Development of Functional Herbal Pomegranate Aquelate Enriched with Coffea arabica and Cymbopogon citratus

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ABSTRACT

Coffea arabica (Green coffee bean) and Cymbopogon citratus (Lemongrass) are well-known medicinal herbs found in India and possess bioactive components which attribute to a strong antioxidant activity which could modulate many diseases. In view of the above aspects the present work comprised to determine antioxidant potential of developed pomegranate aquelates incorporated with Coffea arabica and Cymbopogon citratus singly and their equi-proportioned mixture. The pomegranate aquelate of 5% incorporation enriched with Cymbopogon citratus was found to be insignificant at P 0.05 level when compared with standard aquelette. Therefore, it was acceptable and comparable to standard aquelette in terms of all sensory attributes. In antioxidant activity it was shown that pomegranate aquelette with Coffea arabica extract had higher TPC (16.43±0.34 mgGAE/g) and TFC (0.8±0.64 mgQE/g) whereas tannins content (13.89±0.66 g/100 g) was higher in Cymbopogon citratus extract. DPPH % free radical scavenging activity was high in equi-proportioned mixture (IC50=18 µg/mL) which was followed by Coffea arabica (IC50= 24 µg/mL) and Cymbopogon citratus aqueous extract (IC50=40 µg/mL) when compared to standard ascorbic acid. It is concluded that extracts of the above herbs can be used as a valuable ingredient for the production of herbal beverage with powerful antioxidant properties.

1. Introduction

Oxidative stress plays a key role in the pathogenesis of aging and can be caused by various negative impacts such as gamma or UV radiations, environmental factors, polluted and poor-quality food, stress, some medications or treatments, smoking, alcoholism, etc. Prolonged oxidative stress inevitably leads to dangerous diseases such as cancer, cardiovascular diseases, or diabetes and premature aging. Oxidative stress can be reduced by antioxidant therapy, i.e., by consumption of certain amounts of natural antioxidants contained in vegetables, fruits, berries, vegetable oils, honey, tea, cocoa, juices, wine, sprouted grains, and other foods [1]. However, in order to control consumption of antioxidants, it is necessary to know their content in foods and beverages. In this regard, quantitative measurement of antioxidants in foods and beverages and compilation of a corresponding databank becomes a highly important goal.

The pomegranate fruit is processed commercially into various forms mainly juice, squash and RTS drinks which provide moderate quantity of vitamin C, potassium, bioflavonoid and folic acid and also reported to be a rich source of antioxidants such as anthocyanins, phenolics, etc. which have considerable health-promoting properties with antimicrobial, antiviral, anticancer, antioxidant and anti-mutagenic effects. It is refreshing, thirst quenching and energizing drink that improves health and nutritional requirements [2]. Hence, in the light of the above research facts, the present investigation was undertaken with the objective to incorporate antioxidant rich herbal extracts of Coffea arabica and Cymbopogon citratus in the development of delicious and nutritious aquelates that could therapeutically help in improving the health of consumers. Green coffee is an unroasted, green coffee bean which belongs to the Rubiaceae family, genus Coffea. It has been used as an ingredient in sports food products for weight control. Prospective and epidemiologic studies stated that green coffee quickly absorbed and increased in plasma concentrations and is a strong cardiovascular stimulant that increases epinephrine output to a greater extent to enhance performance of athletes [3].

Various scientific evidences have demonstrated that regular green coffee beverages present high antioxidant properties and have indicated that soluble extracts of green coffee were effective against the high blood pressure and exert a greater ergogenic effect.

