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REVIEW ARTICLE

A COMPREHENSIVE STUDY ON THE NATURAL PLANT PHENOLS: PERCEPTION TO CURRENT SCENARIO

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Phenolic compounds are secondary metabolites of plants that are widely distributed throughout the plant kingdom. Secondary metabolites possess structural diversity that provide flavor and color to fruits, vegetables, and grains. They precipitate various pharmacological and toxicological effects on living beings. Extraction of the bioactive plant constituents has always been a challenging task for the researchers. In the present study, an attempt has been made to give an overview on chemistry, distribution, extraction and isolation techniques of various plant phenolics.

Key words: Polyphenols, Plant kingdom, Plant phenolics, Distribution, Extraction techniques.

INTRODUCTION

All plants create numerous organic amalgams that are always not related to the basic metabolism like progress, growth and development and the functions of these natural compounds in plants have only been noticed recently in a diagnostic perspective. Although these organic amalgam produced by the plants do not help in growth, they are effective in other ways. While some of these protect plants from predators and pathogens, others help in the reproductive process by fascinating pollinating agents or scattering the seeds. Many of them are also useful as they help to produce poisons that protect the plants. Most of the organic products found in plants may be grossly put into three broad categories-alkaloids, phenylpropanoids and terpenoids. While phenylpropanoids are mostly phenolic amalgams, terpenoids are made up of five carbon elements that are blended through the acetate or mevalonate method or the glyceraldehydes 3-phospate/pyruvate process (Robbins, 2003). Many of the terpenoids produced by the plants are a sort of contaminants that protect the plants from being devoured by herbivores creatures or functionally attractants for pests and animals. Basically, all phenols and phenolic amalgams are widely found in nature and can also be blended artificially. They form a separate group of chemical substances that comprises a member of hydroxyl cluster linked to an element of hydrocarbon set. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Phenolic substances tend to be water soluble, since they most frequently occur in combined form with sugar, as glycosides and they are usually located in the cell vacuole (Anonymous, 1992; Madaan *et al* 2011). Among the natural phenolic compounds, of which several thousand structures are known, the flavonoids form the largest group but simple monocyclic phenols, phenylpropanoids and phenolic quinones all exits in considerable numbers. A number of vital vegetation ingredients including tannins, phenolic amalgams, flavones, coumarins, anthraquinones and all their glycosides comprise of phenolic

molecules (Trease and Evans, 1997; Shah and Quadry 1990).

Classification of phenolic compounds

- Simple phenol and phenolic acids
- Tannins
- Quinone pigments
- Flavonoids and related classification
- Anthocyanins and Anthocyanidines
- Xanthone and stilbene

Simple phenol and phenolic acids

Chemistry and distribution

Phenols particles are created when the hydrogen content in a pungent nucleus is swapped with an element from the hydroxyl cluster and this process may happen several times making the isomers include three probable di-hydroxyl-benzenes as well as three tri-hydroxyl-benzenes. While the di-hydroxyl-benzenes are hydroquinone, catechol and resorcinol, the tri-hydroxyl-benzenes include benzenitrol, pyrogallol and phloroglucinol (Robbins, 2003). Interestingly, the hydroxyl set in phenol molecule imparts features of alcohol and tends to classify phenols as tertiary alcohols; basically the hydroxyl group in phenol is linked to a dilute carbon nucleus. Thus, phenols are not alcohols, but actually classified under weak acids. The free phenols and phenolic acids are best considered together, since they are usually identified during analysis of plant tissue to release a number of ether soluble phenolic acids. These acids are either associated with lignin combined as ester groups or present in the alcohol soluble fraction bound as simple glycosides. 4-Hydroxy benzoic acid, protocatechuic acid, vannilic acid and syringic acids, and gentisic acid are widely distributed among the angiosperms (Singh, 2011).

Phenolic compounds are mostly found in vascular plants (tracheophytes) *i.e.* Lycopodiophyta, Pteridophyta (ferns and horsetails), Angiosperms (flowering plants or Magnoliophyta) and Gymnosperms (conifers, cycads, Ginkgo and Gnetales). Beside this, they are also found in non-vascular land plants (bryophytes) (Erickson and Miksche, 1974). Dihydrostilbenoids and bis(bibenzyls) are found in liverworts (Marchantiophyta), rosmarinic acid and a rosmarinic acid 3′-O-*β*-D-glucoside can be found in the hornwort *Anthoceros agrestis* (Vogelsang *et al* 2006).

Phenolic acids are abundant in a balanced diet. Examples of foods rich in phenolic acids include mangos, berries, apples, citrus fruits, plums, cherries, kiwis, onions, tea, coffee, red wine, and flour made from whole wheat, rice, corn or oats (Vasco, 2009).

There are many different phenolic acids found in nature and they can be divided into two categories: benzoic acid derivatives, such as gallic acid; and cinnamic acid derivatives, including caffeic acid and ferulic acid. The cinnamiac acids are more common.

Gallic acid is found in tea and grape seeds. Coffee contains caffeic acid and chlorogenic acid. Blueberries, kiwis, plums, cherries and apples contain large amounts of caffeic acid. Red wine and citrus fruits contain cinnamic acid. Ferulic acid is esterified to hemicelluloses in the cell wall found in the outer coverings of cereal grains. Corn flour possess maximum ferulic acid whereas whole grain wheat, rice, and oat flours are good sources of ferulic acid (Liu, 2004).

Hydroxy-benzoates or benzoic acid derivatives

The hydroxyl-benzoates directly derived from benzoic acid, have the C_6-C_1 skeleton and are universally distributed among the angiosperms. Variations in the structures of the individual hydroxyl-benzoates lie in the hydroxylation and methylation patterns of the aromatic ring. The four most common ones are *p*-hydroxy-benzoic, vanillic, syringic and protocatechuic acids. They may be found either associated with lignin (*i.e.* in cell wall formation) or present in alcohol-insoluble fractions of leaves or alternatively in alcohol soluble portions conjugated as glycosides. Gallic acid is worth mentioning as it is found in many woody plants. This tri-hydroxyl derivative is known to participate in the formation of hydrolysable gallotannins. Its dimeric condensation product (hexahydroxy diphenic acid) and related di-lactone, ellagic acid, are common metabolites. Also known are the aldehydes corresponding to these acids: vanillin (most wide spread) anisaldehyde (in most essential oils) and salicylaldehyde among others.

Phenyl-propanoids

Phenyl-propanoids are naturally occurring phenolic compounds having an aromatic ring to which a three-carbon side chain is attached. The most widespread are the hydroxyl-cinnamic acids and the four most common ones are *p*-coumaric, caffeic, ferulic and sinapic acids that play a pivotal role in phenolic metabolism. Hydroxy-cinnamic acids usually occur in plants

in combined forms as esters. Those with quinic acid are particularly common and chlorogenic acid is almost universal in distribution. An interesting point to be noted is that the systematic distribution of hydroxyl-cinnamate conjugates may show correlation with the systematic arrangements of plant families. In the pharmaceutical sector, for instance, caffeic acid derived from *Cyanora scolymus* L. is used to treat digestive disorders. Rosmarinic acid, another phenolic acid derivative from *Rosmarinus officinalis* L. has diuretic activities and antioxidant properties. Moreover, all these compounds show antibacterial, antifungal and antitumoral activities. Most wide spread are the Hydroxyl cinnamic acids, hydroxyl coumarins, phenyl propenes and lignans.

Hydroxy-cinnamic acids

L-Phenylalanine and L-tyrosine, as C_6-C_3 building blocks, are precursors for a wide range of natural products. In plants, a frequent first step is the elimination of ammonia from the side-chain to generate the appropriate *trans*-(*E*)-cinnamic acid. In case of phenylalanine, this would give cinnamic acid, whilst tyrosine could yield 4-coumaric acid (*p*-coumaric acid). All plants appear to have the ability to deaminate phenylalanine *via* phenylalanine ammonia lyase (PAL) enzyme, but the corresponding transformation of tyrosine is more restricted, being mainly limited to members of the grass family (Graminae/Poaceae). The most representative cinnamic acid is caffeic acid, which occurs in fruits, vegetables and coffee, mainly as an ester with quinic acid (chlorogenic acid or 5-caffeoylquinic acid).

Coumarins

The most widespread plant coumarin is the parent compound coumarin itself, around 1300 coumarins are known, which occurs in over 27 plant families. It is common in many plants grasses and fodder crops. More complex coumarin - furanocoumarins *i.e.* Psoralen are generally restricted to few families such as Rutaceae and umbelliferae**.** With all of them being derivatives of 5, 6-benzo-2-pirone (*α*-chromone) (with OH, OCH_3 or CH_3 substituents on the benzoic ring.). In addition to simple coumarins, *C*-prenylated and *O*-prenylated forms also exist. As derivatives of simple coumarins, other compounds are known, such as furano-coumarins, which include a furanic ring, linear pyrano-coumarins, angular pyrano-coumarins, dimeric coumarins, of which dicoumarol is an example and also furano-chromones. (Proença da Cunha, 2005) Historically, the ability of dicoumarol to inhibit blood clotting, that later led to the development of the anticoagulant drug warfarin, was the first call to this class of compounds biological properties. Several biological activities have been reported in natural-occurring coumarins, from photo sensitizers to vasodilatation. Recently, the interest has been given to synthetic derivatives of coumarins, such as fluorinated and 1-azo coumarins, which displayed moderate analgesia properties, and excellent anti-inflammatory and anti-microbial activities (Kalkhambkar *et al* 2008) (**Figure 1**).

Lignin and Lignan

Cinnamic acids also feature in the pathways to other metabolites based on C6-C3 building blocks. An important example is the plant polymer lignin, a strengthening material for the plant cell wall which acts as a matrix for cellulose microfibrils. Lignin is formed by phenolic oxidative coupling of hydroxyl-cinnamoyl alcohol monomers, brought about by peroxidase enzymes. The most important of these monomers are 4-hydroxycinnamoylalcohol (*p*-coumaroyl alcohol), coniferyl alcohol and sinapoyl alcohol, though the monomers used vary according to the plant type.

Lignans are organic compounds resultant from the establishment of a link between *β* carbons of the side chain of two 1-phenylpropane derivatives (8-8’ link). Lignans are dimeric compounds formed essentially by the union of two molecules of a phenyl propene derivative, mainly found in heartwoods. One other group of phenyl propanoids- the phenyl propenes contribute to the volatile flavour and odour of plants. They are lipid soluble, distinct from other phenolic compounds. Some are widespread, such as eugenol. Others are anethole and myristicin (Pereira *et al* 2009).

Extraction of plant phenols

A general method of extraction commences with the extraction of either the fresh or dried plant material with a non-polar solvent (*e.g.* petroleum ether, benzene, chloroform). This will remove all phenolic, non-polar substances. The residue is extracted with acetone, methanol or water, the choice of solvent depending upon the number of hydroxyl and sugar groups in the molecule to be extracted. The solvent number is reduced and

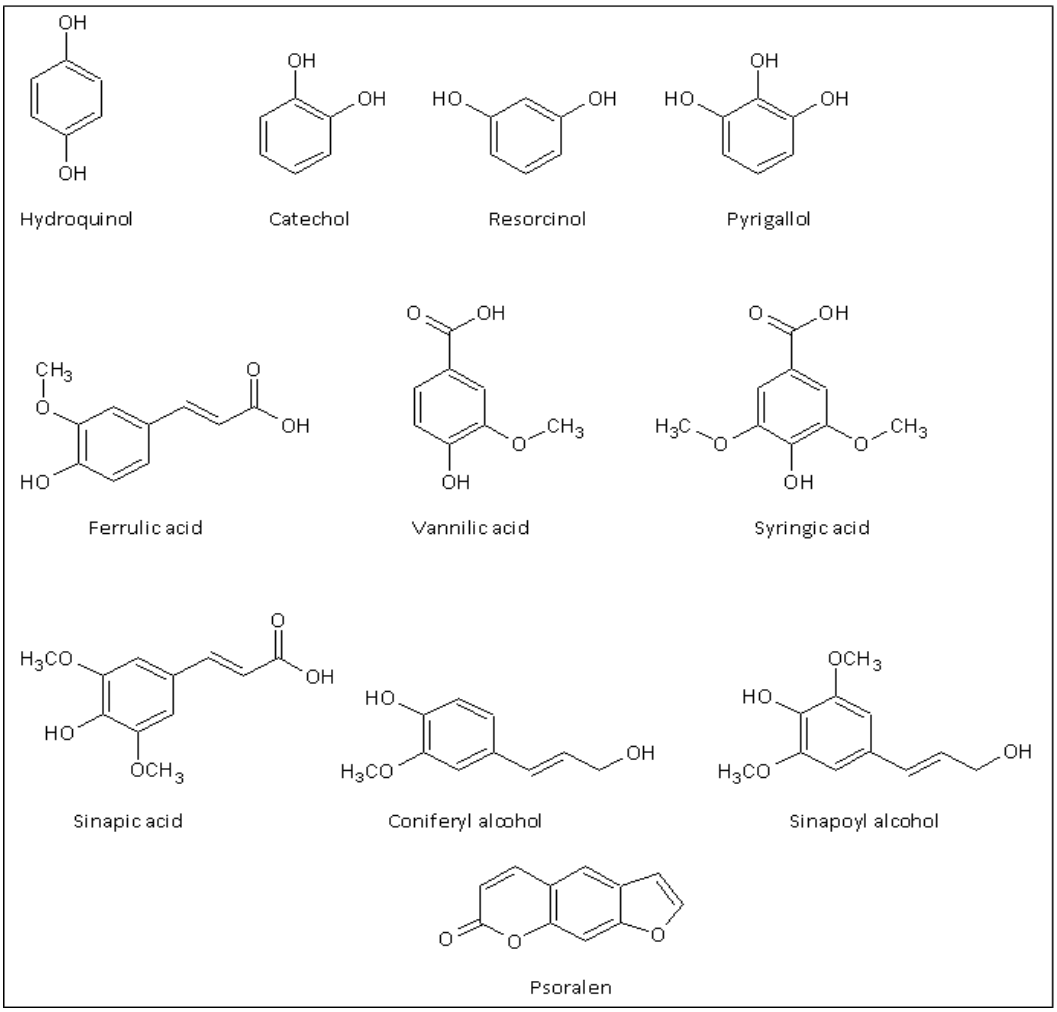

Fig. 1. Structures of some simple phenol and phenolic acids

mixture is extracted with extract transferred to an immiscible solvent (*e.g.* ether, butanol, or ethyl acetate) and mixture is extracted with successive dilute solution of progressively strong bases (passing from sodium acetate to $NaHCO_3$ to Na_2CO_3 to NaOH). The aqueous extracts are acidified and those containing the required phenolics are reextracted with butanol, ether, or ethyl acetate.

Isolation techniques of plant phenolics

Phenolic acids are extracted according to the method of Bate-Smith (Bate-Smith, 1954). About 10-15 g of fresh material is extracted with 2 N HCl and digested on a boiling water bath for 20 min. The digest is filtered and the filtrate is shaken with diethyl ether followed by 20 min heating and then shaken several times with ether (Reddy *et al* 1975). All other extracts are combined and the phenolic acids are taken up from the combined extract into 5% sodium carbonate which is acidified and reextracted with ether and separates the phenol constituents with thin layer chromatography (TLC) and paper chromatography.

TLC is performed using silica gel in AcOH-$CHCl_3$ and ethyl acetate-benzene and separately on cellulose MN 300 in benzene-methanol-acetic acid and aqueous acetic acid. Phenol absorbs in the short UV, and can be detected at 253 nm resulting in dark spot on silica gel containing fluorescent indicator.

Paper chromatographic analysis is performed using a two-dimensional ascending technique on Whatman No. 1 chromatography paper. The solvents used are benzene-acetic acid-water (60:70:30 upper phase) in first direction and the sodium formate-formic acid-water (10:1:200) in the second direction (Ibrahim and Towers, 1960).

Detection is performed preferably by using more specific reagents like Folin-Ciocalteu (phenols with catechol or hydroquinone appear as blue spots), vanillin-HCl and vanillin-H_2SO_4 (Formation of a range of pink colours with resorcinol and phloroglucinol derivatives), Gibbs

reagent followed by fuming the plate with 2 M NH_4OH which gives variety of colours with different phenols. It can distinguish vannilic acid (pink) from isovannilic acid (blue), isomers which have rather similar R_f values.
Another method which is performed now a days, is reverse-phase HPLC (using columns of Bondapak C18 and Spherisorb C18 and the solvent water-methanol-acetic acid (12:6:1) and water-acetic acid-*n*-butanol (342:1:14) which has successively resolved mixtures of phenolic acids and phenolic aldehydes (Harborne, 1998).

Isolation and separation of phenyl propanoids
Hydroxyl-cinnamic acids and hydroxyl-coumarins:
These are detected together by chromatography rather by one dimensional on paper or two dimensionally on plates of microcrystalline cellulose. These compounds are very easily detected, since they give characteristics fluorescent colors in UV light, which are intensified by further treatment with ammonia vapour. Cinnamic acid can be differentiated from coumarins by their less intense fluorescence.

Furano-coumarins:
They are easily separated by TLC on silica gel. Suitable solvents include pure chloroform, chloroform containing 1.5% ethanol, ether-benzene (1:1) and ether-benzene-10% acetic acid (1:1:1). Times of development vary between 1 and 2 h. Furanocoumarins are detected by their blue, violet, brown, green or yellow colours in UV light. The colour may be intensified by spraying plates with 10% KOH in methanol, or 20% antimony chloride in chloroform.

Phenyl-propenes:
They are easily separated on silica gel plates in benzene, mixture of benzene with chloroform (10%) or light petroleum (20%) or in *n*-hexane-chloroform (3:2). They give colored spots when sprayed with vanillin-1 M H_2SO_4 or with Gibbs reagent.

Lignans:
Lignan may be separated by TLC on silica gel using such solvent as ethyl acetate-methanol (19:1) or benzene-ethanol (9:1). These can be seen as dark absorbing spots on paper in short UV light or can be revealed by spraying with 10% antimony chloride in chloroform. On TLC plates, they are detected by spraying conc. H_2SO_4. HPLC with its high resolving power is useful when complex mixtures of phenolics are encountered. Retention time of many phenyl propanoids on a Lichosorb RP-18 column had been eluted with varying proportion of water-formic acid (19:1) and methanol (Harborne, 1998).

Estimation of plant phenolics
Alkaline extraction
This method is used for determining the phenolic content of essential oils. For the extraction of essential oil with 3-5% KOH, decrease in volume of oil layer is used to calculate the phenolic content.

Acetylation
The sample containing 0.1% to 1meq of acetyl able hydrogen, is titrated with 1 ml of the reagent (5 vol. Ac_2O and 12 vol. pyridine), heated to boiling and kept in a constant temperature bath (118°C) for 5 min. It is cooled, washed into a 250 ml Erlenmeyer flask with 40 ml of water and titrated with standard base using phenolphthalein as indicator. A blank is prepared, but not heated and titrated in the same manner as the sample. The difference between the titration gives the quantity of acetic acid equivalent to the phenol. One mole acetic acid is one hydroxyl group.

Titrimetry bromide-bromate method
The method is based upon the general assumption that nascent bromine will replace quantitatively only those H-atoms which are ortho or para to the hydroxyl group of phenols. The most general procedure consists of pipetting a certain volume of phenolic solution (25 ml) into a glass-stoppered flask and adding sufficient water to make up volume to 50 ml followed by addition of 5 ml of concentrated HCl. Enough 0.1 N bromides-bromate solution is run in from a burette to give a slight excess of bromine. Shake intermittently for 5 min, then add 10 ml of 10% KI solution. Care is being taken to avoid escape of bromine. After shaking for few minutes, titrate with 0.1 N $Na_2S_2O_3$ using starch as indicator.

Colorimetric method
A colorimetric procedure is based on formation of insoluble dark colored precipitate formed by mixtures of ortho- and meta-dihydroxy phenols in presence of iodine. The precipitates after the excess iodine is destroyed, are dissolved in acetone and estimated colorimetrically. A more general method, which depends on treatments of the phenols with HNO_3 and H_2SO_4 at 100°C to

form nitroso phenols which rearranges in the presence of excess alcoholic NH_4OH that can be used to form the highly coloured quinoid radical. The resulting coloured solutions are compared with standard phenol solution.

Gravimetric method
The classical method of this type is the formation of insoluble bromination products by the action of bromine water on the phenol. This method is satisfactory for concentrated solutions. However, the method has been extended to dilute solutions by measuring the turbidity of phenol solutions treated with bromine water. 2, 4-Dinitro-fluoro-benzene is used as a precipitant for the qualitative determination of phenolics. The 2, 4-dinitrophenyl ethers are filtered off and weighed.

Spectrophotometric methods
The fact that all simple phenols absorb UV light strongly with principal absorbance maxima in the 270 to 280 μm wavelength region, has been utilized for the determination of phenolic compound separated by paper chromatography. The paper containing a given phenol is extracted with alcohol in a micro soxhlet extractor and the concentration determined spectrophotometric estimation therefore is used for determining mixtures of certain phenols.

HPLC analysis
Usually a trial run was performed on a new sample to determine the optimal volume of sample to be injected on the HPLC system. For the best determination of the phenolic content, 25 μl of the sample is used for the initial run. Typically, the injection volumes of leaf extract samples remain around 25 μl while an injection volume of extract samples is around 100 μl. Peak area percentage and extinction co-efficient are calculated from chromatograms of standard detected at 285 nm. This wavelength is chosen for monitoring because most phenolics absorb at this wavelength.

Determination of total phenolics
For determination of total phenolics, put the extract in a 10 ml glass tube and make up to a volume of 3 ml using distilled water. Then, add 0.5 ml folin ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) sequentially in each tube. A blue color appear in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which results in a blue colored complex, molybdenum blue. The test solutions are then warmed for 1 min and allowed to cool. Absorbance is measured at 650 nm against the reagent using a blank. The concentrations of phenols in the test samples are calculated from the calibration plot and expressed as mg catechol equivalent of phenol/g of sample (Khatiwora *el al* 2010).

Tannins

Chemistry and distribution

Tannins occur widely in vascular plants, their occurrence in the angiosperm being particularly associated with woody tissue. They have the ability to react with protein, forming stable water insoluble co-polymers.

Tannins are involved in the bond formation between the collagen fibre which imparts resistance to water, heat and abrasion. This also explains their characteristic astringency and tartness.

In the plant cell, tannins are located separately from the proteins and enzymes of the cytoplasm but when tissue is damaged, *e.g.* when animals feed, the tannin reaction may occur, making the protein less accessible to digestive juices of the animal. In the higher plants, two groups of tannins are generally distinguished, which differ by their structure, as well as their biogenic origin.

Tannins are distributed in species throughout the plant kingdom and commonly found in both gymnosperms as well as angiosperms including the best known families such as aceraceae, actinidiaceae, anacardiaceae, bixaceae, ericaceae, burseraceae, combretaceae, dipterocarpaceae, grossulariaceae, myricaceae in dicot and najadaceae and typhaceae in monocot. Tannins are mainly physically located in the vacuoles or surface wax of plants. These storage sites keep tannins active against plant predators, but also keep some tannins from affecting plant metabolism while the plant tissue is alive; it is only after cell breakdown and death that the tannins are active in metabolic effects.

Tannins are classified as ergastic substances *i.e.* non-protoplasm materials found in cells. Tannins are found in leaf, bud, seed, root and stem tissues (Salunkhe *et al* 1990). Tannins may help regulate the growth of these tissues. Tannins are another major group of polyphenols in our diets and usually subdivided into four groups *viz.* hydrolyzable tannins, condensed tannins, complex tannins and pseudo tannins.

Hydrolyzable tannins
These are ester of sugars and phenolic acid molecules (gallic acid andellagic acid), hydrolyzed by mineral acids, alkalis and tannase enzyme yielding gallic acid or hexahydroxy diphenic acid with sugar (mainly glucose). Hydrolyzable tannins on dry distillation yield pyrogallol and on reaction with $FeCl_3$ produce bluish black precipitate. These are further sub divided into gallotannins and ellagitannins on basis of their product of hydrolysis (**Figure 2**).

Gallotannins:
These are the simplest one also known as galloylglucose, in which a glucosecore is surrounded by five or more galloyl ester groups and on hydrolysis yields gallic acids and glucose molecule. The molecular weight of gallotannins ranges between 1000-1500. *e.g.* nutgal, rhubarb, clove and chest nut etc.

Ellagitannins:
This is composed of phenolic acids as dimer of gallic acid *i.e.* hexahydroxydiphenic acids (HHDP) and attached glucose molecule, mol. wt. ranging between 1000-3000. These on hydrolysis yield HHDP (readily lactonized to give ellagic acid, with glucose molecule. *e.g.* oak, myrobalan, pomegranut bark etc.

Condensed tannins
These are also called flavolans, proantho-cyanidins, non-hydrolyzable tannins, catechol tannins and phlobatannins, molecular weight ranging between 1000-3000. Condensed tannins are resistant to hydrolysis, does not contain sugar molecule, derivatives of flavones like catechin, flavan-3-ol, flavan 3-4 diol etc. Condensed tannins on treatment with acids or enzymes converted to a red water insoluble compound called as phlobaphene which imparts typical brownish color to many of the plant materials mainly bark.
These on dry distillation gives catechol; on treatment with ferric chloride give brownish green color, with dil. HCl yields phloroglucinol that gives red colour with matchstick on reaction with lignin, with vanillin HCl gives red colour and precipitated with bromine water. *e.g.* green tea, cinnamon bark, cinchona bark, wild cherry bark, oak, coca, catechu etc.

Complex tannins
These are formed by the combination of hydrolyzable and condensed tannins. The formation of complex tannins occurs by the union of C-C bond between C1 of glucose unit of ellagitannins and C8 or C6 of flavones-3-ol. These are present in dicot plants mainly in family rosidae and dilenidae etc.

Pseudo tannins
These are also known as prototannins or tannin precursors, high molecular weight compounds do not respond to protein precipitation test (Gold beaters skin test) with the molecular weight ranging between 200-600. *e.g.* gallic acid, chlorogenic acid, nux-vomica seed etc.

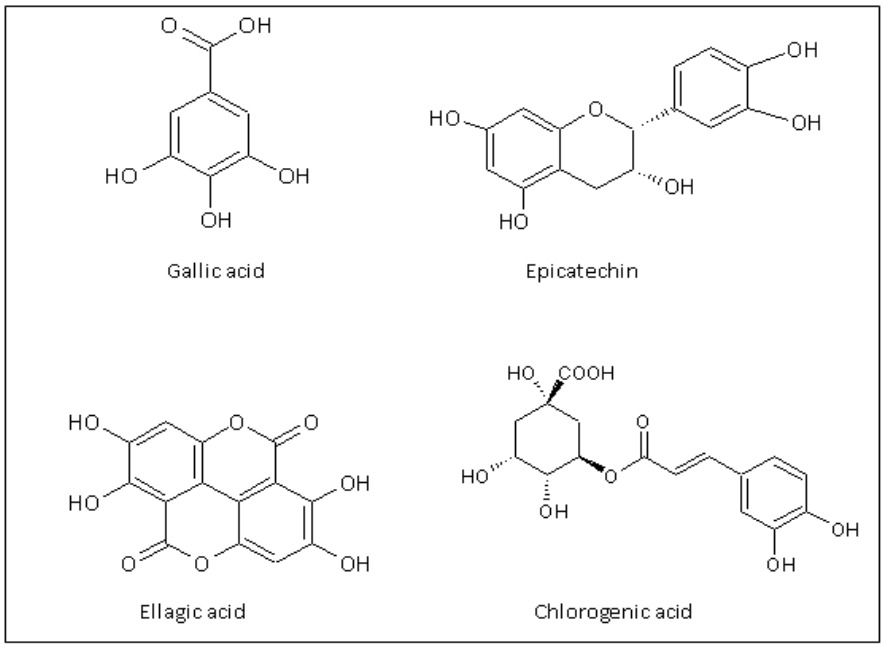

Fig. 2. Structures of some tannins

Extraction of tannins

Tannins are generally extracted with a water and acetone mixture, after eliminating the acetone by distillation. The pigments and lipids are removed from the aqueous solution by a solvent extraction (with dichloromethane), next ethyl acetate extraction of the aqueous solution separates the dimeric proanthocyanidins and most Gallo tannins. The polymeric proanthocyanidine and high molecular weight Gallo tannins remain in the aqueous phase. To obtain pure compound, the appropriate chromatographic techniques are used, most often gel filtration techniques followed by reverse phase chromatography, again in water and alcohol or water, alcohol and acetone mixtures.

Isolation and separation of tannins

Hydrolyzable tannins:

Isolation of hydrolyzable tannins by HPLC is successful. Gradient elution on a RP-18 Lichrosorb 10 μm column, using increasing acetonitrile concentrations in water containing 0.05% H_3PO_4 separates galloyl esters from each other according to the number of galloyl residues.

Condensed tannins:

The isolation of proanthocyanins (condensed tannins) is commonly carried out by employing Sephadex LH-20 column chromatography (Strumeyer and Malin, 1975; Davis and Hoseney, 1979; Asquith *et al* 1983; Nonaka *et al* 1983). The crude extract is applied to the column which is then washed with ethanol to elute the non-tannin substances. Following this, proanthocyanidins are eluted with acetone-water or alcohol-water. An inert glass powder (Pyrex microparticles, 200-400 μm) has been employed for fractionation of grape (seed or skin) proanthocyanidins according to their degree of polymerization (Labarbe *et al* 1999). Purified proanthocyanidins were dissolved in methanol and then applied onto the column filled with glass micro particles and equilibrated with methanol-chloroform (25:75, *v/v*) and massively precipitated on top of the column with chloroform. Proanthocyandins were sequentially eluted from the column by increasing proportions of methanol in a methanol-chloroform solvent system. The molecular weight and composition of mixed polymers can be directly determined by ^{13}C NMR spectral measurement (Naczk and Shahidi, 2004).

Chemical tests for tannins

Tannins show specific chemical reaction like solution of tannins precipitate gelatin, alkaloids, salt of copper, lead and tin and shows color reaction with $K_2Cr_2O_7$ and iron salts.

Test with Iron salts

It show color reaction with iron salt like $FeCl_3$ and potassium ferrrocyanide $K_4Fe(CN)_6$ in presence of ammonia. Addition of $FeCl_3$ solution to solutions of hydrolyzable tannins forms bluish-black precipitate whereas with condensed tannins, it forms greenish-brown precipitate.

Goldbeater's skin test

Goldbeater's skin is the membrane prepared from ox intestine and behaves like untanned hide. Small piece of ox-intestine is dipped in 2% dilute HCl, rinsed with distilled water and then soaked in test solution for few minutes. It is again rinsed with distilled water and transferred to 1% solution of ferrous sulphate. Formation of brownish black color indicates the presence of tannin. This is positive for all true tannins but negative for pseudo tannins.

Gelatin test

To the aqueous solution of gelatin (1% *w/v*), 0.5-1.0% solution of tannin is added. Formation of buff coloured precipitate indicates presence of tannins. Pseudo tannins also show this test positive if tannin is present in sufficient amount.

Phenazone test

Aqueous extract of drug (5 ml) is mixed with 0.5 g of solid sodium acid phosphate ($NaHPO_4$), the solution is heated to boiling, cooled and filtered. Filtrate is treated with 2% solution of phenazone to form precipitate of tannins.

Test for catechin (Matchstick test)

Catechins form phloroglucinol when heated in presence of acids and can be detected by reaction with lignin forming woody red to magenta colour. The paste of test drug (tannin) is applied on the rear end of matchstick and moistened with conc. HCl. Formation of woody pink to magenta colour on heating near the flame indicates presence of condensed tannins.

Test for chlorogenic acid

Extracts of drug containing chlorogenic acid on treatment with aqueous ammonia is converted to green color after exposing to air.

Vanillin HCl test
Solution of test drug is mixed with few drops of vanillin HCl. Pink colour developes in presence of tannins due to conversion of phloroglucinol from catechin.

Bromine water test
Condensed tannins are precipitated in presence of bromine water (De *et al* 2010; Bruneton, 1995).

Quinone pigments
Chemistry and distribution
The natural quinone pigments range in colors from pale yellow to almost black. Although, they are widely distributed and exhibit great structural variation, they make relatively little contribution to color in higher plants. Thus, they are present in bark, heartwood, root, leaves or other tissues where their colors are masked by other pigments. Quinones are oxygen containing compounds which are essentially the oxidized homologous of aromatic derivatives, and are characterized by a 1,4-di keto cyclohexa-2, 5-diene pattern (paraquinones). In naturally occurring quinones, the dione is conjugated to an aromatic nucleus (benzoquinones) or conjugated to a condensed polycyclic aromatic systems: naphthalene (napthoquinones), anthracene (anthraquinone), 1,2-benzaanthracene (anthro-cyclinones), napthodianthrene (napthodi-anthrones) which undergoes reversible redox reaction in the presence of reductase enzymes and the reduced form of quinone (hydroquinone) which occurs as the glycoside arbutin. Many of the more complex compounds including some napthoquinones and anthraquinones have phenolic structures. Quinones form an important component of the electron transport system in plants and mammals *i.e.* ubiquinol; the reduced form of coenzyme Q10 and menaquinone or Vitamin K have significant anti-oxidant properties, playing a major role in protecting cells from free-radical damage (Pengelly, 2004).
Many quinones are known to induce contact dermatitis and repiratory reactions in susceptible people. Napthoquinones including lapachol and others found in the Ebonaceae family have been associated with these reactions, are colored and contain the same basic chromophore, that of benzoquinone itself, which consist of 4 groups the benzoquinones, isoprenoidquinones, napthoquinines and the anthroquinones.
Benzoquinone are characteristics of arthropoda and rather rare in higher plants. Napthoquinone is limited in fungi and sporadic in angiosperms. Anthroquinone are rather widely distributed in fungi lichen and to a lesser extent, Spermatophyta. They are abundant in angiosperm families: rubiacae, fabaceae, polygenaceae. The derivative of anthraquinone present in purgative drugs may be dihydroxy phenols such as emodin, tetrahydroxyl phenol such as carminic acid.
The first three groups are generally hydroxylated with "phenolic" properties and may occur *in vivo* either in combined form with sugar as glycoside or in a colourless, sometimes dimeric, quinol form. In such cases, acid hydrolysis releases free quinones. The isoprenoid quinones are involved in cellular respiration (ubiquinones) and photosynthesis (plastoquinones) and thus universally distributed in plants (Harborne, 1998).

Anthraquinones
It is also known as anthracene glycosides. Anthraquinone are yellow brown pigments contained in many plants. These pigments are used as dyes for textiles *e.g.* Rubia tinctoria-Dyer's Madder. The aglycones consist of two or more phenols linked with a third carbon ring. Hydroxyl groups always occur at positions 1 and 8, hence they are 1, 8 anthaquinones which from O-glycosides. The laxative effect of anthraquinones is used with sennosides A and B. Initially these compounds pass through the stomach and small intestine unaltered, but in the cecum and colon they are converted to dianthrones, which remain unabsorbed, are further transformed into anthrone and anthraquinone, produceing hydragogue and laxative effects in the process.

Napthoquinones
These are dark yellow pigments. The hair dye henna is derived from the plant Lawsonia inermis, which contains the napthoquinone lawsone linked to a sugar (*i.e.* a glycoside). Other napthoquinones have antimicrobial and antifungal properties. These include juglone from the walnut and butternut - *Julglans cineria*, which occurs in leaves and stain derived from the fresh plants.
The leaves are also rich in hydrolyzable tannins; hence they are of benefit for pile and venous insufficiency. Juglone is a laxative and vermifuge agent. Other napthoquinones with potent

antimicrobial properties are plumbagin from the sundew *Drosera rotundifolia* and lapachol from Pau D'arco - *Tabebuia impertigniosa*.

Extraction of quinone

Extraction of napthoquinone

The plant parts are extracted by soxhlet extraction method in which 100 g of plant powder is extracted by using methanol in a soxhlet apparatus in menstruum to drug ratio of 5:1 till extract becomes colorless. The semisolid masses (about 20-25% *w/w*) were obtained after evaporation of solvent. Then, the semisolid masses were separated into phenolic (sodium hydroxide soluble) and neutral fraction by using 3% sodium hydroxide solution. The solution was filtered. The pH of filtrate was adjusted to 4.5 by using 1N hydrochloric acid and extracted with chloroform. Then the organic layer was washed with water, dried and then passed through anhydrous sodium sulfate and evaporated to obtain crude naphthoquinone extract (about 1% *w/w*) (Karpe *et al* 2011). The above extract was subjected to HPTLC analysis using HPTLC LINOMET 5 applicator and CAMAG 3 scanner with the help of Wincat software for characterization. The chromatograms were developed using toluene:acetone:acetic acid (9:1:0.1) as solvent system (Harborne, 1998).

Extraction of anthraquinone

Decoction:

The fresh drug is boiled with 100 ml distilled water for 1 h at 95-98°C. The mixture was filtered through muslin and the pulp is reextracted with water several times until the extraction was exhausted. The decoction extracts is combined filtered again and the filtrate is evaporated to dryness on a boiling water bath to yield a decoction crude extract.

Soxhlet extraction:

The fresh drug is extracted with 300 ml of 70% ethanol in a soxhlet apparatus. The extraction is continued until the extraction is exhausted. The extracts are then combined, filtered and evaporated to dryness on a hot water bath to yield a soxhlet crude extract (Sakulpanich and Gritsanapan, 2008).

Isolation and separation of quinones

Separation of quinones is performed using TLC on silica gel. Since simple benzoquinone and napthoquinone have high lipid solubility, hence they may separate in pure benzene, pure chloroform, pure petroleum or simple mixture of these solvents. As quinones are colored compounds so they can be easily detected in visible light or UV light on TLC plates.

HPLC can also be used for quinone analysis. Benzo- and napthoquinones have been separated on Micropak Si-10 columns in 1% isopropanol in petroleum, while anthraquinone separate on Micropak CH-5 columns in methanol water (1:1) acidified to pH 3. Isoprenoid quinones are generally separated and detected by one or two dimentional TLC on silica gel.

Ubiquinone is separated by using paraffin-coated kieselguhr G plates, acetone-water (9:1) solvent system and antimony chloride in chloroform as detecting agent (Harborne, 1998).

Test for anthraquinone glycosides

Borntrager's test

Boil the test material with 1ml of dilute sulphuric acid in a test tube for 5 min (anthracene glycosides are hydrolyzed to aglycone and sugars by boiling with acids). Centrifuge or filter while hot (if centrifuged hot, the plant material can be removed while anthracene aglycones are still sufficiently soluble in hot water, they are however insoluble in cold water), pipette out the supernatant or filtrate, cool and shake with an equal volume of dichloromethane (the aglycones will dissolve preferably in dichloromethane). Separate the lower dichloromethane layer and shake with half its volume of dilute ammonia. A rose pink to red color is produced in the ammonical layer (aglycones based on anthroquinones give red color in the presence of alkali).

Modified Borntrager's test

Boil 200 mg of the test material with 2ml of dilute sulphuric acid, 2 ml of 5% aqueous ferric chloride solution for 5 min and continue the test as above. As some plant contains anthracene aglycone in a reduced form, if ferric chloride is used during the extraction, oxidation to anthroquinones takes place, which shows response to the Borntrager's test (Trease and Evans, 1997).

Flavonoids

Over 6,000 different substances found in virtually all plants, are responsible for many of the plant colors that dazzle us with their brilliant shades of yellow, orange, and red. Classified as plant pigments, flavonoids were discovered in 1938 when a Hungarian scientist named Albert

Szent-Gyorgyi used the term "vitamin P" to describe them. Flavonoids may also be named directly after the unique plant that contains them. Ginkgetin is a flavonoid from the ginkgo tree, and tangeretin is a flavonoid from the tangerine. Flavanoids are present in all vascular plants. The term flavonoids are virtually universal plant pigments which is usually water soluble and are responsible for the colour of flowers, fruits and sometimes leaves like yellow flavonoid (chalcones, aurones and yellow flavanols) and red blue or purple anthocyanins.

All flavonoids have a common biosynthetic origin, and therefore possess a same basic structural element, namely the 2-phenylchromane skeletons. All contain fifteen carbon atoms in their basic nucleus and these are arranged in a C_6-C_3-C_6 configuration, that is two aromatic rings linked by a three carbons unit which may or not form a third ring. Most of the flavonoids have a carbonyl function situated at one end of the bridge. Chalcone and dihydrochalcone represent the two classes of flavonoids in which the 3-C bridge in “open”. But in remaining classes, the three-carbon bridge is a part of heterocyclic ring, involving a phenolic group on the adjacent ring (**Figure 3**).

Chemistry and distribution

The chemistry of flavonoids is complicated, and within the non-technical term "flavonoids" can be found in many different chemical groups of substances. These groups include flavonols, dihydroflavonols, flavones, isoflavones, flavanones, anthocyanins, and anthocyanidins (Srividya *et al* 2012). For example, well-known flavonols include quercetin, rutin, hesperidin, apigenin and luteolin (**Figure 3**).

Flavones

These molecules represent the majority of known flavonoids. In this, two phenolic hydroxyl groups at C-5 and C-7 established. These are wide spread in the leaves and flowers of angiosperms. Flavones itself is rare, but occurs as a white mealy farina on the leaves and stem of certain species of primula. The commonest flavones are apigenin and luteoline.

Flavonols

These very common compounds differ from flavones in having a 3-hydroxyl substituent. Numerous flavonols have been identified from plant tissue. Kaempferol, quercetin and myricetin are by far the commonest.

Flavonones and dihydroflavonols

Flavonones differ from flavones in lacking the double bond in the 2, 3 position and by the presence of at least one asymmetric centre. Though quite widespread, they are especially common in particular families, such as rosaceae, rutaceae, leguminosae and compositae. They are also found in the fern family polipodiaceae and in the pinaceae.

Isoflavones and isoflavonoids

Isoflavones are isomeric with flavones, having the B ring attached at the 3 position instead of the 2 position. Isoflavonoids have the 2, 3 double bonds reduced, so that they are related to isoflavones in the same ways that are related to flavones.

Biflavonoids

The flavonoids can also bound to one another, particularly through their very reactive C-6 or C-8, the result is a dimer known as biflavonoids. The majority of natural biflavonoids are dimers of flavones and flavonones which are generally 5, 7, 4’ trisubstituted, and interflavonic linkage can be of the carbon-carbon type (3’, 8”). Biflavonoids are characteristics of the gymnosperms and are sporadic in the angiosperms (hypericum, semecarpus, schinus, garcinia).

Chalcones and aurones

There are the anthochlor pigments - yellow flower pigments, which turn orange-red in the presence of ammonia. There is little structural variation in this group, which can be exemplified by the Chalcone- butein and the aurone - sulphuretin. Chalcones do not have a central heterocyclic nucleus and are characterized by a three carbon chain with a ketone function and an α, β unsaturation. Reduction in the double bond in a chalcone gives rise to a dihydrochalcone, a few of which have been found in nature. Phloretin occurs as the glycosides phloridzin in several of Malus (Rosaceae) (Bruneton, 1995).

Glycosyl flavonoids

In bryophyta, the presence of C-glycosyl flavones was reported for the first time in the genera plagiochila and plagiochasma. It is the same situation in gymnospermae for the genera cycas and abies. In ferns, the confirmation of the presence of C-glycosyl flavonoids was provided for three families such as aspleniaceae (*Asplenium sp.*), athyriaceae, and hymeno-

phyllaceae (*Trichomanes sp.*). In monocots, several investigations have concerned two species, zea mays and *Hordeum vulgare*, and two families, restionaceae and velloziaceae. In dicots, the first citations for C-glycosyl flavones concern the families araceae, betulaceae, brassicaceae, capparaceae, chenopodiaceae, droseraceae, geraniaceae, illecebraceae, mimosaceae, oleaceae, orchidaceae, oxalidaceae, pistaciaceae, plumbaginaceae, polygalaceae, sapindaceae, solanaceae, stercullariaceae, turneraceae, urticaceae, and violaceae; important additions to the occurrence of C-glycosyl flavones were given for cucurbitaceae, rosaceae and theaceae (Andersen *et al* 2005).

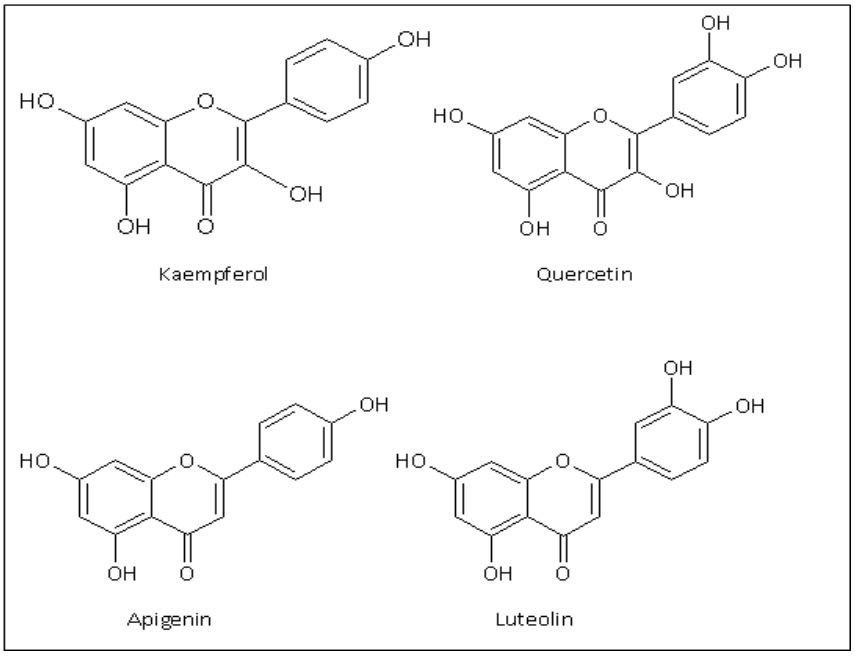

Fig. 3. Structures of some flavonoids

Extraction of flavonoids

For extraction, the solvent is chosen as a function of the type of flavonoid required. Polarity is an important consideration here. Less polar flavonoids (*e.g.* isoflavones, flavanones, methylated flavones, and flavonols) are extracted with chloroform, dichloromethane, diethyl ether or ethyl acetate while flavonoid glycosides and more polar aglycones are extracted with alcohols or alcohol-water mixtures.

Various technique like soxhlet apparatus, sequential solvent extraction, extraction with cold acidified methanol, pressurized liquid extraction, supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction have been reported by scientists (Andersen *et al* 2005).

Isolation and separation of flavonoids

The classical method of separating phenolics from plant extracts is to precipitate with lead acetate or extract into alkali or carbonate, followed by acidification. The lead acetate procedure is often unsatisfactory since some phenolics do not precipitate. There is no single isolation strategy for the separation of flavonoids and one or many steps may be necessary for their isolation. The choice of method depends on the polarity of the compounds and the quantity of sample available. Preparative methods available includes conventional open-column chromatography, modifications of the method (dry-column chromatography, VLC) which are of practical use for the rapid fractionation of plant extracts. Besides, preparative TLC is a separation method that requires the least financial outlay and the most basic equipment. Preparative TLC in conjunction with open-column chromatography remains a straightforward means of purifying natural products, although variants of planar chromatography, such as centrifugal TLC, have found application in the separation of flavonoids.

Identification tests for flavonoids

Shinoda test

Crude extract is mixed with few fragments of magnesium turnings. To this solution, concentrated HCl is added drop wise until pink

scarlet colour appear which indicates the presence of flavonoids.

Alkaline reagent test
Crude extract is mixed with 2 ml of 2% NaOH solution. Intense yellow colour is formed which turned colourless on addition of few drops of dilute HCl which indicates the presence of flavonoids.

Zinc hydrochloride reduction test
To the test solution, mixture of zinc dust and conc. hydrochloric acid is added. Resulting solution gives red color after few min.

Total flavonoid content
Aluminium chloride colorimetric method is used with some modifications to determine flavonoid content. One ml of sample plant extract is mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water respectively and kept at room temperature for 30 min. The absorbance is measured at 420 nm. Quercetin is used as standard (1 mg/ml). All the tests are performed in triplicates and flavonoid contents are determined from the standard curve which are expressed as quercetin equivalent (mg/g of extracted compound) (Khatiwora *et al* 2010).

Anthocyanins and anthocyanidin
Chemistry and distribution
The anthocyanins are the most important and wide spread group of coloring matters in plants. These intensely colored water soluble pigments are responsible for nearly all the pink, scarlet, red, mauve, violet and blue colours in the petals, leaves and fruits of higher plants. The anthocyanins consist of an aglycone (anthocyanidin), sugar(s), and, in many cases, acyl group(s).
The anthocyanidins are derivatives of 2-phenylbenzopyrylium (flavylium cation). There are six common anthocyanidines (anthocyanin aglycones formed when antho- cyanins are hydrolysed with acid) are the aglycone of cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin. Out of these, magenta-coloured cyanidin is most common. Orange-red colours are due to pelargonidin with one or less hydroxyl group than cyanidine, while mauve, purple and blue colours are generally due to delphinidine, which has one more hydroxyl group than cyanidin. Each of these six anthocyanidins occurs with various sugars attached as a range of glycosides (*i.e.* as anthocyanins). The main variation is in the nature of the sugar (often glucose, but may also be galactose, rhamnose, xylose or arabinose), the number of the sugar units (mono, di or tri-glycosides) and the position of attachment of sugar usually to the 3-hydroxyl, or the 3- and 5-hydroxyls. The anthocyanins are nearly universal in vascular plants (they have been detected in a few mosses, in young fern fronds as well as in gymnosperms and angiosperms). Anthocyanins are found in the cell vacuole, mostly in flowers and fruits but also in leaves, stems, and roots. In these parts, they are found predominantly in outer cell layers such as the epidermis and peripheral mesophyll cells. Plants rich in anthocyanins are *Vaccinium* species, such as blueberry, cranberry, and bilberry, rubus berries including black raspberry, red raspberry, and blackberry, blackcurrant, cherry, egg plant peel, black rice, concord grapes, muscadine grapes, red cabbage, and violet petals. Anthocyanins are less abundant in banana, asparagus, pea, fennel, pear, and potato, and may be totally absent in certain cultivars of green gooseberries (Wu *et al* 2004). Red-fleshed peaches are rich in anthocyanins (Cevallos-Casals *et al* 2006).
Nature, traditional agriculture, and plant breeding have produced various uncommon crops containing anthocyanins, including blue or red-flesh potatoes and purple or red broccoli, cabbage, cauliflower, carrots, and corn. Tomatoes have been bred conventionally for high anthocyanin content by crossing wild relatives with the common tomato to transfer a gene called the anthocyanin fruit tomato ("aft") gene into a larger and more palatable fruit (Jones *et al* 2003). Tomatoes have also been genetically modified with transcription factors from snapdragons to produce high levels of anthocyanins in the fruits (Butelli *et al* 2008). Anthocyanins can also be found in naturally ripened olives and are partly responsible for the red and purple colors of some olives (Agati *et al* 2005; Kailis and Harris, 2007) (**Figure 4**).

Extraction of the anthocyanidins and antho-cyanins
Extraction of anthocyanidins
Fresh petals (or other cyanic tissue) are heated in 2 M HCl in a test tube for 40 min for 100°C. The colored extract is cooled and decanted from the plant tissue and the cooled extract is twice washed with ethyl acetate to remove flavones,

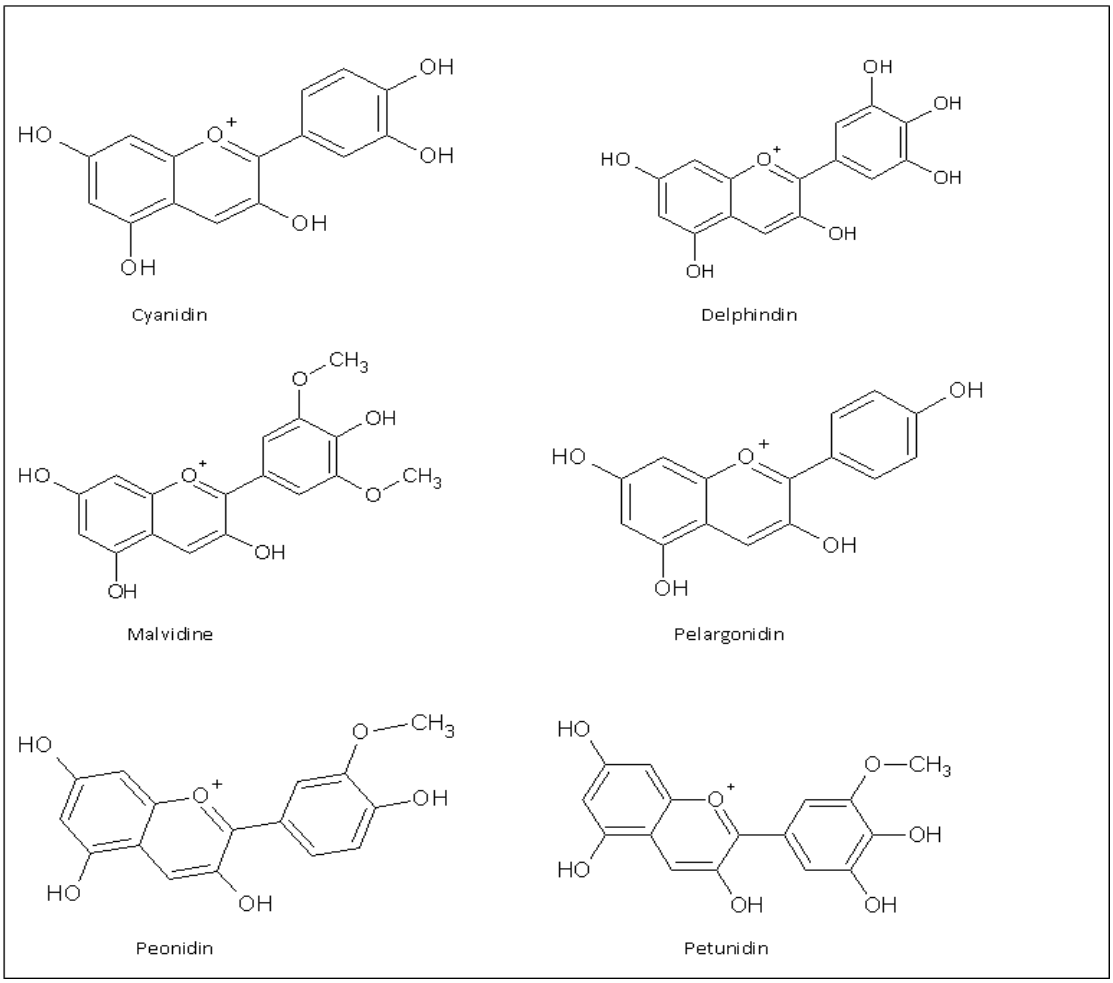

Fig. 4. Structures of some anthocyanidins

the ethyl acetate layer being discarded and the remaining aqueous layer being heated at 80°C for 3 min to remove the last traces of ethyl acetate.

The pigment is then extracted into a small volume of amyl alcohol, which can be pippeted off and concentrated to dryness by heating on a watch glass on a boiling water bath. The anthocyanidin in the residue, is dissolved in 2-4 drops of methanolic HCl and chromatographed one dimensionally on paper in forestall and formic acid solvents.

Extraction of anthocyanins

Extraction of small amounts of fresh coloured petals by crushing in a narrow specimen tube with a minimum amount of methanol containing 1% HCl provides a concentrated extract within 10-15 min.

Alternatively, large amounts of tissue may be macerated for 5 min in methanolic-HCl, the macerate is filtered or centrifuged and the extract is further concentrated in vacuum at 35-40°C, until the volume is reduced to about one fifth of the original extract. The anthocyanins are chromatographed one dimensionally on paper in BAW, Bu.HCl and 1% HCl against one or more marker solutions.

Isolation of anthocyanin and anthocyanidin

Thin layer chromatography of anthocyanin and anthocyanidin

Microcrystalline cellulose is usually used for the separation of anthocyanin and anthocyanidin with solvent system HCl-formic acid-water (24.9:23.7:51.4) and amyl alcohol-acetic acid-water (2:1:1). The anthocyanin content is determined in the petals of red poppy during flower development by six stage polygonal stepwise gradient gel TLC densitometry of the separated zones at 465 nm. Further, in plant extract, anthocyanin is determined by stepwise gradient elution method with various combination of the mobile phase ethyl acetate-isopropanol-water-acetic acid on silica gel 60 plates, Densitogram are recorded at 465 or 580 nm (Fried and Sherma, 2005; Matysik, 1992; Matysik and Benesz, 1991).

HPLC
It is performed using reverse-phase column (Bondapak C_{18} or Li Chrosorb RP-18) for the quantitative analysis of anthocyanins in floral tissues. Acidic solvents are generally preferred like water-acetic acid- methanol (71:10:19) or varying proportions of 1.5% H_3PO_4, 20% acetic acid and 25% acetonitrile in water.

Identification test for anthocyanin
Reactions of the pigments to alkaline conditions
To observe the effect of alkaline conditions on the pigments, 2 ml of extract and 10 ml of water are placed in a flask. Then, while swirling the flask, 1-2 ml of 2 M NaOH is added to the flask drop-wise. Anthocyanins change colour to a blue-green colour that eventually fades.

Xanthone and stilbenes
Chemistry and distribution
Xanthones are yellow poly phenolic compounds that usually occur in plants and have been shown to have widespread biological and pharmacological actions. The xanthone is an organic compound with the molecular formula $C_{13}H_8O_2$. They are comprised of planar-six carbon molecules in a conjugated ring system consisting of a backbone molecule and various chemical groups attached to it. The backbone consists of two benzene rings bridged through a carbonyl group and oxygen. Each ring is connected in a fused formation not allowing free rotation about the carbon-carbon bonds. This unique backbone along with type and position of the attached chemical groups define specific functionality (properties) of xanthones. Xanthones are phenolic pigments which are similar in color reaction chromatographic mobility to flavonoids. Chemically, they are different and they are readily distinguished from flavonoids by their characterstics spectral properties. Almost all the xanthones are confined to families guttiferae, gentianaceae, moraceae, polygalaceae, bonnetiaceae, clusiaceae, podostemaceae (Anonymous, 2003). However, xanthone namely mangiferin which is C-glycosylated, is very wide spread, occurring in ferns as well as in higher plants.
Stilbenes are a sub-group of phytochemicals called polyphenols. They are not as abundant in foods as flavonoids, lignans or phenolic acids, which are also groups of polyphenols. Hydroxystilbenes are biogenetically related to the chalcones, but with one less carbon atom in their basic skeleton, which is C_6-C_2-C_6. The biologically active stilbene is lunularic acid, actually a dihydrostilbene, which is a growth inhibitor in certain liver worts. Hydroxylated derivatives of stilbene (stilbenoids) are secondary products of heartwood formation in trees that can act as phytoalexins (antibiotics produced by plants). Because of the chemical stability of phenyl moiety of 1, 2-diphenyl-ethylene, stilbene is not a suitable starting compound for synthesis of stilbene derivatives. In order to form more complex molecules, it is necessary to introduce more reactive functional groups (**Figure 5**).

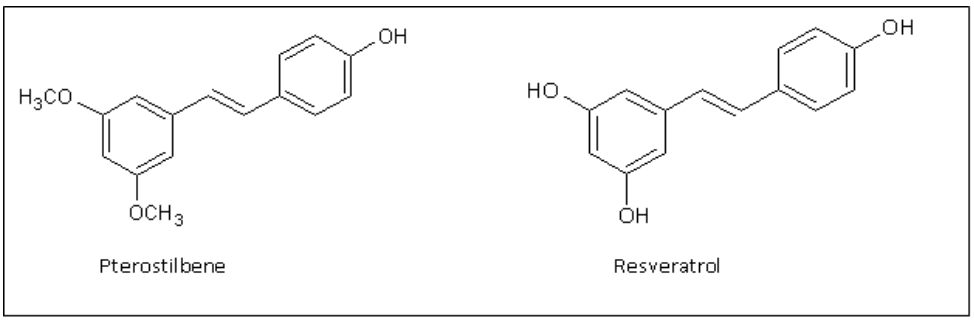

Fig. 5. Structures of some stilbenes

Extraction of xanthones
The air dried and powdered drug is extracted with CH_2Cl_2-MeOH (1:1) for 48 h at room temperature and the extract is subjected to complete dryness to yield the crude extract. The extract is dissolved in CH_2Cl_2 to give the dichloromethane soluble fraction and remaining part is dissolved in water and extracted with butanol to give xanthone fraction (Djemgou *et al* 2010).

Extraction of stilbenes
The freeze dried powdered drug is extracted by ASE (accelerated solvent extractor) with ethanol-water as a solvent. The extract was further hydrolyzed enzymatically after

evaporation of the solvent. After hydrolysis, polyphenolic compounds were purified directly from the hydrolysate mixture with the polymeric resin sorbent and injected to preparative HPLC to collect the pure compounds.

Separation and identification

Xanthones

TLC commonly separates xanthones on silica gel; using chloroform-acetic acid (4:1), chloroform-benzene (7:3) or chloroform-ethyl acetate varying proportions and can be detected by their colours in UV light with or without ammonia.

Stilbenes

Stilbenes show intense purple florescence in UV light changing to blue with NH_3, have spectra maxima at about 300 nm and can be separated on paper by TLC.

CONCLUSION

Herbs and shrubs are useful in treatment of various diseases as they contain bioactive plant phenols like flavonoids, glycosides, saponins, carotenoids. The health benefits of fruits and vegetables are largely due to the antioxidant properties of these phytochemicals.

Although there is a huge data available on phenolic compounds but the available literature does not seem to offer enough information on the structural diversity, mechanisms and the effect of metabolites extracted from different plants etc. Therefore, phenolic compounds are required to screen thoroughly so as to develop potential pharmaceutical and agricultural products.

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