Health-Promoting Phytochemicals: Functions, Properties, Occurrence And Levels In Philippine Fruits And Vegetables

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Bioactive food phytochemicals

- Phytochemicals chemicals produced by plants for protection against pests & diseases
- > 1980s only vitamins & minerals were thought to be important to human health
- > 1990s it became clear that phytochemicals are the ones responsible for prevention & delaying onset of chronic & degenerative diseases

A New Diet-Health Paradigm

- *nutrients for sustaining life & growth
- other microconstituents
 (e.g.phytochemicals) for prevention & delaying onset of chronic diseases

1990s – 2nd golden age of nutrition

- ✓ role of phytochemicals in promoting optimal health & preventing chronic diseases
- no longer sufficient to evaluate foods in terms of nutrient content alone

This paper will discuss:

- Properties & functions of phytochemicals that already have a strong evidence for their health-promoting effects
- ❖ Our research on occurrence and levels of carotenoids, phenolics, phytosterols and glucosinolates in some Philippine fruits & vegetables; bioassay of activities related to their health-promoting properties



Functional Foods (mid 1980s)

– processed foods containing ingredients that aid specific bodily functions in addition to being nutritious

Designer Foods (1989)

 foods that naturally contain or are enriched with nonnutritive, biologically active chemical components of plants that are effective in reducing cancer risk

Nutraceuticals (1989)

 any substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of disease Scientific evidence for efficacy of functional foods/phytochemicals

- Epidemiological studies
 - ✓ correlation or ecological
 - ✓ migrant studies
 - √ case-control (retrospective)
 - √ cohort (prospective)
- Biological and experimental studies
- Intervention (controlled) trials

Overall, epidemiology backed by convincing experimental and biological findings can provide strong causal relationship between diet and disease (World Cancer Research Fund 1997).

Potential disease-preventive mechanisms of fruits and vegetables and their constituents as identified in human dietary studies

- Antioxidant activity
 oxidative damage plays a key role in CVD,
 cancer initiation, cataract formation, aging
 process, inflammatory diseases, variety of
 neurologic disorders
- Modulation of detoxification enzymes responsible for biotransformation of endogeneous compounds and detoxification of venobiotics

Disease-preventive mechanisms - continued

- Stimulation of the immune system
 the immune system plays a central role in protecting against various external disease-promoting factors & probably against malignancies
- Decrease in platelet aggregation
 platelet aggregation is fundamental to a wide range of physiological processess including normal blood coagulation, thrombosis, atherosclerosis and tumor formation and metastasis

Disease-preventive mechanisms - continued

- Alteration of cholesterol mechanism
 elevated total serum cholesterol, LDL-cholesterol
 and triacylglycerol concentrations and reduced HDL cholesterol concentration are risk factors for
 coronary artery disease
- Modulation of steroid hormone concentration and hormone metabolism
 - certain cytochrome P450 (CYP) enzymes alter the potency of hormones via oxidation and hydroxylation; the induction or inhibition of these enzymes via the diet has the potential to modify the biological impact of hormones in humans

Disease-preventive mechanisms - continued

- Blood pressure reduction control of blood pressure is important for prevention of heart disease, kidney disease and stroke
- Antiviral and antibacterial activity
 viruses are now recognized as the second most important known cause of human cancer; some microbial infections are also associated with certain cancers

Phytochemicals with strong scientific evidence for health-promoting properties

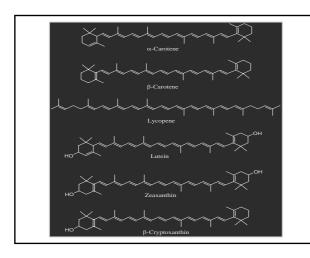
- Carotenoids
- Phenolics flavonoids, phytoestrogens, phenolic acids
- Phytosterols and phytostanols
- Limonoids
- Tocotrienols
- Organosulfur compounds allium compounds & glucosinolates
- Nondigestible carbohydrates dietary fiber & prebiotics

Rodriguez et al. 2006, Segurancia Alimentar e Nutricional 13(1):1-22

Carotenoids

- Natural pigments synthesized by plants, algae, fungi, yeasts and bacteria
- More than 650 carotenoids have been isolated & characterized from natural sources
- $_{\odot}$ Most investigated in terms of human health: $\alpha\text{-carotene},\ \beta\text{-carotene},\ \beta\text{-cryptoxanthin},\ lycopene,\ lutein & zeaxanthin;\ these are also the principal carotenoids encountered in human blood & most commonly found in foods$