Cymbopogon citratus (Lemongrass) belongs to Poaceae family, genus Cymbopogon and is an aromatic perennial grass which has been traditionally used to remediate a plethora of medical conditions. This is due to the broad spectrum of secondary metabolites that it produces. It has been used to treat fever, cough, elephantiasis flue, leprosy, malaria and digestive problems among many other illnesses [4]. Medical conditions like hyperlipidemia, hypercholesteremia and hyperglycemia lead to metabolic disorders like obesity and diabetes mellitus, it has been reported that lemon grass is bestowed with hypolipidemic, hypocholesteremic and hypoglycemic properties; additionally the antagonistic activity of lemon grass towards different pathogenic bacteria, protozoa and fungi has also been reported [5]. Hence, in the light of the above research facts, the present investigation was undertaken with the objective to develop herb based functional fruit aquelette that could therapeutically help in improving the health of consumers.

2. Experimental Methods

2.1 Collection of Samples

Coffea arabica was collected from local fields of Chamrajnagar (Bangalore) under National Horticulture Board and Cymbopogon citratus was collected from Khari badi market (Delhi). Green coffee bean and Lemongrass were washed and dried in open air for 2-3 weeks at 35-40 °C and then dried material was pulverized in an electric grinder and stored in plastic containers in refrigerator (5 °C), for further analysis.

2.2 Aqueous Extract Preparation and Development of Herbal Pomegranate Aquelate

20 g of powdered plant material was kept in 200 mL conical flask and add 100 mL of distilled water. The mouth of the conical flask was covered with the aluminum foil and kept in a reciprocating shaker for 25 min for continuous agitation at 150 rev/min for thorough mixing. Then extract was filtered by using muslin cloth followed by Whatman filter paper No. 42 (125 mm). The content was filtered by using rotator vacuum
The pomegranate aqualetes were prepared by the incorporation of Coffea arabica and Cymbopogon citratus aqueous extract in three different concentrations i.e. equi-proportioned mixture of Coffea arabica and Cymbopogon citratus (5%), Cymbopogon citratus (5%) and Coffea arabica (5%) assigned as PACa, PACa+Cc (5% incorporation of Coffea arabica and Cymbopogon citratus) and PACa+Cc+C (5% incorporation of equi-proportion mixture of C. citratus and C. arabica) aquarrea extract.

3. Results and Discussion

The pomegranate aqualetes were prepared by the incorporation of Coffea arabica and Cymbopogon citratus aqueous extract in three different concentrations i.e. equi-proportioned mixture of Coffea arabica and Cymbopogon citratus (5%), Cymbopogon citratus (5%) and Coffea arabica (5%) assigned as PACa, PACa+Cc, PACa+Cc+C which were incorporated with a beverage having formulation 6% basil and 1.5% T. cordifolia/ orange juice was found to be optimum among the other formulation and also can be used as a valuable ingredient for the production of herbal beverage with all the important properties and medicinal characteristics.

Table 1 | Sensory evaluation of pomegranate aqualetes based on Coffea arabica, Cymbopogon citratus and their equi-proportioned mixture in the term of sensory attributes

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Standard</th>
<th>PACa,</th>
<th>PACa+Cc</th>
<th>PACa+Cc+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>4.4±0.22</td>
<td>3.7±0.68*</td>
<td>3.7±0.68*</td>
<td>3.3±0.27*</td>
</tr>
<tr>
<td>Color</td>
<td>4.12±0.44</td>
<td>3.7±0.60*</td>
<td>3.8±0.93*</td>
<td>3.0±0.22*</td>
</tr>
<tr>
<td>Taste</td>
<td>4.2±0.60</td>
<td>3.9±0.60**</td>
<td>4.1±0.75**</td>
<td>3.3±0.31**</td>
</tr>
<tr>
<td>Consistency</td>
<td>4.26±0.93</td>
<td>3.3±0.71**</td>
<td>4.0±0.82**</td>
<td>3.3±0.50**</td>
</tr>
<tr>
<td>After taste</td>
<td>4.17±0.03</td>
<td>3.8±0.70**</td>
<td>4.0±0.83**</td>
<td>3.2±1.14**</td>
</tr>
</tbody>
</table>

