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Phytochemical Composition, Antioxidant Activity, and Formulation of a Polyherbal Drink into Effervescent Granules from *Hibiscus rosa-sinensis*

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Abstract

Oxidative stress is a major contributor to the pathogenesis of numerous chronic diseases and is identified by the World Health Organization as a leading global health concern. In response, the present study investigates a polyherbal formulation, composed of antioxidant-rich botanicals, for its potential to mitigate oxidative stress and its transformation into a stable solid dosage form. Selected herbs included *Nigella sativa* (black seed), *Hibiscus sabdariffa* (hibiscus flower), *Trigonella foenum-graecum* (fenugreek), *Syzygium aromaticum* (clove), *Zingiber officinale* (ginger), *Foeniculum vulgare* (fennel), *Elettaria cardamomum* (cardamom), *Cinnamomum verum* (cinnamon), *Curcuma longa* (turmeric), and *Beta vulgaris* (beetroot). The aqueous polyherbal decoction was analyzed for phytochemical constituents and quantified for total phenolic content (TPC) using the Folin–Ciocalteu method, expressed in gallic acid equivalents (GAE). Antioxidant potential was assessed via 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The formulation was subsequently converted into effervescent granules via wet granulation. Phytochemical screening confirmed the presence of phenolic, flavonoids, alkaloids, glycosides, coumarins, tannins, and other bioactive. TPC was measured at 117.71 ± 3.48 µg/mg GAE. The extract demonstrated potent free radical scavenging activity (FRSA), with an IC_{50} of 18.59 µg/mL, outperforming standard ascorbic acid (IC_{50} = 30.85 µg/mL). These findings highlight the synergistic antioxidant efficacy and nutraceutical potential of polyherbal blends, supporting their role as functional food ingredients. Moreover, the development of a stable solid dosage form broadens the scope for application in preventive healthcare targeting oxidative stress-mediated disorders.

Keywords

Polyherbal drink, Phenolic content, Antioxidant, Oxidative stress, Effervescent granule

Introduction

Oxidative stress is the outcome of an imbalance between the body's antioxidant defense systems and the production of reactive oxygen species. Several of the top ten leading causes of mortality-including diabetes, stroke, neurological disorders, and various forms of cancer-have been associated with oxidative stress [1, 2]. Free radicals such as reactive oxygen species have the power to start chain reactions that cause damage to the cells of an organism. Long-term cellular damage, inflammation, and the advancement of illness can all result from persistently elevated reactive oxygen species. Antioxidants are substances that inhibit the chemical process of oxidation, which generates free radicals. They are essential because they can neutralize free radicals and prevent oxidative damage [2, 3]. Synthetic molecules such aminoguanidine and alagebrium have potential impacts in treating oxidative-stress-mediated diseases, including diabetes and Alzheimer's diseases, but have displayed limited success in clinical trials [4]. Nutraceuticals, particularly poly-herbal drinks composed of multiple herbs known for their antioxidant activity, have emerged as promising alternatives for disease prevention. These beverages harness the synergistic effects of their constituent herbs to offer substantial protection against oxidative damage [5, 6]. The investigation into natural sources containing antioxidants has increased interest in polyherbal drinks, which are concoctions derived from the mixture of various medicinal plants. Each herb in a polyherbal formulation contributes its unique phytochemical constituents, potentially leading to a synergistic enhancement of therapeutic effects, including antioxidant activity [7]. So, there is need to develop and evaluate affordable, stable, readily available nutraceutical products with antioxidant rich phytochemicals. A blend of *Hibiscus rosa-sinensis* flowers, black seeds, fenugreek, clove buds, ginger, fennel, cardamom, cinnamon bark, turmeric, and beetroot has traditionally been used to prepare polyherbal beverages [8-14]. Based on the review of literature on medicinal herbs incorporated in beverages, it was found that such drinks contain scientifically proven antioxidant phytochemicals, and the inclusion of medicinal herbs in a polyherbal drink enhances its potential as a therapeutic beverage. This formulation may effectively combat oxidative stress, contributing to health maintenance and the prevention of various diseases [15-23]. But the polyherbal drink is aqueous in nature which makes it prone to microbial attack, lesser shelf life and poor stability [24-26]. In contrast, effervescent granules offer advantages such as enhanced patient compliance, rapid disintegration, improved dissolution, better stability, and ease of use [27]. Therefore, in present research polyherbal drinks from *H. rosa-sinensis* and other medicinal herbs were evaluated for their phytochemical profiles, phenolic content, and antioxidant activities. Furthermore, polyherbal drink was converted into effervescent granules and evaluated.

Materials and Methods

Collection of ingredients and chemicals

The ingredients such as fennel, fenugreek, cinnamon, cardamom and clove, were purchased from Yucca enterprise, New Delhi, India. Fresh ingredients such as beetroot, turmeric, ginger, hibiscus and black seed were bought from local market, Tramba, Rajkot, Gujrat. These were washed under running tap water. All the chemical required, including ascorbic acid, gallic acid, DPPH 2, and methanol were purchased from oxford lab fine chem. ltd.

Preparation of poly herbal drink

Preparation of polyherbal drink from *H. rosa-sinensis* and other ingredients were prepared by blending of fresh and boiling of dry ingredients. Quantity of ingredients used to prepare polyherbal drink is mentioned as per table 1. Each herb underwent a meticulous rinsing process, being washed three times under running water. The dried ingredients such as fenugreek, fennel, cinnamon, clove, black seeds and cardamom, were blended and boiled with hibiscus in 500 ml of portable drinking water for 15 min below 45 °C to avoid destruction of phytochemicals. Fresh ingredients ginger, turmeric, and beetroot were blended with an adequate amount of water. The

Table 1: Ingredients of polyherbal drink and its quantity.