Functions of carotenoids in plants

- Two major functions in photosynthetic organisms
 - 1. as accessory pigments for light harvesting
 - 2. prevention of photo-oxidative damage
- Provide distinct pigmentation to flowers and fruits to attract animals for pollination and seed dispersal



" I wandered lonely as a cloud That floats on high o'er vales and hills, When all at once I saw a crowd, A host of golden daffodils; Beside the lake, beneath the trees, Fluttering and dancing in the

William Wordsworth

Food sources of carotenoids considered important to human health

Carotenoid	Food sources
α-carotene	carrot, banana, orange
β-carotene	carrot, melon, green leafy vegetables, red pepper, yellow & orange-fleshed sweet potato, mango, orange
β- cryptoxanthin	red pepper, mango, orange
lycopene	tomato, watermelon, pink-fleshed guava, red-fleshed papaya
lutein	green leafy vegetables, yellow corn
zeaxanthin	yellow corn

Carotenoids and Disease Prevention

- Some carotenoids have provitamin A activity
- Other health-promoting effects: immunoenhancement & reduction of risk of degenerative diseases such as cancer, cardiovascular diseases, cataract & macular degeneration

Carotenoids and Disease Prevention

- Physiological activities attributed to antioxidant property (quench singlet oxygen & free radicals)
- Other mechanisms of action: modulation of carcinogen metabolism, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of cell to cell communication & filtering of blue light

Phenolic compounds

- One of the most numerous & widely distributed groups of substances in the plant kingdom; more than 8000 phenolic structures currently
- Common structural feature: hydroxyl-substituted benzene ring; non-hydroxylated derivatives, e.g. cinnamic acids are considered 'honorary phenolics'
- Classes: phenolic acids, flavonoids, isoflavones, lignans (1,4-diarylbutanes), stilbenes (1,2diarylethenes), tannins

Biological functions of phenolics

- Ubiquitous in higher plants & ferns as structural polymers
- UV screens for terrestial plants
- Flower pigments for pollination
- Defense from pathogens & herbivorous predators, e.g. phytoalexins



Phenolics	Common food sources
Flavonoids	Fruits, vegetables, coffee, tea, wine
Isoflavones	Cereals, pulses
Stilbenes (<i>trans</i> -resveratrol)	Grapes, peanuts
Lignans	Flaxseed, sesame seed
Phenolic acids	Fruits, vegetables, nuts, tea, wine

Protective mechanisms of phenolics

Anti-cancer mechanisms

- Antioxidant activity
- Modulation of Phase I and II enzymes
- Modulation of gene expression, apoptosis (programmed cell death) and malignant transformation
- Upregulation of intracellular gap junctional communication and inhibition of neoplastic transformation
- P-glycoprotein activation

Protective mechanisms of phenolics

Mechanisms for reduction of CVD risk

- Antioxidant activity: inhibition of LDL oxidation
- Prevention of platelet aggregation and adhesion
- Reduction of blood pressure
- Antithrombic effects
- Cholesterol-lowering effect

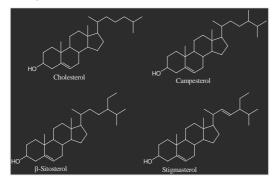
Phytosterols and Phytostanols

- Phytosterols triterpenoids occurring in free, esterified and glycoside forms
- Phytostanols less abundant, saturated forms of phytosterols
- Found in the plasma membrane, endoplasmic reticulum, outer membrane of mitochondria
- Regulate the fluidity of membranes, probably play a role in the adaptation of membranes to temperature

Plant food sources of phytosterols and phytostanols

- Vegetable oils richest natural sources
 - * majority: 100-500 mg sterol/100 g oil
 - * exceptions (g sterols/100 g oil): coffee (2.4), corn (1.4), sesame seed (2.9), rice bran (3.2)
- Vegetables: 1-200 mg sterols/100 g fresh weight food
- Fruits: 2-30 mg sterols/100 g edible fruit
- Cereals: highest rice bran (1325 mg/100 g food)

