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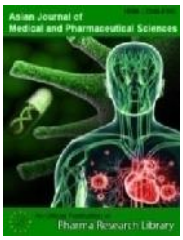

Review Article **Open Access**

Oxidative Stress and Inflammation

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A B S T R A C T

Oxidative stress is an imbalance between prooxidant and antioxidant system, it is associated with upregulation of reactive oxygen species and reactive nitrogen species, these reactive oxygen species causes radical induced inflammatory diseases and cancer. Many synthetic antioxidants are useful for curing radical induced inflammatory diseases, due to side effects of these drugs, ethanopharmacologists focus on development of plant derived antioxidants for formulation of safer and effective antioxidant drugs.

Keywords: Antioxidants, inflammation, reactive oxygen species, oxidative stress, superoxide dismutase, curcumin.

A R T I C L E I N F O

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1. Introduction

Oxidative stress is defined as an imbalance between production of free radical so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of cellular constituents [1]. ROS are products of a normal cellular metabolism and play vital roles in the stimulation of signaling pathways in living organisms in response to changes of intra- and extracellular environmental conditions. Most ROS are generated in cells by the mitochondrial respiratory chain. During endogenous metabolic reactions, aerobic cells produce ROS such as superoxide anion (O2-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), and organic peroxides as normal products of the biological reduction of molecular oxygen [2]. Under hypoxic conditions, the mitochondrial respiratory chain also produces nitric oxide (NO), which can generate other reactive nitrogen species (RNS). RNS can further generate other reactive species, e.g., reactive aldehydes-malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), by inducing excessive lipid peroxidation. Proteins and lipids are also significant targets for oxidative attack, and modification of these molecules can increase the risk of mutagenesis.

During inflammation, mast cells and leukocytes are recruited to the site of damage, which leads to a 'respiratory burst' due to an increased uptake of oxygen, and thus, an increased release and accumulation of ROS at the site of damage [3]. On the other hand, inflammatory cells also produce soluble mediators, such as metabolites of arachidonic acid, cytokines and chemokines, which act by further recruiting inflammatory cells to the site of damage and producing more reactive species. These key mediators can activate signal transduction cascades as well as induce changes in transcription factors, such as nuclear factor kappa B (NF-κB), signal transducer and activator of transcription 3 (STAT3), hypoxia-inducible factor-1α (HIF1-α), activator protein-1 (AP-1), nuclear factor of activated T cells (NFAT) and NF-E2 related factor-2 (Nrf2), which mediate immediate cellular stress responses. Induction of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), aberrant expression of inflammatory cytokines tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6 and chemokines IL-8, CXC chemokine receptor 4 (CXCR4), as well as alterations in the expression of specific microRNAs, has also been reported to play a role in oxidative stress-induced inflammation .

Oxidative stress is a most important phenomenon with clinical significance in a wide variety of disease conditions such as cancer, muscle hypertrophy, Parkinson disease, diabetes, Alzheimer's disease, cardiovascular disease. Oxidative stress is featured in the production of oxygen derived free radicals like hydrogen peroxide, superoxide, nitric oxide, hydroxyl radicals. These free radicals are collectively termed as reactive oxygen species (ROS), apart from oxygenated species, nitric species such as nitric oxide (NO^-) and peroxy nitrite (ONOO) play an important role in causing oxidative stress. These nitric species are collectively termed as reactive nitrogen species (RNS). ROS and RNS exert oxidative stress making each human cell to undergo 10,000 oxidative hits per second [4], which results in alteration of redox potential leading to un-repairable damage to biomolecules such as lipids, nucleic acid, proteins leading to cellular dysfunction and death.