Data are reported as MEAN ± SD (n=30). Test Aqualetes were enriched with 5% Coffea arabica (PACa), 5% Cymbopogon citratus (PACc) & 5% equi-proportion mixture of Coffea arabica and Cymbopogon citratus (PACa+Cc) compared to standard Pomegranate Aqualete. *Significant and NS- non significant at P≤0.05

Table 2 | Preliminary phytochemical screening of pomegranate aqualetes aqueous extracts of Coffea arabica (PACa), Cymbopogon citratus (PACc) and equi-proportion mixture (PACa+Cc)

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Tests</th>
<th>PACa,</th>
<th>PACa+Cc</th>
<th>PACa+Cc+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorf’s test</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Lierman’s test</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Shinoda’s test</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenols</td>
<td>5% ferric chloride</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannin</td>
<td>Ferric chloride test</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Table 2 showed that the phytochemical screening of pomegranate aqualetes aqueous extract of Coffea arabica and Cymbopogon citratus contained all the phytochemicals such as alkaloids, flavonoids, phenols, tannins, were present in both aqueous extracts except terpenoids in Cymbopogon citratus and saponins in Coffea arabica whereas all phytochemicals were found in equi-proportion mixture aqualete. The results of our study agree with the investigations of phytochemicals in Coffea arabica and Cymbopogon citratus [9] who reported that extracts of green coffee contain higher levels of phytochemicals and chlorogenic acids. It has been shown that lemongrass extract contains alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids [10]. Each of these phytochemicals is known to have various protective and therapeutic effects. For instance, phenol was known to be an erythrocyte membrane modulator and have been found to possess an anti-oxidant potential. Reported the roles of these phytochemicals such as anti-inflammatory, anti-hypertensive, and anti-microbial properties and also

Fig. 1 Flow diagram of herbal pomegranate aqualetes incorporated with Coffea arabica and Cymbopogon citratus

The mean score of standard pomegranate aqualete shown in Fig. 1 was found to be optimum among the other formulation and also change.

Fig. 2 Sensory evaluation of pomegranate aqualetes based on Coffea arabica, Cymbopogon citratus and their equi-proportioned mixture

Fig. 3 Overall acceptability evaluation of pomegranate aqualetes aqueous extract of Coffea arabica, Cymbopogon citratus and equi-proportioned mixture in the terms of sensory attributes by 9 point hedonic scale
exhibit cytotoxic effects and growth inhibition making them suitable as tumor inhibiting agents.

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Antioxidant profile of Pomegranate Aqualete (PA) and Test Pomegranate Aqualete with Coffea arabica (PA_{C}) and Cymbopogon citratus (PA_{C}).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidants</td>
<td>PAC_{C}</td>
</tr>
<tr>
<td>Flavonoids (mg QE/g)</td>
<td>8.8±0.04*</td>
</tr>
<tr>
<td>Tannins (g/100)</td>
<td>8.34±0.028*</td>
</tr>
<tr>
<td>Total Phenols (mg GAE/g)</td>
<td>16.6±3.03*</td>
</tr>
</tbody>
</table>

*Values expressed as Mean ± SD, *significant at P≤0.05 (n=3) of triplicate determinations.