Phytosterols



Phytosterols/phytostanols and disease prevention

Reduction of CVD risk

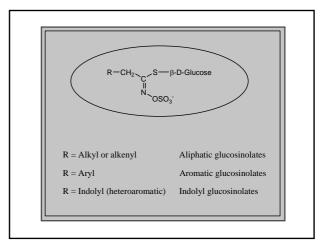
- Lower plasma cholesterol and LDL cholesterol in humans
- Sept 2000: US FDA allowed a health claim for reduction of risk of coronary heart disease for foods containing phytosterols and phytostanol esters

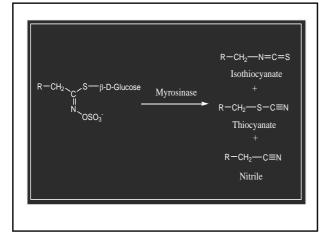
Prevention of cancer

- □ May offer protection against the most common cancers: colon, breast and prostate
- Probable mechanisms of action: effect on signal transduction pathways, inhibition of tumor growth and stimulation of apoptosis

Glucosinolates

- Sulfur-containing glucosides predominantly found in order Capparales, family Cruciferae
- Breakdown products resulting from action of myrosinase act as natural pesticides
- Breakdown products exert a variety of antinutritional and toxic effects in higher animals, e.g. goitrogenic effect
- Breakdown products responsible for characteristic hot flavors of condiments, e.g. mustard and horseradish





Anticancer Properties of Glucosinolates

- The World Cancer Research Fund (1997) concluded that diets rich in cruciferous vegetables probably protected human beings specifically against cancer of the colon, rectum, thyroid
- Breakdown products act as blocking agents (modulation of Phase I & II enzymes) & suppressing agents(induction of apoptosis) in carcinogenesis

Glucosinolate	Food source	Isothiocyanate	Nitrile
Glucoraphanin	Broccoli	Sulforaphane (SF)	Sulforaphane nitrile (SFN)
Gluconasturtiin	Chinese cabbage, radishes, watercress	Phenethyl isothiocyanate (PEITC)	
Sinigrin	Brussels sprouts, cabbage, cauliflower	Allyl isothiocyanate (AITC)	
Glucobracissin	All crucifers	Indole-3-carbinol (I3C)	Indole-3- acetonitrile
Progoitrin	Crambe (oil seed)	Crambene	
Glucotropaeolin	Cabbage, mustard	Benzyl isothiocyanate (BITC)	

Our Research

- Identification and quantification of carotenoids, phenolics, phytosterols, glucosinolates
- Evaluation of antioxidant activity: peroxide value, TBA assay, free-radical scavenging activity
- Evaluation of cholesterol-lowering activity (hamster)
- Evaluation of angiogenic activity (duck embryo)

Our **Approach**

Practical approach for developing countries:

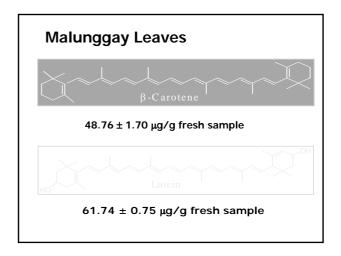
know developments in human health research in developed countries & focus efforts on identification of local sources of phytochemicals for which scientific evidence is strong

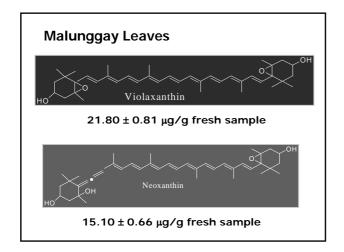
Our Approach

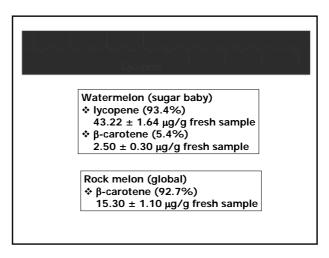
Emphasis on traditional/underutilized fruits & vegetables - conservation & sustainable use of these species offer a powerful tool for addressing food security in terms of both quality & quantity

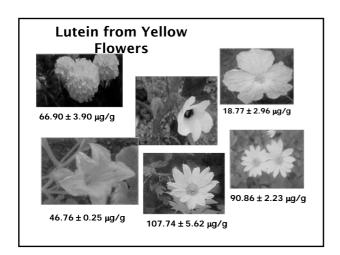
Carotenoid Analysis

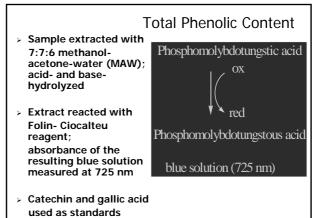
- Extraction: maceration w/ cold acetone, then partitioning to petroleum ether
- Isolation: OCC using MgO/Hyflosupercel
- Identification and quantification: VIS spectrophotometry, chemical tests

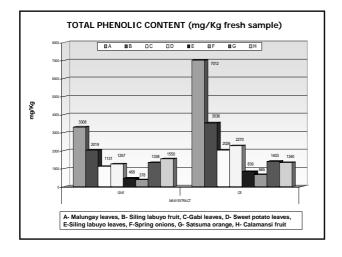






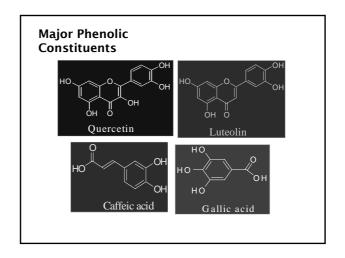


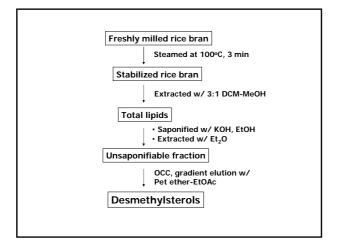




Sample	Total phenolic content (per kg fresh sample)						
	MAW extract		Acid-hyd extract	Acid-hydrolyzed extract		Base-hydrolyzed extract	
	mg GAE	mg CE	mg GAE	mg CE	mg GAE	mg CE	
Malunggay leaves	3308	7012	8565	18156	1819	3856	
Siling labuyo fruits	2019	3536	2420	4091	1253	217	
Gabi leaves	1131	2026	784	1269	597	896	
Sweet potato tops	1257	2270	5723	5148	455	842	
Siling labuyo leaves	468	839	816	1133	103	131	
Spring onions	378	665	2076	3524	123	217	
Satsuma orange	1338	1403	623	1307	134	291	
Calamansi fruits	1550	1340	436	456	122	150	

Sample	Major phenolic constituents				
	MAW extract	Acid-hydrolyzed	Base-hydrolyzed		
Malunggay leaves	Quercetin-3- <i>O</i> -glucoside	Quercetin	Caffeic acid		
Siling labuyo fruits	Quercetin-3- <i>O</i> -glucoside; luteolin-3- <i>O</i> -glucoside	Quercetin, luteolin	A cinnamic acid		
Gabi leaves	Quercetin-3-O-glucoside	Quercetin	Caffeic acid		
Sweet potato tops	Cyanidin-3- rhamnosylglucoside; bound ferulic and caffeic acids	Cyanidin, ferulic acid, caffeic acid	-		
Siling labuyo leaves	Apigenin-7- <i>O</i> -glucoside; Quercetin-3- <i>O</i> -glucoside	Quercetin, apigenin	A cinnamic acid		
Spring onions	Quercetin	Quercetin	A cinnamic acid		
Satsuma orange	Hesperidin	Hesperitin	A cinnamic acid		
Calamansi fruits	Naringin, hesperidin	-	-		

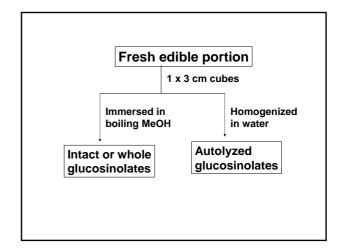


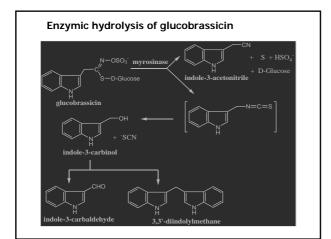


Sample	% oil	% unsaponifiable matter	Total sterol content (g sterol/100 g oil)
Rice bran	10.4	3.8	3.044
Sesame seeds	43.9	1.1	0.733
Corn kernel	3.6	1.1	0.903

HPLC Analysis of Sterols: Identity and % Composition

	RBO	Corn oil	Sesame oil
stigmasterol	13.93	7.61	10.44
campesterol	15.78	17.85	15.50
β-sitosterol	28.86	54.39	41.51