Pro-oxidant and antioxidant system in living organism

In 1954, Geshman and Gilbert discovered that the lethal effects of ionizing radiation seen in Japanese people may be due to the formation of reactive oxygen species (ROS), this discovery gave rise to prominent research on reactive oxygen and nitrogen species [5]. Denham Harman, trailblazer of free radical research first discovered the link between ageing and free radical chemistry in early 1950. In his theory on free radicals, he clearly highlighted that cellular ageing is associated with oxidative stress. Harman contribution gained worldwide recognition with the discovery of superoxide dismutase by McCord and Fridovich, a natural anti-oxidant enzyme which is capable of destroying the free radicals formed in the body. Forty years later Diplock *et al.,* 1998 published a far reaching review strongly supported the fact that oxidative damage is an important causative factor in the development of diseases and antioxidants are capable of preventing and curing the diseases. Diplock *et al.,* 1998 concluded that maintenance of well-being and health depends on the intake of antioxidants through diet, which can modulate the free radical process resulting in the removal of free radicals formed in the body. Current research is focused on the collaborative role of free radicals and antioxidants in diseases such as Atherosclerosis, Alzheimer's disease and diabetes. [8]

2. Origin of free radicals

Free radicals are atoms or molecules with unpaired electrons which are capable of independent existence. The unpaired electrons create an unstable and highly reactive molecules which, to stabilize itself, will take an electron away from a stable molecule. On the loss of an electron, this previously stable molecule becomes damaged (a free radical), setting up a destructive chain reaction i.e. one radical begetting another. These free radicals are collectively known as Reactive Oxygen Species (ROS) or oxygen derived species and accomplishes both oxygen radicals and certain non-radicals that are oxidizing agents, or are easily converted into radicals. ROS may oxidize cellular components changing their structure and causing them into malfunction [6]. Free radical reactions occur continuously in cells and tissues as a consequence of various enzymatic and non-enzymatic reactions. Enzymatic reactions which are sources of free radicals are involved in phagocytosis, prostaglandin biosynthesis and in cytochrome P450 system. Non-enzymatic reactions mainly involve ionizing radiations. Apart from biological source non-biological sources such as tobacco smoke, automobile exhaust, ionizing radiation, hydrogenated oils, toxic metals are considered as richest source of free radicals [7].Nitric oxide (NO^-) is another well-known free radical found in various living systems. It is generated by the vascular

endothelium and other cells. Nitric oxide reacts with superoxide anion to produce a highly reactive free radical peroxy nitrite intermediate ($ONOO^-$) which is capable of destroying biomolecules and causing them to malfunction

3. Types of free radicals

A wide variety of free radicals cause oxidative destruction in the biological system they include reactive oxygen species such as superoxide anion (O_2^-), peroxyl (RO_2^-), hydroxyl (OH^-). Reactive nitrogen species include nitric oxide (NO^-), peroxy nitrite ($ONOO^-$), peroxy nitrous acid (ONOOH), nitrogen dioxide (NO_2) and super oxide anion radical (O_2^-). The commonly produced free radicals (Reactive oxygen species) in the living organisms are shown in the **table 2.** The reduction product of oxygen is most often involved in the initiation of oxidative stress. Phagocytic cells like macrophages extensively produce superoxide anion radical to destroy the invading microbes such as bacteria, viruses [9]. It can be produced in the electron rich environment of the mitochondria membrane in the respiratory chain. In addition to this it can be produced endogenously by enzymes such as xanthine oxidase and NADPH oxidase pathways. NADPH oxidase pathway facilitates superoxide production to destroy pathogens. Superoxide radicals are highly capable of damaging red blood cells, lungs, joints [10]

.Free radical associated inflammatory diseases

Oxidative damage to various cells, tissues and organs by free radical results in various chronic diseases affecting various organs and systems. Free radicals and their formation are highly associated with chronic inflammatory diseases. Free radicals form one of the defense mechanism employed by the immune system to expel the invaders by recruiting inflammatory cells to the site of injury [11]. Free radicals activate NF-κB, a nuclear transcription factor resulting in increased synthesis and release of IL-1β, IL-6 and TNF-α (Tumor necrosis factor), all these proinflammatory mediators provoke tissue damage.

Cardiovascular disease

Degenerative blood vessels and heart disease is one of the prominent and the serious effects of free radicals. Free radical attack results in build-up of plaques in the walls of coronary arteries. The plaques will rupture and superimposed with thrombosis. This is the actual basis for the acute syndrome of myocardial infarction and unstable angina. Calcifying of plaques increases the risk of cerebral infarction (stroke) leads to severe disability or death [12].

Cancer

Cancer is the uncontrolled autonomous growth of abnormal cells that can arise in any organ or tissue of the body. The Cancer cell is once normal cell which continues to grow and multiply without any limit. This may be due to various endogenous and exogenous factors including oxidative stress, which brings a pathological change in the cell's DNA resulting in a tumor.Transformed cells evading immune system defense will only become tumors. Free radicals can deteriorate the immune system which can allow the abnormal cells to grow continuously [13]. Harman suggested that the risk of cancer increases with age this is due to increased levels of endogenous free radical reactions and decreasing antioxidant defenses.

DNA is a major target of free radical damage. Free radicals target and attack the nucleic acid bases, resulting in various forms of base damage, yielding products such as thymine glycol or basic sites and 8-hydroxy guanosine. Apart from these, free radicals can attack and damage deoxyribose sugar as well as DNA protein cross-links. These damages to DNA result in mutations that yield cancer in germ cells and somatic cells. Free radical reactions with inadequate antioxidant defenses results in increased rates of mutations in proto-oncogenes, which are involved in normal cell growth and development, tumor suppression genes which suppress cell proliferation. Cancer causing agents either initiating or promoting the carcinogenesis. Initiating agents include UV rays, radiation, chemical pollutants, tobacco consumption, and viruses. Promoting agents include hormones such as androgens for prostate cancer, estrogens for breast cancer and ovarian cancer. Inflammation induces iNOS (inducible nitric oxide synthase) as well as COX and LOX, which are capable of initiating carcinogenesis.

Role of ROS in carcinogenesis

Oxidative damage to DNA proved *in vivo* and *in vitro* confirming that ROS damage to cells contributes to carcinogenesis in many ways. They may cause translocations and base pair mutations [14], abnormal cell to cell communication that favors unrestricted cell proliferation structural changes in DNA such as gene sequence amplification interference with genes that modulate cell growth preventing programmed cell death by necrosis and apoptosis [15] and damage to DNA repair enzymes results in existence and survival of mutations. White cells of the immune system are highly capable of removing altered cells, which have the potential to cause cancer. ROS decrease the membrane fluidity of white blood cells. Loss of membrane fluidity decreases the ability of lymphocytes to eliminate the altered cells and invading agents. Free radicals also damage the DNA of immune cells, resulting in mutations and reduced cell function [16].

Osteoarthritis

Osteoarthritis, a joint disease is one of the common disorder affecting humans. At present osteoarthritis is considered as dynamic repair process of synovial joints triggered by a various mechanical and metabolic factors. All the tissues of the joint, such as bone, synovium, cartilage, ligament and muscle depend on each other for health and function. Damage to any one affects other, resulting in osteoarthritis affecting the whole joint.

Role of ROS in osteoarthritis

Reactive oxygen species and the products of their reaction shown to decrease the fluidity of synovial fluid, thus affecting its function. Free radicals in excess destroy the synovium, loss of joint fluid and support between bones. High levels of superoxide radicals in the exudates of patients suffering with active synovitis supported the fact that free radical damage may be the main cause of osteoarthritis [17] propose that chondrocyte lipid peroxidation in both physiological and pathological conditions plays a key role in cartilage destruction by

oxidizing cartilage collagen resulting in brittleness of cartilage, thereby initiating osteoarthritis.