S. No.	Ingredients of polyherbal drink	Quantity (gm)
1	*H. rosa-sinensis* petals	30
2	*B. vulgaris* root	20
3	*F. vulgare* fruits	4
4	*S. aromaticum* flower bud	4
5	*Cinnamomum zeylenicum* bark	4
6	*E. cardamomum* fruit	4
7	*Z. officinale* rhizome	10
8	*C. longa* rhizome	8
9	*T. foenum-graecum* seed	5
10	*N. sativa* seed	8

two herbal mixtures were then combined and allowed to stand for 30 min at room temperature before undergoing a filtration process with a muslin cloth. The filtrate was then mixed with microcrystalline cellulose (MCC), which was then carefully dried in an oven set at a low temperature below 45 °C to produce powdered material.

Phytochemical screening of polyherbal drink

The polyherbal drink then was analyzed for presence of the phytochemical class present in the polyherbal drink. The presence of phytochemical class like phenol, flavonoids, protein, amino acids, carbohydrates, coumarin, anthraquinones, cardiac glycoside, alkaloids, fixed oil, lipids, tannin, volatile oil, saponin, and phytosterols a phytochemical screening was carried out as per standard literature [28, 29].

Determination of TPC

Total phenol content in polyherbal drink was measured using Folin-Ciocalteu method [30]. Using a calibration curve that was created from standards for gallic acid, the amount of phenolic content was measured. First, 50 mg of gallic acid were dissolved in 500 ml of water to create a stock solution with a concentration of 100 µg/ml. Gallic acid concentrations ranging from 10 - 100 µg/ml were achieved by transferring different volumes of this stock solution (1, 2, 3, 5, 6, 7, 8, 9, and 10 ml) into 10 ml volumetric flasks and filling them up to the mark with methanol in order to construct the calibration curve. 500 µl of this stock was subsequently put into a 25 ml volumetric flask, along with 1.5 ml of Folin-Ciocalteu reagent and 10 ml of water. 4 ml of a 20% sodium carbonate solution were added following a 5 min break. Distilled water was added to the capacity until it reached 25 ml. A UV-visible spectrophotometer was used to test the mixture's absorbance at 765 nm after it had stood for 30 min. The concentrations in the test samples were then determined using this calibration curve of standard solutions.

DPPH-FRSA

Antioxidant potential of polyherbal drink was measured by DPPH-FRSA [31, 32]. Preparation of standard solution: 10 mg of ascorbic acid was dissolved in 10 ml of methanol to give the concentration of 1 mg/ml. Preparation of test sample:

100 mg of extract of drink was dissolved in 100 ml of methanol to give 1 mg/ml concentration. 4.3 mg of DPPH were dissolved in 3.3 ml of methanol to create the DPPH solution. It was then wrapped in aluminum foil to keep the light off of it. A volume of 50 µl of the DPPH solution was added to 3 ml of methanol, and the absorbance was promptly measured at 517 nm for control reading. Different volume levels of test sample and standard solution (60, 70, 80, and 90 µl) were screened and made 200 µl of each dose level by dilution with methanol. Diluted with methanol with up to 3 ml. Absorbance was taken after 15 min at 517 nm in UV-visible spectrophotometer using methanol as a blank. Ascorbic acid was taken as reference. The FRSA% was determined using the following equation:

$$FRSA\% = \left(\frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}}\right) \times 100$$

Preparation and evaluation of effervescent granules

Effervescent granules of the polyherbal extract were formulated by the wet granulation method described by Xiang-Zhou and Sheng [24] and Forman et al. [33] with some modifications. According to Forman et al. [33], the best effervescent vehicle ratio of citric acid ratio for citric acid, tartaric acid, and sodium bicarbonate is 1:2:3.44. The dried polyherbal drink powder (extract), tartaric acid, sodium bicarbonate and citric acid were accurately weighed according to calculation and mixed in ascending order of their weights by trituration. A porcelain dish was set on a water bath and heat the water bath, to boiling point and place the mixture into the hot porcelain dish which is kept on hot water bath and stir it with spatula for 1 - 5 min. The damp mass was removed from the dish and immediately passed through sieve number 20 by placing on butter paper [34], the resultant granules were dried by spreading and putting in hot air oven at temperature not exceeding 40 °C.

Evaluation parameters for polyherbal effervescent granules such as angle of repose, tapped density, bulk density, Carr's index, and Hausner's ratio were determined as per standard literature [35]. Effervescent time was measured by pouring one dose of granules (3 g) in a beaker containing 50 ml of water. Each batch's granules were selected at random, and the duration of the *in vitro* effervescence was recorded [36]. About 1 gm of effervescent granules were added in 50 ml of water at 25 °C until it completely dissolved. The pH was measured using a digital pH apparatus [37].

Results and Discussion

Preliminary phytochemical screening of the polyherbal drink

The phytochemical screening confirmed the existence of multiple classes of phytochemicals within the polyherbal drink. Phytochemical class found were flavonoids, alkaloids, carbohydrates, coumarin and phenolic acids, indicating a rich phytochemical profile. The results of phytochemical screening are depicted in table 2.

TPC of polyherbal drink

The drink's TPC was measured using the Folin-Ciocalteu reagent method [30]. