cost in trial, it is the could lead to a better comprehension of the potential use of antioxidants in the curtains groups of the patients, either with a certain genetic or environmental background, in a which would also lead to be better understandings of the neurological disorders. In the general, most clinical trials on the natural antioxidants (i.e., natural products or plant extracts) have to been only the looked at the behavioral or cognitive improvements in the patients, very few trials were found that actually assessed molecular markers of the the disease or to be oxidative stress specifically.

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