4. Antioxidants

Antioxidants are a group of molecules which are present in small concentrations in the cell to protect, can prevent or reduce the extent of oxidative destruction of cellular components. These antioxidants inhibit the oxidative reactions and create a barrier from free radical damage. Antioxidants halt the chain-breaking mechanism, by which the primary oxidants donate an electron to the free radical present in the system forming a radical, more stable than the initial one. Primary oxidant includes compounds such as flavonoids, tocopherol and ascorbic acid. Another mechanism can be accomplished by deactivation of high energy species like O_2^- absorption of UV light, chelation of metal catalyzing free radical reactions, or by inhibition of peroxides such as Xanthine oxidases or lipoxygenases. Any compounds that can react with the initiating radical inhibits the initiating enzyme or reduce the O_2 level without generating ROS can be considered as secondary antioxidants.

Types of Antioxidants

Antioxidants may intervene at different levels in the oxidative process, antioxidants can be grouped into synthetic and natural antioxidants [18]. Many synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT) and propyl gallate (PG) have been used to retard the oxidation process. In recent years dietary antioxidants used in preventing many human diseases like diabetes, rheumatoid arthritis, cancer and cardiovascular diseases. Antioxidants may be classified as endogenous antioxidants, which are of physiological origin that involve intracellular essential enzymes viz,. Superoxide dismutase (SOD), catalase, glutathione peroxidase. Exogenous antioxidants are administered as supplements to protect against pro-oxidant forces as they are not produced by the human body. Although endogenous antioxidants scavenge free radicals, they may be overwhelmed by excess free radicals. So, for an effective protection against oxidative species that we encounter in our daily lives, regular consumption of at least some antioxidants in the diet or as supplements appears to be very crucial. Ascorbic acid (Vitamin C) and Tocopherol (Vitamin E) are considered very potent in protecting the body against the destructive effects of free radicals. A number of plants derived substances collectively termed phytonutrients or phytochemicals gained prominence for their antioxidant activity. Polyphenol compounds such as flavonoids serve protection against a variety of free radicals formed as a result of oxidative stress [19]. Plants secondary metabolites such as phenolic compounds, flavonoids, tannins, alkaloids, cardiac glycosides and phytosterols acts as natural antioxidants.

Phenolic acids

Polyphenolic compounds widely distributed in plants, these polyphenolic compounds can neutralize free radicals thus prevent free radical related diseases. The phenolic compound exhibit antioxidant activity by acting as reducing agent, Hydrogen donor and singlet oxygen quenchers [20]. Phenolics such as flavonoids, Benzoic acids, Coumarins, Lignins and Tannins act as natural anti oxidants.

Flavanoids

Flavanoids are heterogenous group of phenolic compounds that occurs naturally in free form or conjugated form. Flavanoids maintain the membrane integrity by protecting from lipid peroxidation. Flavanoids prevents generation of free radicals by stabilizing the reactive oxygen species, hydroxyl group of flavonoids are responsible for inactivation of free radicals [21].

Alkaloids

Alkaloids are low molecular weight nitrogenous compounds present in the plants as secondary metabolites. These alkaloids prevents the free radical generation by activating antioxidant enzymes such as superoxide dismutase and catalase. [22]

Terpenoids

These are natural bioactive compounds present in plants, these compounds are built from isoprene units. These compounds inhibit the lipid peroxidation and scavenging the free radicals such as O_2^-, NOO^- then prevents the free radical related diseases. [23].

Table 1: Free radical reactions: Free radicals are signified by a superscript dot, which indicates the presence of one or more unpaired electrons (Halliwell, 1994).

ADDITION	$X^{\cdot}+Y \rightarrow [X\text{-}Y]^{\cdot}$
Hydrogen abstraction	$X^{\cdot}+yH \rightarrow XH+Y^{\cdot}$
Electron donation	$X^{\cdot}+Y \rightarrow Y^{\cdot}+X^{+}$
Electron transfer	$X^{\cdot}+Y \rightarrow X+Y^{\cdot}$
Electron removal	$X^{\cdot}+Y \rightarrow X^{\cdot}+Y^{\cdot +}$
Termination	$X^{\cdot}+X^{\cdot} \rightarrow X2$ $X^{\cdot}+Y^{\cdot} \rightarrow XY$

Table 2: Commonly produced free radicals (ROS) in living organisms

Type of ROS	Name	Illustration
O_2^-	Superoxide radical	One- electron reduction state of O_2 formed in the respiratory chain
OH	Hydroxyl radical	Three-electron reduction state, formed by Fenton reaction and decomposition of peroxynitrite and it is extremely reactive. $H_2O_2+Fe^{2+} \rightarrow OH+OH^-Fe^{3+}$(Fenton reaction)
RO & ROO	Alkoxyl and peroxyl	Produced in the presence of Oxygen by radical addition to double

	radicals	bonds or hydrogen abstraction and participate in lipid per oxidation reactions.
H_2O_2	Hydrogen peroxide	Two electron reeducation states, formed by dismutation ofO_2^- or by direct reduction of O_2. Lipid soluble and thus able to diffuse across membranes.
O_2	Singlet oxygen	It is less stable than the normal triplet oxygen. Singlet oxygen is usually generated with a photo sensitizer pigment.
NO	Nitric oxide	Nitric oxide (NO) is an intracellular messenger, produced from oxygen by the various nitric oxide syntheses.
ONOO	Peroxynitrite	Formed in a rapid reaction between O_2^- and NO.
HOCl	Hypochlorous acid	Formed from H_2O_2 by myeloperoxides. Lipid soluble and highly reactive. Will readily oxidize protein constituents, including thiol groups, amino groups and methionine.

Table 3: Oxidative damage involved in pathogenesis of human diseases

Category	Examples
Neuronal diseases	Parkinson's diseases (Jenner *et al*,1992), schizophrenia (Smythies, 1998; Reddy, 1999)
Respiratory diseases	Asthma, lung cancer, cystic fibrosis, especially during exacerbation, exposure to environmental pollutants (O_3,NO_2,SO_2, auto exhaust), Emphysema (van der vliet ,2000)
Digesitve system diseases	Inflammatory bowel diseases, ulcerative colitis(Grisham,1993)
Infection diseases	AIDS, malaria (Halliwell ,1999; Mollace *et al*, 2001)
Endocrine diseases	Diabetes (Paolisso *et al*,1995)

Table 4: Enzymatic and non enzymatic antioxidant system in the cell

Anti oxidant system	Description
Antioxidant Enzymes	
copper/zinc and manganese-dependent superoxide dismutase (SOD)	Scavenging in superoxide radical
iron-dependent catalase	Scavenging Hydrogen peroxide
Selenium-dependent glutathione peroxidise	Scavenging Hydrogen peroxide
Cyctochrome–oxidase system	Scavenging of 90% of single O_2 in cell
Peroxidase	Scavenging hydrogen peroxide
Non Enzymatic antioxidants	
α –tocopherol (Vitamin E)	Neutralizes hydrogen peroxide, lipid peroxides
β – carotene (Vitamin A)	Neutralizes lipid peroxides, hydrogen peroxide
Ascorbic acid (Vitamin C)	Neutralizes superoxide radicals, hydrogen peroxide
Dietary Antioxidants	Beta carotene and other carotenoids and oxycarotenoids
Uric acid	Scavenges O_2,OH
Cysteine	Scavenges O_2,OH
Ubiquinone (coenzyme Q10)	Neutralizes lipid peroxides
Flavonoids	Scavenges H_2O_2,OH,O_2,lipid peroxides
Lipoic acid	Scavenges H_2O_2,OH^-
Bilirubin	Scavenges OH^-

