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Development of Functional Herbal Pomegranate Aqualete 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 herb 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 aqualetes incorporated with *Coffea arabica* and *Cymbopogon citratus* singly and their equi-proportioned mixture. The pomegranate aqualete of 5% incorporation enriched with *Cymbopogon citratus* was found to be insignificant at P≤ 0.05 level when compared with standard aqualete. Therefore, it was acceptable and comparable to standard aqualete in terms of all sensory attributes. In antioxidant activity it was shown that pomegranate aqualete with *Coffea arabica* extract had higher TPC (16.43±0.34 mgGAE/g) and TFC (8.8±0.04 mgQE/g) whereas tannins content (13.89±0.06 g/100 g) was higher in *Cymbopogon* citrates extract. DPPH % free radical scavenging activity was high in equi-proportioned mixture (IC_{50}=18 µg/mL) which was followed by *Coffea arabica* (IC_{50}= 24 µg/mL) and *Cymbopogon citratus* aqueous extract (IC_{50}=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, coffee, 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 aqualetes 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 increases 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 flu, 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 aqualete 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 baoli 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 Aqualete

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

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evaporator with the water bath temperature of 65 °C and finally the residues were collected and used for the analysis [6]. The aqualetes were developed with standard procedure is given in Fig. 1. The prepared pomegranate herbal aqualetes were divided into 3 batches coded as PACc (5% incorporation of *Cymbopogon citratus*), PACa (5% incorporation of *Coffea arabica*) and PACc+Ca (5% incorporation of equi-proportion mixture of *C. citratus and C. arabica* aqueous extract.

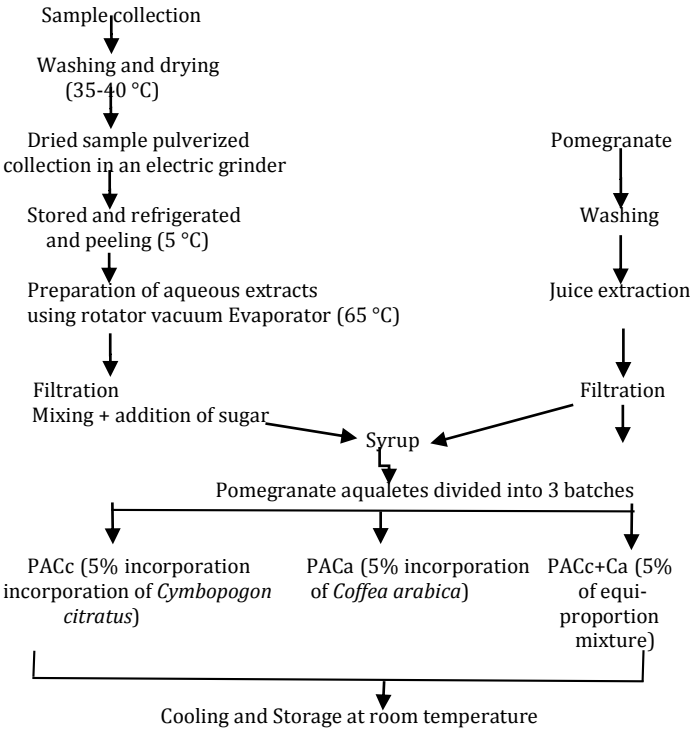

Fig. 1 Flow diagram of herbal pomegranate aqaletes incorporated with *Coffea arabica and Cymbopogon citratus*

2.3 Determination of Antioxidant Profile

Antioxidants such as total flavonoids [7] content by aluminum chloride colorimeter assay, tannin by modified vanillin-HCl method, Total phenolic content (TPC) by Folin-Ciocalteau reagent and DPPH scavenging activity by spectrophotometric method [8] were carried out.

2.4 Statistical Analysis

The results obtained were expressed as mean ±SD and student t-test of three determinations and also statistically analyzed to ascertain its significance. The significance was estimated at ($p \leq 0.05$ level).

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 PAC_c+C_a, PAC_c and PAC_a respectively which were compared with standard pomegranate aqualete illustrated in Table 1 and Fig. 2. The mean score of standard pomegranate aqualete obtained varied from 4.12±0.44 to 4.47±0.22 in all sensory attributes. The mean score secured for the all attributes of test pomegranate aqualetes were ranging from 3.0±0.22 to 4.1±0.75. However, PAC_c showed the maximum score 4±0.82, 4.1±0.75 and 4±0.83 in terms of consistency, taste and after taste in comparison to standard 4.26±0.93, 4.22±0.60 and 4.17±0.03 which was non-significant at $p \leq 0.05$ that means pomegranate aqualete enriched with 5% *Cymbopogon citratus* was comparable to standard aqualete and highly acceptable to semi trained panel. The score registered for the overall acceptability done by 9-point Hedonic scale was 8.8±0.75 for standard pomegranate aqualete shown in Fig. 3 as PAC_c showed the maximum score (8.5±0.51) followed by PAC_c+C_a (8.0±0.51) and then PAC_a (6.2±0.97) resulting due to bitter taste of Green coffee bean as shown in Fig. 3.

Thus, Sensory analysis revealed that the most acceptable aqualete was PAC_c and registered as insignificant difference at $0.05 \leq p$ level in terms of all attributes. Similar study observed by Chauhan [2] that herb mixed beverage having formulation 6% basil and 1.5% *T. cordifolia*in 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

Attributes	Standard	PAC_a+C_c	PAC_c	PAC_a
Appearance	4.47±0.22	3.7±0.68*	3.7±0.88*	3.3±0.27*
Color	4.12±0.44	3.7±0.60*	3.8±0.93*	3.0±0.22*
Taste	4.22±0.60	3.9±0.60^{NS}	4.1±0.75^{NS}	3.3±0.31*
Consistency	4.26±0.93	3.3±0.71*	4.0±0.82^{NS}	3.3±0.50*
After taste	4.17±0.03	3.8±0.70^{NS}	4.0±0.83^{NS}	3.2±0.14*

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

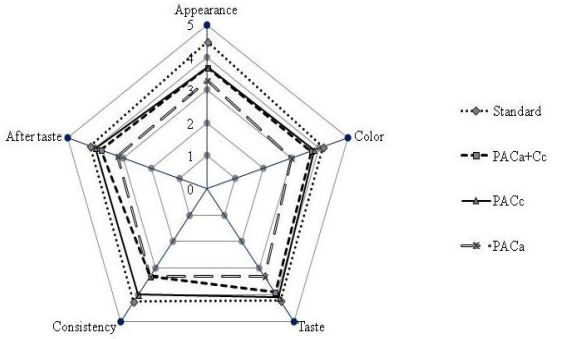

Fig. 2 Sensory evaluation of pomegranate aqualetes based on *Coffea arabica*, *Cymbopogon citratus* and their equi-proportioned mixture

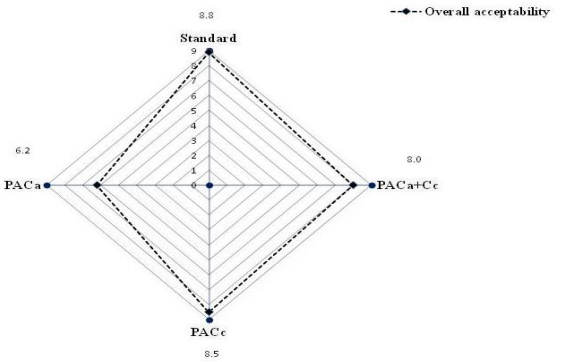

Fig. 3 Overall acceptability evaluation of pomegranate aqualetes aqueous extract of *Coffea arabica, Cymbopogon citratus* and equi-proportioned mixture in the terms of sensory attributes by 9 point hedonic scale

Table 2 Preliminary phytochemical screening of pomegranate aqualetes aqueous extracts of *Coffea arabica* (PAC_a), *Cymbopogon citratus* (PAC_c) and equi-proportioned mixture (PAC_a+C_c)

Phytochemicals	Tests	PAC_a	PAC_c	PAC_a+C_c
Alkaloids	Dragendroff's test	+ve	+ve	+ve
Terpenoids	Lieberman's test	+ve	-ve	+ve
Flavanoids	Shinod's test	+ve	+ve	+ve
Phenols	5% ferric chloride	+ve	+ve	+ve
Saponins	Foam test	-ve	+ve	+ve
Tannin	Ferric chloride test	+ve	+ve	+ve

- Absence, + present

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-proportioned 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 modifier 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

exhibit cytotoxic effects and growth inhibition making them suitable as tumor inhibiting agents.

Table 3 Antioxidant profile of Pomegranate Aqualete (PA) and Test Pomegranate Aqualete with *Coffea arabica* (PAC_a) and *Cymbopogon citratus* (PAC_c)

Antioxidants	PAC_a	PAC_c	PA
Flavonoids (mg QE/g)	8.8± 0.04*	6.9±0.04*	1.4±0.01
Tannins (g/100 g)	8.34± 0.028*	13.89± 0.06*	2.9±0.04
Total Phenols (mg GAE/g)	16.43± 0.34*	13.23± 0.03*	3.8±0.03

*Values are 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* (PACa) and *Cymbopogon citratus* pomegranate aqualetes (PACc) were were 3.8±0.03, 16.43±0.34 and 13.23±0.03 respectively. The data depicted in Table 3 shows that PACa had significantly higher value when compared with PACc and PA. According to Affonso [11] stated that green coffee bean contains 35.39 ± 3.69 mg GAE/g phenols content which is higher value than present data in the study. Similar data was observed by Aloqbi [12] that content of polyphenols was 118.56 µg 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* is 8.8±0.04 and 6.9±0.04 respectively. This shows that *Coffea arabica* had 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 anti-oxidant 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 8.34±0.02 and 13.89±0.06 mg/100 g respectively. This shows that *Cymbopogon citrates* 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 accessibility to the digestive enzymes.

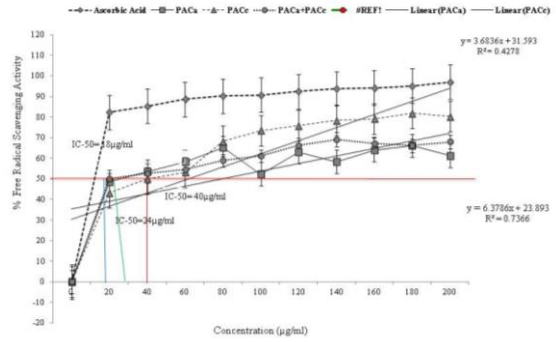

Fig. 4 % free radicals scavenging activity of Pomegranate Aqualetes Aqueous extract of *Coffea arabica* (PAC_a), *Cymbopogon citrates* (PAC_c) sand equi-proportioned mixture (PAC_a+C_c)

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, 1diphenylpicrylhydrazine [14]. The extent of color change depends on hydrogen donating ability of the antioxidants. DPPH assay is based on the ability of DPPH which is 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 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 citrates* and their equi-proportioned mixture are shown in Fig. 4. The antioxidants activities of *Coffea arabica* and *Cymbopogon citratus* with IC_{50} (Inhibitory concentration) values ranged 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 *Cymbopogon citratus* had highest score among all three aqualetes in terms of overall acceptability and acceptable by semi trained panel. This study also showed that pomegranate aqualetes 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 profile and can be used as a promising drink supplement or may be useful in pharmaceutical applications.

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