Sample	Total indole glucosinolates (mg/100 g fresh weight)
Cabbage	9.92 ± 1.22
Petchay	8.84 ± 0.59
Mustasa	6.94 ± 1.23

Antioxidant Activity

- measurement of peroxide value and thiobarbituric assay (TBA) on corn oil autooxidation
- 2. free-radical scavenging (DPPH) assay
- 3. TBA assay on metal-catalyzed lipid (lecithin liposome) peroxidation
- 4. TBA assay on Fenton-induced oxidation of calf thymus DNA

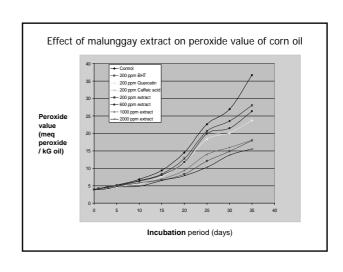
What are free radicals?

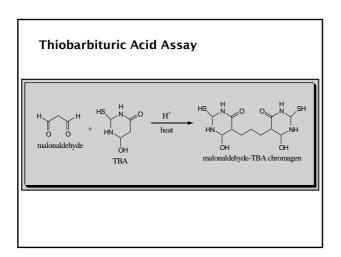
- Reactive molecular species w/ unpaired electrons
- Include reactive oxygen species (ROS): O₂-, NO-, RO-, ROO-, OH
- Increased levels in the body lead to oxidative stress – impair metabolism; cause damage to lipids, proteins, nucleic acids; eventually cell death
- 3 main sources
 - normal oxidative mechanism
 - infection
 - inflammation

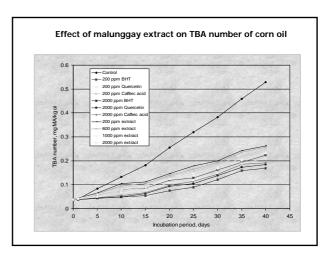
What are antioxidants?

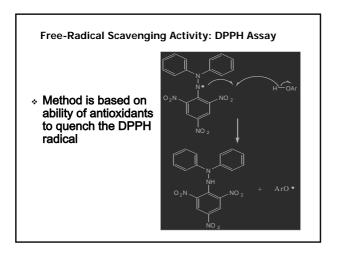
Substances that:

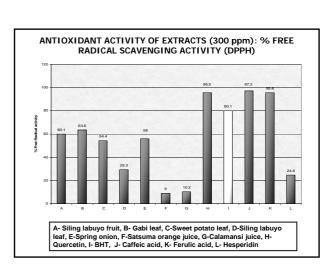
- metabolize free radicals to non-radical
- stop the chain reaction or propagation of free radicals
- take the damaging hit









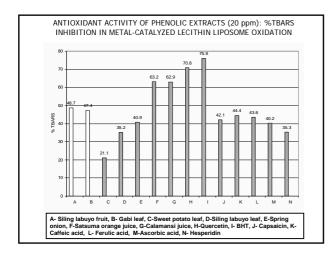


Antioxidant activity of phenolic extracts: % free radical scavenging activity (DPPH)

Sample (300 ppm)	% Free radical scavenging activity
Siling labuyo fruit phenolic extract	60.1
Gabi leaf phenolic extract	63.6
Sweet potato leaf phenolic extract	54.4
Siling labuyo leaf phenolic extract	29.3
Spring onion phenolic extract	56.0
Satsuma orange juice phenolic extract	9.0
Calamansi juice phenolic extract	10.2
Quercetin	95.5
внт	80.1
Caffeic acid	97.2
Ferulic acid	95.6
Hesperidin	24.8

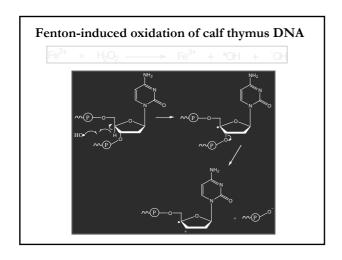
Antioxidant activity of glucosinolate extracts: % free radical scavenging activity (DPPH)

Sample (300 ppm)	% Free radical scavenging activity
Cabbage whole glucosinolate extract	27.3
Petchay whole glucosinolate extract	37.2
Mustasa whole glucosinolate extract	46.1
Cabbage autolyzed glucosinolate extract	18.6
Petchay autolyzed glucosinolate extract	14.3
Mustasa autolyzed glucosinolate extract	13.6



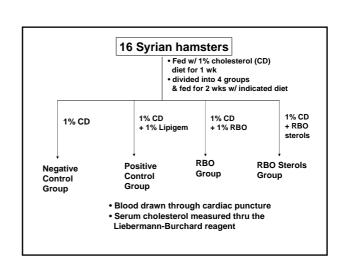
Antioxidant activity of phenolic extracts: % TBARS inhibition in metal catalyzed lecithin liposome oxidation

Sample (20 ppm)	% TBARS inhibition
Siling labuyo fruit phenolic extract	48.7
Gabi leaf phenolic extract	47.4
Sweet potato leaf phenolic extract	21.1
Siling labuyo leaf phenolic extract	35.2
Spring onion phenolic extract	40.9
Satsuma orange juice phenolic extract	63.2
Calamansi juice phenolic extract	62.9
Quercetin	70.8
внт	75.9
Capsaicin	42.1
Caffeic acid	44.4
Ferulic acid	43.6
Ascorbic acid	40.2
Hesperidin	35.3



Antioxidant activity of phenolic extracts: % TBARS inhibition in Fenton-induced DNA oxidation Sample (8 ppm) % TBARS inhibition Siling labuyo fruit phenolic extract 40.5 Gabi leaf phenolic extract 40.5 Sweet potato leaf phenolic extract 37.1 Siling labuyo leaf phenolic extract 32.9 Spring onion phenolic extract 47.0 Satsuma orange juice phenolic extract 45.5 Calamansi juice phenolic extract 43.9 Quercetin 47.0 BHT 43.9 Capsaicin 36.5 Caffeic acid 44.7 Ferulic acid 53.8 Ascorbic acid 00.0 Hesperidin 21.2

Sample	0.5 ppm	1.0 ppm	5.0 ppm	8.0 ppm
Cabbage, whole	10.22	12.56	12.83	17.53
Petchay, whole	10.05	11.83	12.37	24.11
Mustasa, whole	13.65	16.30	16.39	17.94
Cabbage, autolyzed	10.82	13.33	13.61	15.07
Petchay, autolyzed	16.50	19.50	20.96	22.05
Mustasa, autolyzed	20.37	24.79	24.93	25.48
Quercetin	23.79	31.42	40.32	44.11
BHT	21.64	26.58	31.51	34.86

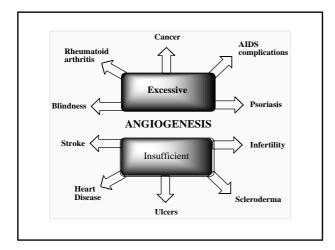


Cholesterol-lowering activity

Test substance	% serum-cholesterol lowering
Lipigem	22.34
RBO	8.56
RBO sterols	18.78
Sesame oil	10.26
Sesame oil unsaponifiable fraction	18.20
Corn oil	8.84
Corn oil unsaponifiable fraction	18.99

What is angiogenesis?

- Formation of new blood vessels; an important natural process that occurs in the body, both in health & disease
- Takes place for healing wounds, restoring blood flow to tissues after injury; in monthly period & pregnancy
- Healthy body controls angiogenesis through growth factors & inhibitors – a perfect balance is maintained



Angiogenesis Assays

■ In Vitro

- vendothelial cell proliferation, migration and tube formation
- ✓ rat aortic ring, chick aortic ring

■ In Vivo

- √ chick chorioallantoic membrane (CAM)
- √ Matrigel plug
- ✓ mouse corneal assay
- √ duck embryo assay

Siling labuyo fruit phenolic extract	Anti-angiogenic	Caffeic acid	Anti-angiogenic
Gabi leaf phenolic extract	Anti-angiogenic	Ferulic acid	Anti-angiogenic
Siling labuyo leaf phenolic extract	Anti-angiogenic	Ascorbic acid	Anti-angiogenic
Sweet potato leaf phenolic extract	Anti-angiogenic	Hesperidin	Anti-angiogenic
Spring onion phenolic extract	Anti-angiogenic	Lutein	Anti-angiogenic
Satsuma orange phenolic extract	Anti-angiogenic	β-Carotene	Pro-angiogenic
Calamansi juice phenolic extract	Anti-angiogenic	Mixture of carotenoids from malunggay	Extreme angiostatic effect
Quercetin	Anti-angiogenic		

Corn oil	Anti-angiogenic	Cabbage whole glucosinolate extract	Anti-angiogenic
Corn oil unsaponifiable fraction	Anti-angiogenic	Petchay whole glucosinolate extract	Anti-angiogenic
RBO	Anti-angiogenic	Mustasa whole glucosinolate extract	Anti-angiogenic
RBO sterols	Anti-angiogenic	Cabbage autolyzed glucosinolate extract	Anti-angiogenic
Sesame oil	Anti-angiogenic	Petchay autolyzed glucosinolate extract	Anti-angiogenic
Sesame oil unsaponifiable fraction	Anti-angiogenic	Mustasa autolyzed glucosinolate extract	Anti-angiogenic

Anti-Angiogenic Activity

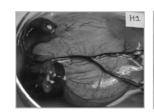


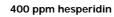


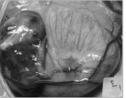


400 ppm ascorbic acid

Anti-Angiogenic Activity







400 ppm calamansi phenolic extract

Herb	Constituent	
Artemisia annua (Chinese wormwood)	95% artemisinin, other related terpenes and flavonoids	
Viscum album (European mistletoe)	Mistletoe lectin III (ML3A)	
Curcuma longa (turmeric)	95% curcumin	
Camellia sinensis (green tea)	95% phenolics (50% epigallocatechin)	
Vitis vinifera L. (grape seed extract)	95% proanthocyanidins	
Angelica sinensis (Dong quai)	4-hydroxyderricin	
Taxus brevifolia (Pacific yew)	taxol	
Scutellaria baicalensis (Chinese Baical skullcap)	95% baicalin and flavonoids	
Polygonum cuspidatum (Japanese knotweed)	20% resveratrol	
Silybum marianum (Milk thistle)	80% silymarin	
Magnolia obovata	90% honokiol	
Zingiber officinale	6-gingerol	

Importance of Our Results

Show which fruits & vegetables (due to their unique chemical constituents) warrant a special place in the normal diet or perhaps in the diet of people with certain diseases or those with high risk for certain diseases, such as cancer & heart diseases

Importance of Our Results

- A basis for further research in this area (there is a dearth of information on phytochemical contents of Philippine fruits & vegetables)
- Useful for policy makers in agriculture profile of phytochemicals that may protect against certain diseases will be very important consideration in deciding what plant varieties should be grown

Prospects for Developing Countries

- © Each country will have to identify & promote its own sources of phytochemicals
- Developing countries diversity of unstudied or understudied foods with higher levels of bioactive phytochemicals

Challenges for Developing Countries

- Accurate quantification of phytochemicalsneeded for product development
- To exploit beneficial effects of phytochemicals – understanding of their behavior at different stages of the food chain

Functional Foods From Plants

- Epidemiological, biological & experimental studies, clinical intervention trials: a plant-based diet reduce risk of degenerative diseases, esp. cancer & cardiovascular disease
- Plant-based diets prevent 20-50% of all cases of cancer (World Cancer Research Fund 1997)
- The single compound approach has given way to the concept that overall protection against diseases is provided by the additive synergistic effects of phytochemicals in whole foods

Eating a variety of colorful fruits and vegetables

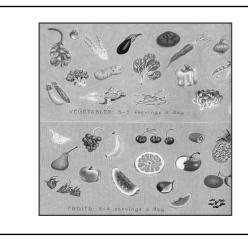
- gives your body a wide range of nutrients/phytochemicals that are important for good health
- each color offers something unique - different vitamins, minerals, and disease-fighting <u>phytochemicals</u> - that work together to protect your health
- only fruits and vegetables, not pills or supplements, can give you these nutrients/phytochemicals in the healthy combinations nature intended



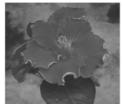


The 5 A Day For Better Health Program

- Developed in 1991 as a partnership between the National Cancer Institute and the Produce for Better Health Foundation
- The first national health promotion focusing on the importance of eating more fruits and vegetables
- Over the last decade, it has succeeded in increasing both the awareness of the health benefits of fruits and vegetables and their consumption in the United States



Acknowledgement



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