Evaluation of medicinal and nutritional components from the *Eleagnus conferta* fruit

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ABSTRACT

Most of the wild plants from konkan (M.S.) India are being used for medicinal purposes. The present study deals with the evaluation of bioactive phytochemicals present in the fruits of the *Elaeagnaceae* family, especially *Eleagnus conferta,* commonly called as berry. The ripen fruit material was collected from the place Radhanagari; district Kolhapur, in the month of April. The objective of study is getting relevant information about the bioactive compounds having medicinal and nutritional importance. The various bioactive components such as 6.08 % phenolics, 11.68 % flavonoids, lipids from seed 2.9 % and from pulp 3.5%, carotenoids from seeds 1.35 % and from pulp 16 %, 39 % carbohydrates, 8.2 % ascorbic acid and 2.37 % of titratable acidity was quantified here.

Keywords: Elaeagnaceae, phenolics, flavonoids, carotenoids, bioactive.

INTRODUCTION

The *Elaeagnaceae* family contains many plants of interest to the pharmaculturist apart from producing edible fruits; most species also have a wide range of other uses. Not all of the species in the family have edible seeds. All the species have a symbiotic relationship with certain soil bacteria. These bacteria form nodules on the roots and fix atmospheric nitrogen. Some of this nitrogen is utilized by the growing plant but some can also be used by other plants growing nearby. All members of the family can increase the yields of fruit trees by up to 10%.

Fruits of this family rich source of vitamins and minerals, flavanoids and other bioactive compounds. It is also a good source of essential fatty acids, which is fairly unusual for a fruit. These plants produce insignificant but exquisitely scented flowers in the autumn (October to December) very attractive fruits in early April Unless fully ripe, these fruits can be quite astringent, but as they ripen they develop a very acceptable flavour and at their peak of ripeness they become very pleasant, almost delicious in fact. They are also very easy to pick.

Flavonoids and cinnamates are widespread phenolics secondary metabolites which are particularly bioactive and have pronounced effects on mammalian cells, like intracellular damages due to free radicals formed in the body. (Burda and Oleszek 2001).

Along with these components the fruit also contains vitamin C (ascorbic acid) in considerable amount. It is required in the synthesis of collagen in connective tissue (Tiedtke and Marks 2007), neurotransmitters, steroid hormones, carnitine, and conversion of cholesterol to bile acids and enhances iron bioavailability (Kamp *et al* 2003). Ascorbic acid is a great antioxidant and helps protect the body against pollutants. *An Elaeagnus conferta fruit also contains* some minerals like Ca, Mg, K, Mn, P etc (Valvi and Rathod 2011).

MATERIALS AND METHODS

Fruits Eleagnus conferta

The fruit material (ripen fruit) was collected from the place Radhanagari District Kolhapur in the April. The fruit material is stored at freeze until it was analyzed in tightly pack containers. After the fruits were cleaned with tap water, chopped and a weighed portion was lyophilized for 48h. Whenever necessary the product was ground to a fine powder by mortar and pestle and it was stored and packed at 4^0C. The average ripe fruit weight found was 4.20 g, length 3.18 cm, breadth 1.54 cm.

a) Extraction of Free and Total Phenols

A 50-mg aliquot of lyophilysate was accurately weighed in a screw-capped tube. For free phenols, 5 ml of 50% methanol/ water was used and the sample was vortexed for one minute and heated at 90 °C for 3 h with vortexing every 30 min. After the samples were cooled, they were diluted to 10 ml with methanol and centrifuged for 5 min at 5000 rpm with a bench-top centrifuge to remove solids. Total phenols were extracted with 5 ml of 1.2 M HCl in 50% methanol/water and treated as above and it was stored at 4 ^{o} C until it was analyzed (Vinson *et al*. 2001).

b) Determination of Phenolic Content

The total and free phenolic content of the extracts was determined using the FC colorimetric method. A 125 μL aliquot of the extract was mixed with 0.5 ml of distilled water and subsequently with 125 μL of FC reagent. After 6 min, 1.25 ml of a 7% aqueous sodium carbonate solution was added. Water was added to bring the total volume to 3 ml, and samples were allowed to stand for 90 min. The samples were then read at 760 nm vs a prepared blank with a spectrophotometer and compared with a known

concentration range of gallic acid standards similarly prepared. All results were expressed as milligrams of gallic acid equivalents per 100 g fresh weight of berry.

c] Determination of Total Flavonoid Content

The extract of total phenols was used to determine the total flavonoid content using a colorimetric method described previously. A 250 μL aliquot of the extract was mixed with 1.25 ml of distilled water and subsequently with 75 μL of 5% $NaNO_2$ solution. After 6 min, 150 μL of a 2% $AlCl_3$,6H2O solution in methanol was added and allowed to stand for 5 min before the further addition of 0.5 ml of 1 M NaOH. Water was added to bring the total volume to 2.5 ml, and the samples were read immediately at 510 nm against a prepared blank using a spectrophotometer. All values were expressed as milligrams of quercitin equivalents per 100 g dry weight of berry. (Meyers *et al*. 2003)

d] Method of estimation of ascorbic acid

Sample solution equivalent to 0.2 mg ascorbic acid/ml was prepared in water containing 3% w/v meta-phosphoric acid, added to increase the stability of ascorbic acid. It was titrated against standard 2,6-dichlorophenol indo-phenol (2,6-DCPIP) solution of concentration 0.5 mg/ml, until the pink colour developed completely. The operation was repeated with a blank solution omitting the sample being examined. From the difference, the ascorbic acid in each mg of sample was calculated from the ascorbic acid equivalent of the standard DCPIP solution. (*Indian Pharmacopia* 1996)

e] Soluble carbohydrates

Soluble carbohydrates (sugars) were twice extracted from lyopolised samples with boiling ethanol (80% v/v). Pooled alcohol extracts were used for determination of total and reducing sugar. Total sugars were estimated by phenol-H_2So_4 method and reducing sugars were estimated by DNSA method. Starch was estimated in the residue left after sugar extraction by phenol-H_2So_4 method.

f] Determination of Total Carotenoids

1 ml aliquot from the lipid extract was added to 0.5 ml of 5% NaCl, vortexed for 30 s, and centrifuged for 10 min at 4500 rpm. The supernatant (100 μl) was diluted with 0.9 ml of hexane and measured at 460 nm. *ß*-Carotene was used as a standard and total carotenoids were expressed as mg/100 g *ß* -carotene equivalents (Gao Xiangqun *et al*. 2000).

g] Quality Attributes

Titratable acidity was measured by homogenizing thoroughly 10 g of pulp with distilled water and filtering under vacuum. The filtrate was made up to 30 ml and titrated against 0.1 N NaOH to pH 8.2 using phenolphthalein as an indicator and expressed as the units of citric acid (milligrams per gram of fresh weight) on a fresh weight basis (Shivashankara *et al.* 2004)

h] Lipid Extraction

Samples (1 g) of seeds (powdered) and lyophilized pulp was crushed in a mortar and the lipids were isolated using a methanol-chloroform extraction procedure as described by (Yang *et al*.2001). The sample was homogenized in methanol (10 ml) for 1 min in a blender, chloroform (20 ml) was added, and homogenization continued for a further 2 min. The mixture was filtered, and the solid residue was re-suspended in chloroform/methanol (2:1, v/v, 30 ml) and homogenized for three minutes. The mixture was filtered again and washed with fresh solvent (chloroform/ methanol, 2:1, v/v, 30 ml). The combined filtrates were transferred into a measuring cylinder, one-fourth of the total volume of 0.88% potassium chloride water solution was added, and the mixture was shaken thoroughly before being allowed to settle. The lower layer was removed and washed with one fourth of its volume of methanol/water (1:1 v/v). The washing procedure was repeated, and the bottom layer containing the purified lipids was filtered before the solvent was removed on a rotary film evaporator. The lipids were weighed, and the oil contents (percentages) in seeds and pulp were calculated. Lipids were stored in chloroform at -20°C. All data was analyzed by using MS excel computer program and results are obtained in Mean ± SD manner.

RESULTS AND DISCUSSION

a) Quantification of free and total phenol

The free and total phenolic content obtained is given in the table. These are determined by a colorimetric method and quantification was done on the basis of a standard curve of gallic acid. Results were expressed as milligrams of gallic acid equivalents per 100 g fresh weight of berry.

b) Flavonoids content

The total flavonoids are determined by using the colorimetric method. The results are given in the table and are expressed in terms of milligrams of quercitin equivalents per 100 g dry weight of berry. Quantification done on the basis of a standard curve of quercitin.

c) Ascorbic acid content

The ascorbic acid content determined from the fruit in comparison with standard was given in the table and expressed in mg of ascorbic acid.

d) Titratable acidity

The titratable acidity determined from the fruit is given in the table and expressed in mg of citric acid equivalent. These are estimated by a simple titrimetric method.

e) Quantification of total lipids

The total lipid content obtained from both the pulp and seed are given in the table. The total lipids obtained from pulp are slightly higher than the total lipids obtained from the seeds. These are extracted from the lyophilized sample of pulp and dry sample of seeds.

f) β carotene content

The β carotene content obtained from seed and pulp is given in the table. These are estimated from the total lipids obtained and quantified by using standard β carotene curve, by using colorimetric method. Total carotenoids were expressed as mg/100g β-carotene equivalents.

Table: Nutritional attributes of *Eleagnus conferta* fruit

PARAMETER	RESULTS OBTAINED
Phenolics	
Free phenolics	2.88 ± 0.06 mg/100mg of lyophilized sample
Total phenolcs	6.08 ± 0.85 mg/100mg of lyophilized sample
Flavonoids	11.68± 0.43 mg/100mg of lyophilized sample
Ascorbic acid	8.2± 0.40 mg/100g of fruit sample
Titratable acidity	2.3703 ± 0.0004 /100g fruit sample
Total lipids	
From seeds	2.9g± 0.02 /100g sample
From pulp	3.5g± 0.04/100g sample
β carotene content	
From seeds	1.35± 0.05 mg/100mg of lyophilized sample
From pulp	16.00± 0.45 mg/100mg of lyophilized sample
Sugars	
Total Carbohydrates	39.00± 0.40 mg/100mg of lyophilized sample
Total reducing sugars	35.00± 0.40 mg/100mg of lyophilized sample
Non reducing sugars	4.0± 0.05 mg/100mg of lyophilized sample
Total starch content	8.25± 0.03 mg/100mg of lyophilized sample

G) Quantification of total sugars

Total carbohydrates

The total carbohydrates obtained are given in the table. The total carbohydrates (total reducing and total non reducing sugars) were estimated by the phenol-H_2SO_4 method. These are expressed in terms of glucose concentration in mg per 100g of lyophilized sample.

Total reducing sugars

The total reducing sugars were determined by the DNSA method. The results obtained are given in the table. These are expressed in terms of glucose concentration in mg per 100g of lyophilized sample. The fruit contain comparatively higher concentration of reducing sugars.

Total non reducing sugars

The total non reducing sugars are given in the table and are quantified from the total carbohydrates and the total reducing sugars obtained.

Total starch content The total starch content was estimated by the phenol-H_2SO_4 method. The results obtained are given in the table. These are expressed in terms of glucose concentration in mg per 100g of lyophilized sample.

Literature data shows that the plant is reputed to have considerable medicinal value being useful for the treatment of skin disorders resulting from bed confinement, stomach and duodenal ulcers, cardiovascular diseases, and perhaps growth of some tumors. *E. conferta* is the only species from family Eleagnaceae shows presence in western Ghat which is one of the 18th biodiversity hotspot in world. From this study it is being explored that the fruits from species *E. conferta* is full of nutritional attributes and can be considered as “fruit with marketing potential”. Systemic approach towards *E. conferta* fruit for consumer acceptance will certainly be a hope in life of tribals as their economic sources are limited.

Fruits and berries contain phenolic acids and Flavonoids. The phenolic compounds are combined with sugars or other polyols via O-glycosidic bonds (flavonols, anthocyanidins, and hydroxycinnamic acids) or ester

bonds (hydroxycinnamic acids). The distribution of these conjugated forms of phenolic compounds is typical for the plant species, which might be utilized to study the authenticity of the raw material in manufactured jams, jellies, juices, and wines (Macheix *et al*. 1990). The contents of phenolic compounds in plant foods have also received much attention during recent years because of their biological properties imparting possible benefits to human health (Parr and Bolwell. 2000). In consideration of berries as a source of phenolic compounds as well as in the characterization of the composition of individual conjugates, a simultaneous determination of all major phenolic classes is needed.

Berries are rich in wide range of flavonoids and phenolic acids that show antioxidant activity. Main flavonoid subgroups in berries and fruits are anthocyanins, proanthocyanins, flavonols, and catechins. Phenolic acids present in berries and fruits are hydroxylated derivatives of benzoic acid and cinnamic acid (Macheix *et al*. 1990). Small berries constitute one of the important sources of potential health promoting phytochemicals. The content of phenolics in berries is affected by the degree of maturity at harvest, genetic differences (cultivar), preharvest environmental conditions, postharvest storage conditions, and processing (Prior *et al.* 1998). Phenolic acids constitute about one-third of the dietary phenols, and they are present in plants in free and bound forms. Bound phenolics may be linked to various plant components through ester, ether, or acetal bonds (Robbins 2003 and Clifford 1999) estimated that daily consumption of phenolic acids ranges from 25 mg to 1 g. An increasing interest in determining the antioxidant activities exhibited by phenolic acids and their derivates should also be noted (Wang *et al*. 2000).

Yet, there is an urgent need to promote these locally grown berries as a crop which is rich in nutritional value. Underutilized plant species like *E. conferta* can play a crucial 'safety net' function in poor communities and are well accepted because of their traditional use and cultural value. Long familiarity means that rural populations may hold extensive germplasm and knowledge on these species, which can facilitate community empowerment and encourage self-reliance. Many species have excellent nutritional profiles, with high protein, vitamin and/or mineral contents, and can contribute to alleviate 'hidden hunger' in wealthier as well as poor communities. Furthermore, they are generally not overly competitive and are able to fit well within particular 'micro-environments' in farming ecosystems.

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