The total phenols (mg GAE/g) content was expressed in Gallic acid equivalents (GAE). The total phenols content of pomegranate aqualete (PA), test Coffea arabica (PA_{C}) and Cymbopogon citratus pomegranate aqualetes (PA_{C}) were 3.8±0.03, 16.4±3±0.34 and 3±1.0±0.03 respectively. The data depicted in Table 3 shows that PAC_{C}a has significantly higher value when compared with PAC_{C} and PA. According to Affonso [1] stated that green coffee bean contains 35.39 ± 5.69 mg GAE/g phenols content which is higher value than present data in the study. Similar data was observed by Aloi [12] that the content of polyphenols was 11.56 ± 6.49 mg of gallic acid/40 mg and the total flavonoid content of the pomegranate juice was 31.5 µg of catechin/60 mg of dry weight of pomegranate Arils. The contents of total flavonoids that were measured by aluminum chloride technique in term of quercetin equivalent, the content of flavonoids (mg QE/g) aqueous extracts expressed in QE of Coffea arabica and Cymbopogon citratus was 8.8±0.04 and 6.9±0.04 respectively. This shows that Coffea arabica has significantly higher flavonoids at P≤0.05 levels when compared with Cymbopogon citratus aqueous extract. Similar study was seen by Hasim [13] that Lemongrass contains flavonoids compounds that serve as flavoring ingredients of plant leaves and possesses an antioxidant potential which is agreement with the present study. It has been approved that flavonoids showed significant antioxidant action on human health and fitness through scavenging or chelating process.

The tannins content was measured by potassium ferrocyanide technique and found that pomegranate aqualete with Coffea arabica and Cymbopogon citratus were 3.4±0.02 and 3.09±0.06 mg/100 g respectively. This shows that Cymbopogon citratus had significantly higher tannins content at P≤0.05 levels when compared with Coffea arabica aqueous extract. Tannins are known anti-nutritional factor that reduce the uptake of blood glucose by binding with calcium which is needed to stabilize amylase activity. They can equally bind with starch to influence its degree of gelatinization or its access ability to the digestive enzymes.

The DPPH is a stable nitrogen centered free radical with absorption maximum band around 515 to 528 nm. In this assay, the antioxidants reduce the DPPH radical [purple color] to a yellow colored compound, 1, 1 diphenylicrylhydrazine [14]. The extent of color change depends on hydrogen donating ability of the antioxidants. DPPH assay is based on the ability of DPPH to convert a stable free radical which decolorize in the presence of antioxidants [15]. It is a direct and reliable technique for determining radical scavenging action. The DPPH radical scavenging activities of aqueous extract were evaluated by using the parameters IC_{50} which means the concentration of antioxidant required for 50% scavenging of DPPH radical in the particular time period [16]. In vitro activities of both Coffea arabica and Cymbopogon citratus extracts were measured with the standard antioxidant (Ascorbic acid). Here, the DPPH free radical scavenging activities of pomegranate extracts with Coffea arabica, Cymbopogon citratus and their equi-proportioned mixture are shown in Fig. 4. The antioxidants activities of Coffea arabica and Cymbopogon citratus with IC_{50} (Inhibitory concentration 50%) were determined from [20-210 µg/ml] from which equi-proportioned mixture pomegranate aqualete exhibits highest antioxidant activity with IC_{50} value (18 µg/ml) followed by Coffea arabica (24 µg/mL) and Cymbopogon citratus (40 µg/mL). Thus, aqueous extract of equi-proportioned mixture pomegranate aqualete showed strongest antioxidant activity as compared to ascorbic acid as standard. This result was also observed by Gunalan [3] that DPPH scavenging activities were found in aqueous extract of Coffea arabica with IC_{50} values ranging from 24 µg/mL to 38 µg/mL. A similar study demonstrated that DPPH scavenging activities of Cymbopogon citratus with IC_{50} values ranging from 7.13 µg/mL to 89.6 µg/mL [17].

4. Conclusion

Herbs can be used as a valuable ingredient for the production of herbal beverages with all the remedial properties and medicinal characteristics. Sensory analysis of the present study revealed that pomegranate aqualete of 5% incorporation enriched with Coffea arabica had highest score among three aqualetes in terms of overall acceptability and acceptable by semi trained panel. This study also showed that pomegranate aqualete aqueous extract of Coffea arabica and Cymbopogon citratus have powerful antioxidant potential as compared to pomegranate aqualete Thus, it was noticed that both the herbs showed strong hydrogen donating abilities to act as an effective antioxidant potential and can be used as a promising drink supplement or may be useful in pharmaceutical applications.

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References