Table 5: Antioxidant activity of Medicinal plants

S. No	Name of the species\Family	Local name	Plant part\s	AO activity (%)
1	*Alocasia fornicata(Roxb.) schott\Araceae*	Baibing	Rhizome Aerial part	41.06 21.63
2	*Alpiniamalaccensis rose. Zingiberaceae*	Aiphal	Aerial part	5.69
3	*Alpinia officinarun Hance Zingiberaceae*	Aichal	Rhizome Aerial part	94.02 74.97
4	*Aquilaria malaccensis lam Thymelaeaceae*	Agar	Steam Leaf	47.30 92.03
5	*Artocarpus chaplasha roxb Moraceae*	Harikothong	Steam Leaf	47.70 76.94
6	*Mitragyna rotundifolia*	Viteaval	Steam	91.51

	O.Kuntze Rubiaceae		Twig	93.58
7	*Melastoma malabathricum linn Melastomataceae*	Builukham	Aerial part	36.58
8	*Mallotus tetracoccus(Roxb.) kurz euphorbiaceae*	Thingkhei	Steam Leaf	23.02 31.42
9	*Litsea glutinosa(Lour.) C.B. Robinson Lauraceae*	kalimendi	Steam Twig	90.57 41.53
10	*Grewia sapida Roxb. Ex DC. Tiliaceae*	Yongkomla	Steram Twig	95.46 97.01
11	*Hydnocarpus kurzii(King) Warb. Flacoutiaceae*	Khavitur	Steam Twig Fruit	45.96 53.27 47.95
12	*Callicarpa arborea Roxb. Varbenaceae*	Hnahkiah	Stem Leaf	53.65 47.20
13	*Cassia nodosa Bush.-Ham. Ex Roxb. Caesalpiniaceae*	Tisibi	Stem Twig	78.96 71.44
14	*Cassia renigera wall.ex Benth. Caeslpiniaceae*	Radhachura	Stem Twig	36.11 17.65
15	*Clerodendrum indicum (Linn.) Kuntze*	Kuthap	Aerial part Root	47.07 1.0

5. Conclusion

Oxidative stress is a attribute in the production of both oxygen and nitrogen derived radical species. Normally reactive oxygen species involved in signal transduction and phagocytosis, where as in up regulated state they cause membrane damage by inducing lipid peroxidation and cause cancer by inducing mutations in genetic material. Both enzymatic (Superoxide dismutase and catalase) and non enzymatic (Tocopherol, carotene and ascorbic acid) systems regulates the over production of free radicals Plant derived antioxidant compounds from traditional medicinal plants with high phenolic groups are ideal candidate for development of both antioxidant and anti-inflammatory drugs. Antioxidant drugs have side effects on organ functions and causes uncontrolled growth of cells hence ethanopharmacologists focus on the medicinal plants having high free radical scavenging activity and are further validated and developed potent antioxidant drugs with high therapeutic index, low toxicity and economic viability.

6. References

[1] Durackova Z. Some current insights into oxidative stress. *Journal of Physiological Research*, **2010,** Vol 59, pp 459–469.

[2] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Journal of Free radical Biology and Medicine*, **1991,** Vol 11, pp 81–128.

[3] Coussens LM, Werb Z. Inflammation And Cancer. *Nature*. **. 2002,** Vol 420, pp 860-7

[4] Maeda, H. and Akaike T. Nitric oxide and oxygen radicals in infection, inflammation, and cancer. *Journal of Biochemistry*, **1998**, Vol.63, pp 854-865.

[5] Govindarajan R., Rastogi S., awat.A K.S., Vijayakumar M., Shirwaikar A., Mehrotra S. and

[6] Palpu P. Studies on the Antioxidant Activities of Desmodium gangeticum. *Biological Pharmaceutical Bulletin*. **2003,** Vol 26, pp 1424-1427.

[7] Diplock AT, Charleux JL, Crozier-Willi G, Kok FJ, Rice-Evans C, Roberfroid M, Stahl W, Vina-Ribes J (1998). Functional food science and defence against reactive oxidative species. *British Journal of Nutrition*, **1999,** Vol 80, pp 77-112.

[8] Prieto P, Pineda M and Aguilar M Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, **1999**, Vol 269, pp 337-341.

[9] Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxy radical production by peroxinitrite: Implications for endothelial injury from nitric oxide and superoxide *Proceedings of National Academy of Sciences U.S.A*, **1990**, Vol 87, pp 1620-4.

[10] Basu, J., Bousbaa, H., Logarinho, E., Li, Z., Williams, B.C., Lopes, C., Sunkel, C.E., and Goldberg, M.L. Mutations in the essential spindle checkpoint gene bub1 cause chromosome missegregation andfail to block apoptosis inDrosophilaJ. *Cell.* Biol, **1999,** Vol. 146, pp 13-28.

[11] Lin SC, Lin CR, Gukovsky I, Lusis AJ, Sawchenko PE, Rosenfeld MG Molecular basis of the little mouse phenotype and implications for cell type-specific growth. *Nature* **1993,** .Vol 364 (6434), pp 208-13.

[12] Ames, B.M., Shigena, M.K. and Hagen, T.M.. Oxidants, antioxidants and the degenerative diseases of aging. *Proceedings of National*

Academy of Sciences U.S.A, **1993**, Vol. 90, pp 7915-7922.

[13] Brown, J. The Ribonuclease P database. *Journal of Nucleic Acids Research*, **1999**, Vol 27, pp312-314.

[14] Balk will, F., Charles, K. A., and Mantovani, A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Journal of Cancer Cell*, **2005**, Vol. 7, pp 211–217.

[15] Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC and Gaydou EM Antioxidant properties of hydroxylflavones. *Journal of Free radical Biology and medicine*, **1996**, Vol 20, pp 35-43.

[16] Robbins, P. F., Morgan, R. A., Feldman, S. A., Yang, J. C., Sherry, R. M., Dudley, M. E., et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1, *Journal of Clinical Oncology*, **2011**, Vol 29, pp 917–924.

[17] Fabiani R, De Bartolomeo A, Rosignoli P, Servili M, Montedoro GF, Morozzi G. Cancer chemoprevention by hydroxytyrosol isolated from virgin olive oil through G1 cell cycle arrest and apoptosis. *European Journal of Cancer Prevention*, **2002**, Vol 11, pp.351-358.

[18] Zhang S1, Mantel C, Broxmeyer HE. Flt3 signaling involves tyrosyl-phosphorylation of SHP-2 and SHIP and their association with Grb2 and Shc in Baf3/Flt3 cells. *Journal of Leukocyte Biology*, **1999**, Vol 65(3), pp 372-80.

[19] Shree jayan and Rao MNA, Curcuminoids as potent inhibitors of lipid peroxidation, *Journal of Pharmacology*, **1994,** Vol 46, pp 1013-1016.

[20] Cotelle N, Bernier JL, Catteau JP, Pommery J, Wallet JC and Gaydou EM Antioxidant properties of hydroxylflavones. *Journal of Free radical Biology and medicine*, **1996**,.Vol 20, pp 35-43,

[21] Evans - Rice CA, Sampson J, Bramley PM, et al. Why do we expect carotenoids to be antioxidants in vivo? *Journal of Free radical Research*, **1997**.Vol26, pp 381–98,

[22] Hendra Rudi, Syahida Ahmad, Aspollah Sukari, M. Yunus Shukor, and Ehsan Oskoueian Flavonoid Analyses and Antimicrobial Activity of Various Parts of Phaleria macrocarpa (Scheff.) Boerl Fruit *International journal of Molecular Science*, **2011**, Vol 12(6), pp 3422–3431.

[23] Mensor, L. I., Menezes, F. S., Leitao, G. G., Reis, A. S., dos Santos, T., Coube, C. S. and Leitao, S. G.. Screening of Brazillian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, **2001**, Vol 15, pp 127-130.

[24] Talhouk R, Karam C, Fostok S, El-Jouni W, Barbour E. Anti-inflammatory bioactivities in plant extracts. *Journal and Medicine and Food*, **2007**, Vol 10, pp 1–10.