ejpmr, 2019,6(3), 205-214

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

A REVIEW ON EVALUATION OF ANTIOXIDANT ACTIVITY OF POMEGRANATE

Lirin Mary M. K.*[1], Nipu Sam. P. George[2] and Renjitha Krishnan[3]

[1]Assistant Professor, Department of Pharmaceutical Chemistry, KVM College of Pharmacy, Cherthala.
[2]Assistant Professor, Department of Pharmaceutics, KVM College of Pharmacy, Cherthala.
[3]Student, KVM College of Pharmacy, Cherthala.

***Corresponding Author: Dr. Lirin Mary M. K.**

Assistant Professor, Department of Pharmaceutical Chemistry, KVM College of Pharmacy, Cherthala.

Article Received on 30/12/2018 **Article Revised on 19/01/2019** **Article Accepted on 10/02/2019**

ABSTRACT

The aim of present study was to evaluate in-vitro antioxidant properties of *Punica granatum* fruit (pomegranate fruit) peel. Antioxidants are molecules involved in defense mechanisms against the deterious effects of free radicals in most organisms. Antioxidants are the agents responsible for currently being used for the evaluation of the antioxidants and free-radical scavenging properties of natural and synthetic antioxidants, including the DPPH method. The *Punica granatum* fruit (pomegranate fruit) peel powder suspension was the method adopted to determine antioxidant potentials of aqueous suspension of pomegranate peel powder. Results revealed that DPPH aqueous solution gave compared free radical activity 24 hours post preparation compared with the freshly prepared solution. After 24 hours, activity was greatly reduced. It is therefore recommended that freshly prepared DPPH solution should be used at all times, however for prolonged experimental schedules, the DPPH solution should be used within 24 hours post prepation, so as to give comparable results with the freshly prepared solution and avoid doubtfulness in results interpretataion. Aqueous suspension of peel powder showed good antioxidants effect. Percentage of inhibition increased with the increased concentration of extracts. The present study provides evidence that the *Punica granatum* fuits peels is a potential source of natural antioxidants.

KEYWORDS: Antioxidant, *Punica granatum***,** DPPH method, Flavanoids.

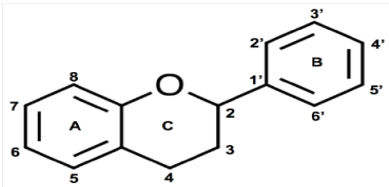
INTRODUCTION

Free radical contributes to more than hundred disorders in human being including artherosclerosis, arthritis, respiratory injury of many tissues, cancer, AIDS. Free radical due to the environment pollutant, chemicals and deep fried spicy food as well as physical stress cause depletion of immune system antioxidants and change in gene expression and induce abnormal proteins.

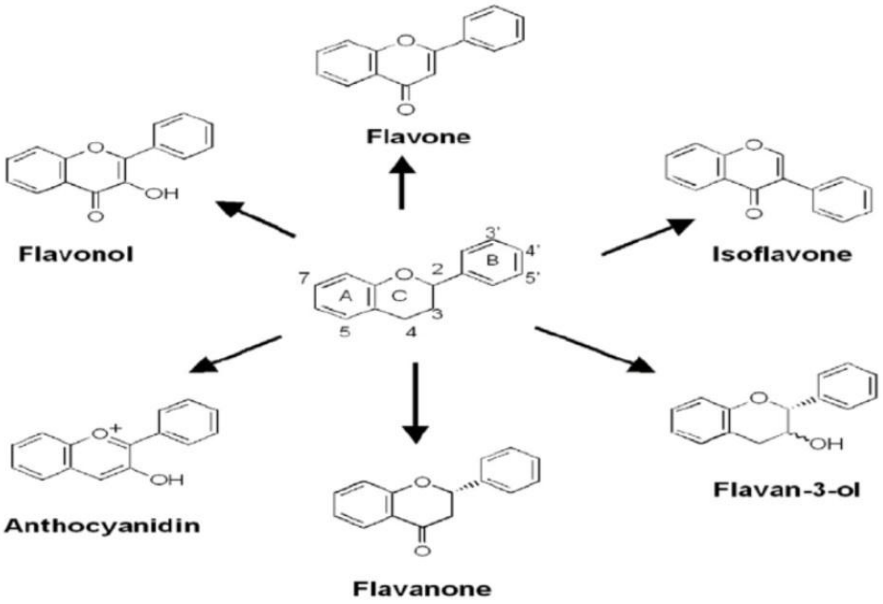
Oxidation is one of most important routs for producing free in food, drugs and living system. *Catalase* and hydrogen *peroxidase* enzyme convert hydrogen peroxide and hydrogen peroxide in to non-radical form and function as natural antioxidant in human body. Current available synthetic antioxidants like butylated hydroxy anisole, butylated hydroxyl toluene and gallic acid ester have suspended to cause or promote negative health effect.[1]

Flavanoids are low molecular weight bioactive polyphenols. Polyphenols are an important secondary metabolites present in plants and are also responsible for their antioxidant action and various beneficial effects in a multitude diseases. The word flavanoids come from the latin word '*flavus*' which means yellow however some flavanoids are red, blue or purple in colour. Most of the flavanoids are isolated from natural sources. The original flavanoid research apparently began in 1936, when Hungerian scientist Albert Szent-Gyorgi was uncovering a synergy between pure vitamin c and as yet unidentified cofactors from the peels of lemons which he first called "citrin" and later "vitamin P". Recent interest in these substances has been stimulated by potential health benefits arising from the antioxidant activities of these polyphenolic compounds.[2]

FLAVANOIDS



STRUCTURE OF FLAVANOID

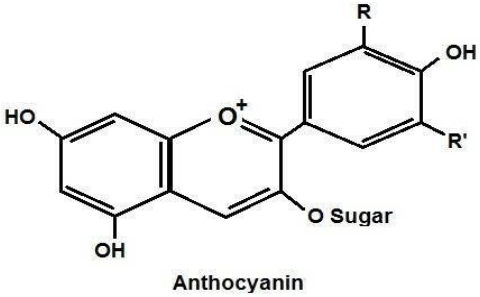


ANTHOCYANIN

Anthocyanins are water soluble vacuolar pigments that depending on their pH may appear red, purple or blue. Food plants rich in anthocyanins include the blueberry, raspberry, black rice, and black soyabean, among many others that are red, blue-purple or black. Some of the colors of autumn leaves are derived from anthocyanins.

Anthocyanins belong to a parent class of molecules called flavonoids synthesized via the phenyl - propanoid pathway. Anthocyanins occur in all tissues of higher plants, including leaves, stems, roots, flowers, and fruits. Anthoxanthins are clear, white, to yellow counterparts of anthocyanins occurring in plants. Anthocyanins are derived from anthocyanidins by adding sugars. They are odorless and moderately astringent. Although approvedto color foods and beverges in the European Union, anthocyanins are not approved for use as a food addictive. There is no high - quality evidence anthocyanins have any effect on human biology or diseases.[3]

CHEMISTRY OF ANTHOCYANIN



Anthocyanin

IUPAC NAME	(2S,3R,4S,5S,6R)-2-(3-chromenylium-2-ylphenoxy)-6-(hydroxymethyl)oxane-3,4,5- triol
CHEMICAL NAME	Anthocyanin 3`- O-beta-D-glucoside
MOLECULAR FORMULA	$C_{12}H_{21}O_7$
MOLECULAR WEIGHT	385.392g/mol
MELTING POINT	84.5◦C

Anthocyanins are water-soluble glycosides of polyhydroxyl and polymethoxyl derivatives of 2-phenyl benzopyrylium or flavylium salts. Individual anthocyanins differ in the number of hydroxyl groups present in the molecule; the degree of methylation of these hydroxyl groups; the nature, number and location of sugars attached to the molecule; and the number and the nature of aliphatic or aromatic acids attached to the sugars in the molecule. Hundreds of anthocyanins have been isolated and chemically characterised by spectrometric tools; cyanidins and their derivatives are the most common anthocyanins present in vegetables, fruits and flowers.[4]

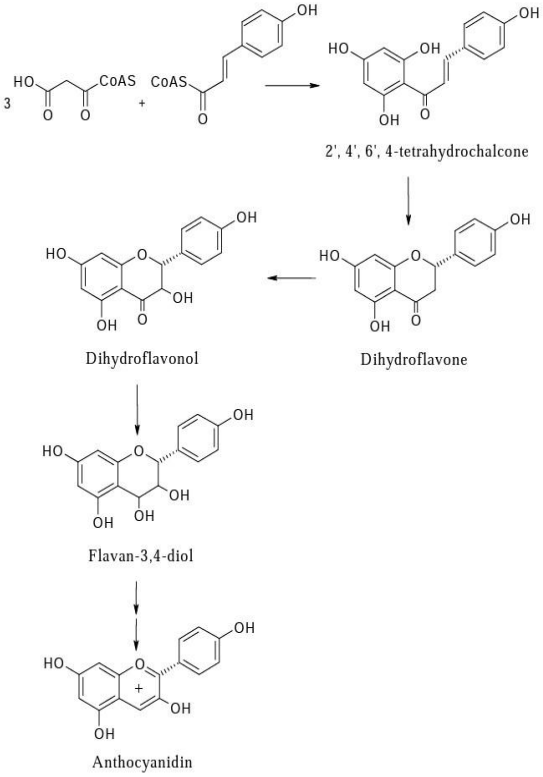
Anthocyanins share a basic carbon skeleton in which hydrogen, hydroxyl or methoxyl groups can be found in six different positions. In fruits and vegetables, six basic anthocyanin compounds predominate, differing both in the number of hydroxyl groups present on the carbon ring and in the degree of methylation of these hydroxyl groups. The identity, number and position of the sugars attached to the carbon skeleton are also variable; the most common sugars that can be linked to carbon-3, carbon-5 and, sometimes, carbon-7, are glucose, arabinose, rhamnose or galactose. On this basis, it is possible to distinguish monoxides, dioxides and trioxides.[5]

Another important variable that contributes to the chemical structure of anthocyanins is the acylating acid that may be present on the carbohydrate moiety. The most frequent acylating agents are caffeic, ferulic, sinapic and p-coumaric acids, although aliphatic acids such as acetic, malic, malonic, oxalic and succinic acids may also occur. Up to three acylating acids may be present simultaneously. The natural shielding offered by the three-dimensional structure of anthocyanins protects them from aqueous attack, thus preventing hyper chromic and batho-chromic shifts on the phenolic hydroxyl ion present in the carbon skeleton. Such an effect is lost at high temperatures such as those that occur during industrial processing of anthocyanin-rich vegetables. Therefore, anthocyanins are often partially degraded in many foods stored for long periods of time.[6]

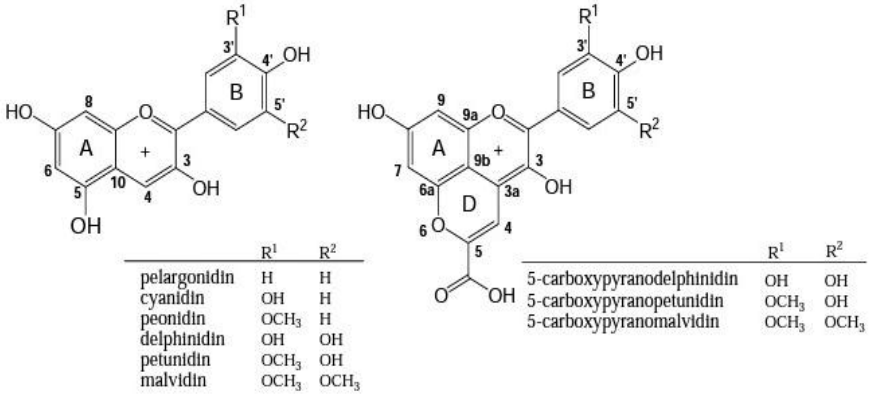
This variability in the chemical structure of anthocyanins accounts for the large number of compounds belonging to the anthocyanin family, and allows researchers to assign chemical/molecular fingerprints to many different plant species on the basis of their anthocyanin composition.

BIOSYNTHESIS OF ANTHOCYANIN

The initial step in biosynthesis of all flavonoids is the condensation of 4-coumarate coenzyme A (shikimate derived, B ring) to give 2, 4`,6`,4- tetrahydroxychalcone, which is catalysed by the enzyme *chalcone synthase*. The chalcone is then isomerised to the flavanone naringenin, a key intermediate, which can be converted to several end-product including anthocyanin.



NATURALLY OCCURRING ANTHOCYANIN



	R^1	R^2
pelargonidin	H	H
cyanidin	OH	H
peonidin	OCH_3	H
delphinidin	OH	OH
petunidin	OCH_3	OH
malvidin	OCH_3	OCH_3

	R^1	R^2
5-carboxypyranodelphinidin	OH	OH
5-carboxypyranopetunidin	OCH_3	OH
5-carboxypyranomalvidin	OCH_3	OCH_3

Biological Activities Of Flavanoids And Its Derivative Anthocyanin

Natural and synthetic flavanoids have attracted considerable attention because of their interesting biological activities.

Antioxidant activity
Hypolipidemic activity
Anti-carcinogenic activity
Anti- microbial activity
Anti-inflammatory activity
Anti-diabetic activity
Anti-malarial activity
Anti-miotic activity
Anti-artherosclerotic activity[9,10]

ANTIOXIDANTS

Antioxidants are any substance that inhibits oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are

capable to neutralize free radicals by donating one of their own electrons, ending the carbon stealing reaction. Thus antioxidants prevent cell and tissue damage and act as scavenger. Cells produce defence against excessive free radicals by their preventative mechanisms, repair mechanisms, physical defences and antioxidant defences. When an antioxidant destroys a free radical, this antioxidant itself becomes oxidized. Therefore, the antioxidant resources must be constantly restored in the body thus while in one particular system an antioxidant is effective against free radical, in other systems the same antioxidant could become ineffective. Also in certain circumstance an antioxidant may even act as pro-oxidant. eg. It can generate toxic reactive oxygen species (ROS).

The antioxidant process can function in one of two ways; Chain breaking or prevention. For the chain breaking when a radical releases or steals an electron a second radical is formed. The last one exerts the same action on another molecule and continues until either the free radical formed is stabilized by a chain breaking antioxidant (Vitamin C, E, carotenoids, etc.) or it simply disintegrates in to an in offensive product.[11]

Polyphenolic antioxidant's recent studies have shown that many flavonoid and related polyphenols are actually better antioxidants than vitamins. Fruits and vegetables are high in flavonoid content; flavonoids impart colour and taste to flowers and fruits, and it is estimated that humans consume between a few hundred milligrams and one gram of flavonoids every day. Flavonoids appear in blood plasma at pharmacologically active levels after eating flavonoid - rich foods but do not accumulate in the body. Consuming flavonoid regularly increases longevity by reducing inflammation and contributing to the amelioration of atherosclerosis from CHF. The range of flavonoid biological activity is large; in addition to scavenging free radicals and ROS, flavonoid actions include anti-inflammatory, antiallergenic, antiviral, antibacterial, antifungal, antitumor and anti-hemorrhagic. The anthocyanins are the most important flower and fruit pigments; they attract pollinators and seed dispersers and protect plant tissues from ultraviolet (UV) radiation damage. Some flavonoids act as antifeedants to herbivorous pests. The isoflavones are responsible for the chemical signalling involved in legumous root node formation. It is well established that the efficacy of flavonoids as antioxidants stems from the number and position of the hydroxyl substitutions on the basic structure; an increase in number of hydroxyl groups is directly correlated with increasing activity, and the 3, 4-dihydroxy substitution is significant.[12]

TYPES OF ANTIOXIDANT

1. Primary antioxidant (Scavenger anti-oxidant): These antioxidant can neutralize free radicals by donating one of their own electrons ending the electron "stealing" reaction.
2. Secondary or preventive antioxidants. They act through numerous possible mechanisms like,

a) Sequestration of transition metal ions
b) Removal of peroxides that *catalyse* and *glutathione peroxidase* they can react with transition metal ions to produce reactive oxidants species.

3. Tertiary antioxidant defences: they are the repair processes which remove damaged biomolecules before they are accumulate and their presence results in altered cell metabolism and viability. Eg: Damaged DNA repaired by enzyme methionine sulphaoxidereductase.[13]

CLASSIFICATION OF ANTIOXIDANT

1) Enzymatic antioxidant
2) Non enzymatic antioxidant

ENZYMATIC ANTIOXIDANT

It benefit by breaking down and removing free radicals. They can flush out dangerous oxidative products by converting them into hydrogen peroxide then into water. This is done through a multistep process that requires a number of trace metal cofactors such as Zinc, Copper, Manganese, and Iron. An enzymatic antioxidant cannot be found in supplements, but instead are produced in our body. The enzymatic antioxidants in our body are:

Superoxide dismutase (SOD) can break down superoxide into hydrogen peroxide and oxygen, with the help of Copper, Zinc, Manganese and Iron. SOD enzymes are present in almost all aerobic cells and in extracellular fluid. In humans, the copper or zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion.

Catalase (CAT) works by converting hydrogen peroxide into water and oxygen using iron and manganese cofactor. It finishes up the detoxification process started by SOD. This protein is localized to peroxisomes in most eukaryotic cells.[14]

Glutathione peroxides (GSHpx) and **Glutathione reductase** (GR) break down hydrogen peroxide and organic peroxides into alcohol. They are most abundant in liver. GSH is a tri-peptide and powerful antioxidant present within the cytosol of cells and is the major intracellular non protein thiol compound (NPSH).

NON- ENZYMATIC ANTIOXIDANT

Non- enzymatic antioxidants classified into; metabolic antioxidant and Nutrient anti-oxidants.

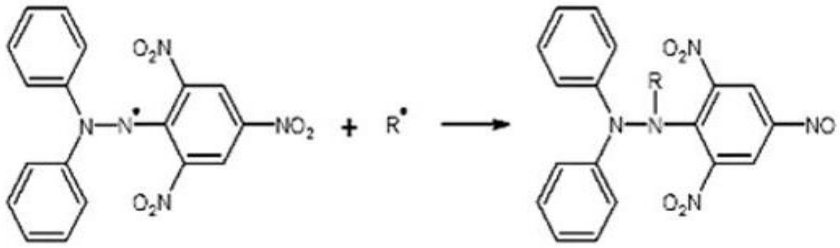
Metabolic antioxidant belonging to the endogenous antioxidants is produced by metabolism in the body such as lipoid acid, glutathione, L-arginine, coenzyme Q_{10}.

Nutrients antioxidants belonging to exogenous antioxidants are compound which cannot be produced in the body must be provided through food supplements such as vitamin E, vitamin C, carotinoid, flavonoids, etc.

DETERMINATION OF ANTIOXIDANT ACTIVITY

Dpph Method

Antioxidant activity of fruit or root extract was determined on the basis of their scavenging activity of the stable 1,1- diphenyl -2- picryl hydrazyl (DPPH) free radical . DPPH is a stable free radical containing an odd election in its structure and usually utilized for detection of radical scavenging activity in chemical analysis. 1ml of the solution of the extract was added to 3ml of 0.004% ethanolic DPPH free radical solution. After 30 min absorbance of the mixture were taken at 517nm by a UV spectrophotometer which was compared with ascorbic acid concentration.



Mode of action of DPPH radical compound RH

Advantage

This technique is easy, effective, and rapid way to study plant extract profile. No sample separation is needed. Potency of sample can be known.

Disadvantage

Time consuming, costly.[15]

% radicle scavenging activity =

$$\frac{\text{Absorbance of blank} - \text{absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Frap Method

The ferric reducing antioxidant potential (FRAP) assay performed according to the method described by benzene and strain, direct measurement of antioxidant to the method (reducing) ability through reduction of the complex Fe^{3+} to Fe^{2+} at acidic pH 3.6. It was taken at 620nm using uv-vis spectrophotometer during monitoring period (2hr). FRAP values were expressed in µmoltrolox equivalent (TE) per g of fresh material for the extracts and µmol TE per 500µmol of pure compound.

Advantage

It is simple, speedy, and inexpensive.
It can be performed using automated, semi- automated or manual method.

Disadvantage

FRAP cannot detect species that act by radical quenching, particularly SH- group containing antioxidant like thiol.[16]

Abts Method

The ABTS cation radical which absorbs at 743nm (giving a bluish green colour) is formed by the loss of an electron by the nitrogen atom of ABTS (2,2-azino- bis(3-ethylbenthiazoline-6-sulphonic acid). In the presence of Trolox (or of another hydrogen donating antioxidant), the nitrogen quenches the hydrogen atom yielding the solution decolourisation.

ABTS can be oxidised by potassium persulphate or manganese dioxide, giving rise to the ABTS cation radical whose absorbance at 743nm was monitored in the presence Trolox chosen at standard.

Pfrap (Potassium ferricyanide reducing power method)
An absorbance increase can be correlated to the reducing ability of antioxidant.The compounds with antioxidant capacity react with ferricyanide to form potassium ferrocyanide. The latter react with trichloride, yielding ferric ferrocynide a blue coloured complex, with a maximum absorbance at 700nm.[1]

Pomegranate

Pomegranate (*Punica granatum*) is a fruit bearing deciduous shrub or small tree in the family *Lythraceae*.

Scientific classification

Kingdom: Plantae
Clade: Angiosperms
Order: Myrtales
Family: Lythraceae
Genus: Punica
Species: Punica granatum

History

Pomegranate origin begins in Egypt thousands of years ago. It was the fruit of kings, is thought to be the fruit that Eve fed Adam in the Garden of Eden, and was one of the fruits that would have been seen hanging in the gardens of Babylon of ancient days. There are differing ideas however as to where it originally showed up. Some say it's the native fruit of Persia, whereas others say it's native to Iran and India. It was cultivated in these countries and a few other countries as well such as the East Indies, Asia, Africa, and Malaysia. One of the most detailed historic recordings of pomegranate origin indicates that the fruit was not native to China like some people thought at one time, but actually it was brought to the country around 100 B.C.A man by the name of Jang Qian, a representative of the Han dynasty, is credited with bringing the pomegranate in India.

You might think that the name of the pomegranate might be just that, but in fact different countries have called it by various names over the years. *Romans* called the pomegranate fruit, the Punic apple. *Latin translation* – granata, *English translation* – grenade /pomeapple. *Other names* – apple of amber, apple fruit, little fruit, little apple. The official name of the pomegranate fruit is *Punicum granatum*.[18]

Cultivation And Collections

Pomegranate crop prefers dry climate grows up to an elevation of 1800 meter and especially during fruit development stage, prolonged hot and dry climate is mandatory for better growth and yield the optimum temperature for fruit development is 36^0C-38^0C. Pomegranate fruits are easily damaged in humid climatic condition by pomegranate butterfly and will not develop sweetness in the fruit. Pomegranate can be cultivated in wide range of soils. However well drained, deep loamy soil rich in organic matter are best for its cultivation. Optimum pH range is 5.5-7. Propagation by cuttings is common and cuttings should be taken from suckers with spring from the base of the main stem and should be mature about 20cm-30cm (length) and 6mm-12mm (thickness). Rainy season is the best period to achieve maximum success in establishing a pomegranate plantation. Plant bear fruits in 3-4 years after planting. Usually pomegranate flowers take 5-7 months to be ready as a mature fruits. Fruits should be harvested at mature stage which can be judged by change in skin colour to slightly yellow and metallic sound when tapped.[19]

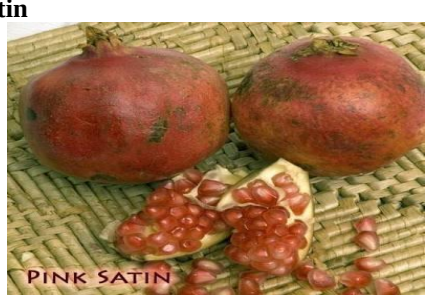
Varieties of Pomegranate

Several new varieties have been developed and new orchards have come will well-known improved varieties in many countries. As there are many improved varieties available for each region. It is better to find out hybrid cultivar which results in high yielding. While selecting pomegranate seedlings from reputed nursery, make sure to get high quality, commercial high yielding and disease tolerant plants.

Kashmir Blend

Medium size pomegranate with light pink red exterior. Ruby red seeds have intense flavor with no overbearing acidic taste.tree has a slightly spreading growth habit and also be grown as a shrub. Keep any height with summer pruning. Excellent source of antioxidants-eat fresh or use in cooking. Requires 150-200 chill hours. Self-fruitful.

Pink Satin



Medium to large size, bright red fruit with small, light-pink edible seeds. Light-coloured juice is non-staining, with a sweet, fruit- punch flavor. Tree is vigorous and can also be grown as a shrub. Eat fresh, juice or use in salads. Excellent source of antioxidants. Chill requirement150-200 hours. Self- fruitful.

Redsilk

Medium to large size fruit with a pinkish - red exterior. Firm yet edible sweets have a sweet berry flavor and a great acid or sugar balance. Naturally semi-dwarf tree has a slightly spreading grown habit and sets large crops.

Grow as a tree or shrub and keep any height by summer pruning. Excellent source of antioxidants. Eat fresh or use cooking. 150-200 hours self-fruitful.

Sharp Velvet

Large sized pomegranate with a very appealing, unique refreshing flavor. Fruit has a dark red exterior and dark seed, the colour of crushed-red velvet. Upright growing tree sets huge crops of highly ornamental fruit and can be kept any height with summer pruning. Eat fresh or use in cooking. An excellent source of antioxidants. Requires 150-200 chill hours, self- fruitful.[20]

Chemical Constituents Of Pomegranate

It is about 50% of the total fruit weight corresponds to the peel, which is an important source of bioactive compounds such as flavonoids like anthocyanin, pro-anthocyanidin, phenolics, ellagitannins and minerals mainly potassium, nitrogen, calcium, phosphorous, magnesium sodium and complex polysaccharides. Pomegranate fruit consist of 40% arils and 10% seeds. Arils contain 85% water, 10% total sugars, mainly fructose and glucose. And also contain 1.5% pectin, organic acid, such as ascorbic acid, citric acid, malic acid. It contain 12-20% of total seed weight of pomegranate comprises seed oil and is self-possessed with more than 70% of the conjugated linolenic acids. The fatty acid component of pomegranate seed oil comprises over 95% of the oil, of which 99% is triglycerols.

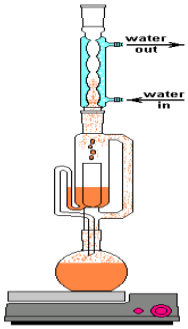
EXTRACTION OF ANTHOCYANIN FROM POMEGRANATE

Fruit or peel extraction with ethanol or methanol and acidified water

100g of the blended whole fruit or the manually separated peels were extracted with 400ml ethanol or methanol or with 400ml ethanol: acidified water (10% acetic acid) in 1:1 or 3:1 ratios. Samples were stirred for 24hrs at room temperature in the dark.

The extraction mixture was well decanted and filtered on paper, and then evaporated at 40^0c in the dark obtaining a purple-red sticky residue from peels that were immediately analysed.

Peel extraction in soxhlet apparatus



100g of the blended peels were extracted with 400ml ethyl acetate in soxhlet extractor for 3, 6 and 15 hrs in the dark (4-5 extraction cycles per hour). The obtained solution was evaporated at 40^0c in the dark to a dry yellow-brown residue that was immediately analysed or stored at -18^0c.

In-Vitro Antioxidant Properties Of Pomegranate

The aim of this work was to determine the total phenol (TPC), total flavonoid (TFC), tannins contents (TCs) and antioxidant properties of pomegranate (Punicagranatum) peel powder co-product (PPP) in view to its application in the food industry as ingredient. The antioxidant activity of the PPP was determined by means of four different antioxidant tests (2,2′-diphenyl-1-picrylhydrazyl, ferric reducing power, ferrous ion-chelating and thiobarbituric acid reactive substance) and the TPC, TFCs and TC were also determined. The PPP showed a content of TPC of 54.84 mg gallic acid equivalents/g sample; a TFC content of 42.36mg rutin equivalent /g sample and a TC of 21.25 mg catechin equivalent/g sample. As regards the antioxidant properties, PPP has good antioxidant activity at all concentrations tested (0.2, 0.5, 1 and 10 g/L) and all methods used, but in general terms, this activity is lower than that shown by the positive control (butylated hydroxyl toluene) used.[21]

Identification Test For Anthocyanin

Low temperatures is used to avoid hydrolysis of potential acyl groups in the anthocyanin structure and degradation.

1. ANALYTICAL PROCEDURES

Confirmatory Test for Flavonoids

A. Ferric chloride test: 1ml of the flower extract of the studied plant was added with a pinch of ferric chloride. Brown colour formation indicates the presence of flavonoids.

B. Aluminium chloride test: 1ml of the flower extract of the studied plant was mixed with few drops of 5% Aluminium chloride solution and the colour formation indicates the presence of flavonoids.

Confirmatory Test for Anthocyanins

The presence of anthocyanin in the extract was confirmed by performing the following tests.
A. 1ml of the flower extract of the studied plant was mixed with 2M HCl and heated for 5min at 100^0C. When the extract remains the stable purple (magenta) colour confirms the presence of anthocyanins.
B. 1ml of the flower extract mixed with 2M NaOH and the formation of green colour indicates the presence of anthocyanins.
C. To the flower extract, aluminium chloride addition will give a shift of 120nm in spectrophotometer which confirms the anthocyanins.
D. The flower extract was analysed using UV-Visible spectrophotometer, absorbance between 200 – 700nm indicates the presence of anthocyanins.

2. ANTIOXIDANT ASSAYS
The antioxidant analysis of Crude Anthocyanins of Sesbaniasesban flower petal extracts (CASFP) were performed by the following methods.

Scavenging Activity of DPPH Radical
Scavenging activity of anthocyanins against 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals. Briefly, 0.1 ml of CASFP extracts were mixed with 1ml of 0.1mM DPPH, methanol solution and incubated for 30 min at 25ºC in dark. After incubation the absorbance were measured at 517nm. Methanol was used as blank and methanol and DPPH were used as control. Ascorbic acid and BHT were used as standards. The inhibition of DPPH radicals by the samples were calculated according to the following equation:

DPPH - scavenging activity (%) = [1- (absorbance of the sample / absorbance of the control] × 100

Ferric Reducing Antioxidant Power (FRAP) Assay
The FRAP assay reagent was prepared by adding 10 volumes of 300mm acetate buffer (pH 3.6), 1 volume of 10mm TPTZ and 1 volume of 20mm $FeCl_2$. The mixture was diluted to 1/3 with methanol and pre-warmed at 37^0C.

This reagent (3mL) was mixed with 0.1ml of different concentrations of CASFP (1,10, 50 mg/ml) samples similar to those used for the ABTS assays. The mixture was shaken and incubated at 37^0C for 8 min and the readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 593nm along with the blank methanol. The results were expressed as µmol Trolox equivalents (TE) per gram dry weight of sample.[22]

Marketed Preparations
CARLSON- ACES+Zn antioxidant- 360 soft gels
EVOTONE SYRUP-200ml
GRANATUM PLUS+ SYRUP- 100ml
TOVUZNAR SYRUP- 50ml
VIGOROUS-MUSCLE MAXIMIZER- 60mg

DISCUSSION

Chemical analysis of Punica granatum fruit peel and it's in- vitro and in- vivo biological properties
The medical application of pomegranate fruits and its peel is attracted human beings. The aim of the present study was to evaluate the in- vitro α-Glycosidase inhibition, antioxidant activity and in-vivo determination of *Punica granatum* (pomegranate) fruit peel extract using Caenorhabditis elegans. Various in-vitro antioxidant activities of fruit peel extracts was determined by standard protocol, from the results ethyl acetate extract of P.granatum fruit peel (PGPEa) showed the α-Glucosidase inhibition upto 50% at the concentration of IC50 $285.21 \pm 1.$ 9µg/ ml compared to hexane and methanol extracts. PGPEa showed more scavenging activity on 2,2- diphenyl- picryl hydrazyl (DPPH) with IC_{50} value 302.43 ± 1.9µg/ ml and total antioxidant activity with IC_{50} 294.35 ± 1.68µg/ ml. In the present investigation we observed various biological properties of pomegranate fruit peel .The results clearly indicated that pomegranate peel extract could be used in preventing the incidence of long term complication of diabetics.

Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in in-vitro models
Antioxidant- rich fractions were extracted from pomegranate (*Punica granatum*) peels and seeds using ethyl acetate, methanol, and water. The extracts were screened for their potential as antioxidants using various in-vitro models, such as β-carotene-linoleate and 1,1-diphenyl-2-picryl hydrazyl (DPPH) model systems. The methanol extract of peels showed 83 and 81% antioxidant activity at 50ppm using the β-carotene-linoleate and DPPH model systems, respectively. Similarly, the methanol extract of seeds showed 22.6 and 23.2% antioxidant activity at 100ppm using the β-carotene-linoleate and DPPH model systems respectively. As the methanol extract of pomegranate peel showed the highest antioxidant activity among all of the extracts, it was selected for testing of its effect on lipid peroxidation, hydroxyl radical scavenging activity, and human low-density lipoprotein (LDL) oxidation. The methanol extract showed 56,58 and 93.7% inhibition using the thiobarbituric acid method, hydroxyl radical scavenging activity, and LDL oxidation, respectively, at 100ppm. This is the first report on the antioxidant properties of the extracts from pomegranate peel and seeds. Owing to this property, the studies can be further extended to exploit them for their possible application for the preservation of food products as well as their use as health supplements and neutraceuticals.

Evaluation of antioxidant capacity and flavour profile change of pomegranate wine during fermentation and aging process
Antioxidant properties and flavor characteristic profile of pomegranate wine during wine making were investigated. The total phenol content and radical scavenging activity exhibited a slightly decrease in the

end edge. Punicalagins and gallic acid were revealed to be the most abundant phenolic compounds, followed by ellagic acid and vanillic acid. These constituents were mainly responsible for the effective antioxidant capacity of pomegranate wine. The major changes of flavour qualities occurred in the initial stage; particularly 0- 4 day of aldehydes, ketones, heterocyclic and aromatic compounds, but promoted the generation of esters and alcohols. This is the first time of using E-nose and E-tongue to monitor odour and taste changes in the brewing process of pomegranate wine. The study may provide a promising instruction for improving functional features and quality control of the pomegranate wine.

Evaluation of antioxidant properties of pomegranate (*Punica granatum* L.) seed and defatted seed extracts

Basiri S.J was performed the pomegranate seeds are by-products of the pomegranate juice industries that contains functional compounds such as phenols. This study was aimed to evaluate the effect of solvents on extraction from pomegranate seed and pomegranate defatted seed and to measure the yield extract and phenolic content and antioxidant properties. For this purpose, the seeds and defatted seeds were directly isolated from fruits and seeds by cold pressing respectively, then were crushed and extracted with different solvents, including water, methanol, acetone, ethyl acetate and hexane and finally the extracts of them were evaluated.

Phenolic compounds, ferric reducing- antioxidant power and radicals scavenging property of extracts were measured. The results showed in extraction efficiencies were for hexane and acetone solvents in extraction of seed and defatted seed respectively. The highest phenolic content was obtained from methanol seed extract. Reducing activity test proved that the methanol extracts of pomegranate seed and pomegranate defatted seed had the highest reducing strength. Results of radical scavenging activity were similar to reducing activity results.

The order of antioxidant capacity of pomegranate seed and pomegranate defatted seed were found to be methanol> butanol> ethyl acetate> hexane. It can be concluded, pomegranate seed, which possesses high levels of polyphenols, can be one of the sources of the natural antioxidants. The methanol extract had higher antioxidant efficiency than seed and defatted seed extracts.

Evaluation of antioxidant properties and phenolic composition of fruit tea infusions

Sahin S. was performed by the popularity of fruit tea is increasing in the world because of its antioxidant properties and attractive taste. The aim of this study was to determine and compare the antioxidant property and phenolic composition of 16 different fruit teas. The antioxidant property and total phenol content of fruit teas depending on the extraction condition (water temperature) were examined using the ABTS (2,2-azinobis[3-ethylbenzothiazoline-6- sulphonic acid] method and the Folin-ciocalteu method, respectively. The contents of total flavonoid and total anthocyanin of fruit teas was determined by using the UV/Vis spectrophotometric method. The phenolic composition was determined and quantified by using high performance liquid chromatography and photodiode array detection (HPLC-PDA). The highest total phenol content and antioxidant were determined in pomegranate. The highest contents of total flavonoid and total anthocyanin were determined in peach and blackberry respectively. Chlorogenic acid, quercetin, myricetin, rutin, rosmarinic acid and ferulic acid were determined in fruit teas. A water temperature of 100^0C was the most effective to extract the highest contents of total phenols, total flavonoids, total anthocyanins and the highest antioxidant capacity in 16 different fruit teas. The purpose of this study was determined the effect of water and temperature on the extraction and quantify the various phenolic compounds in fruit teas by HPLC method for industrial application in producing the extracts.[21]

Several studies reported that the pomegranate contain flavonones, flavonol glycosides, Proanthocyanidins, anthocyanidins, triterpenoids, chalcones and volatile terpenoids and these bioactive compounds are the major antioxidant components. Polyphenolic compounds and tannins performed the antioxidant activities, improved immune function, and prevented heart diseases.

Method used to measure antioxidant activity are DPPH (1,1-diphenyl-2-picryl hydrazyl), FRAP(Ferric reducing antioxidant potential).
Results of DPPH reveals that methanolic root extracts which (88.021%) than the aqueous and ethyl acetate extracts at 40µg/ml extract concentration.

DPPH method elicits that fruit extract contain flavonoid and shows that antioxidant activity. IC50 values of DPPH compared with ascorbic acid as 15.87 standards. Highly antioxidant strength was observed in red followed by pink cultivar than green cultivar.

The treatment with 50mg GA3 increased the fruit set, growth, size, juice and k^+ content. In addition, TSS, total sugar, sugar acid ratio, vitamin-C, anthocyanin, carotenoid, phenolics and flavonoids and antioxidant activity in the fruits were also significantly increased.

CONCLUSION

Antioxidants are any substance that inhibits the oxidative damage to a target molecule. Thus antioxidants prevent cell and tissue damage as they act as scavenger. When an antioxidant destroys a free radical, this antioxidant itself becomes oxidized. There are so many compounds are found to have antioxidant property. Pomegranate is one of a kind which is also very rich in antioxidants.

Anthocyanin is the antioxidant that present in pomegranate that shows the activity.

ACKNOWLEDGEMENT

This work was supported in part by KVM College of pharmacy, Cherthala.

REFERENCES

1. F. Pourmorad, S. J. Hosseinimehr, N. Shahabimajd. Antioxidant activity of phenol and flavonoid content of some selected Iranian medicinal plant. *African Journal of Biotechnology*. 2006; 5(11): 1142-1145.
2. Harleen K. S, Bimlesh K. Sunil P, Prashnant.T, Manoj.S, Pradeep. S. A review of phytochemistry and pharmacology of flavonoid: *An overview. Int.J. Pharma Sci.*. 2011: 1(1): 25-38.
3. Davies. M. J, Judd. J. T, Baer D. J, Clevidence B.A, Paul D.R, Edwards A.J. Chemistry and biological activities of flavonoids: *An overview. Int. J pharma. Sci.*, 2014; (12): 663-669.
4. Joseph. L, Georg. M, Kassaye. G. One pot method for the synthesis of arylidine flavonoids and some of its activities. *AFR.J.EXPER.MICROBIOL*, 2008; 9(3): 147-151.
5. Hui.W, Xiao.N, Min.Z. Solvent free synthesis of flavonoid over new hybrid mesoprous base catalyst. *Chem. Res. Chinese Universities*, 2011; 27(4): 664-668.
6. Pramod. K, Pradeep.W, Zubaidha. P. An improved and eco-friendly method for the synthesis of flavanone by the cyclisation of 2-hydroxy chalcone using methane sulphonic acid as catalyst. *Chemistry Journal*, 2012; 2(3): 106-110.
7. Saumik. D. Synthesis and pharmacological evaluation of flavonoids. *Rajiv Gandhi University of Health Science*, 2011; 1394-1398.
8. Aibogami.A. S, Karama. U, Mousa. A. A, Khan. M, Abdulla. S. M. Simple and efficient one step synthesis of functionalised flavonoids and chalcones. *Orient Journal of Chemistry*, 2012; 28(2): 619-626.
9. Agarwal.A. D. Pharmacological activity of flavonoids: *A review int. J* Pharma. *Sci. Nano tech.*, 2011; 4(2): 1394-1398.
10. Vatkar. B. S, Pratapwar. A. S, Tapas. A. R, Butle. S. R, Tiwari. B. Synthesis and antimicrobial activity of some of flavonoid derivatives. *Int. J. Chem. Tech. Res.*, 2010; 2(1): 504-508.
11. Sen. S, Chakrabarty. R. Free radical antioxidants diseases and phytomedicine: current status and further prospect. *International Journal of Pharmaceutical Science Review and Research*, 2010; 3(1): 91-99.
12. Pharm-Hug LAHEH. *Free radicals antioxidant disease and health. Int. JBS*, 2008; 4(2): 89-96.
13. Vijayakumar. S, Saritha.G. Role of antioxidant and oxidative stress in cardiovascular diseases. *ISSN*, 2010; 1(3): 158-173.
14. Holton T. A, Cornish E. C. Genetics and biochemistry of anthocyanin biosynthesis. The plant cell., 1995; 7: 1071.
15. Lo Piero A. R, Puglisi I, Rapisarda. P, Petrone. G Anthocyanins accumulation and related gene expression in red orange fruit induced by low temperature storage. *J Agri food chem.*, 2005; 53: 9083-9088.
16. Kong J. M, Chian L. S, Goh N. K, Chia T. F, Brouillard. R. Analysis and biological activities of anthocyanins. Phytochemistry, 2003; 64: 923-933.
17. Grotewold. E. The genetics and biochemistry of loral pigments. *Annu. Rev. Plant. Biol*., 2006; 57: 761-780.
18. Joseph. J, Arendash. G, Gordon. M, Diamond. D, Shukitt - hale B, etal., Blueberry supplementation enhances signalling and prevents behavioral deficits in an Alzheimer disease model. *Nutr Neurosci., 2003;* 153-162.
19. Andersen O. M, Markham K. R (2010) flavonoids: chemistry, biochemistry and applications. *CRC Press*. 2000.
20. Harborne J. B, Williams C. A Analytical determination in flavonoid research since. Phytochemistry, 1992; 55: 481-504.
21. Quintana. A. M; Santos-Buelga, C.; Rivas- Gonzalo, J. C. Anthocyanin- dervied pigments and colour of red wines, *Anal. Chim. Acta*, 2002; 458: 147-152.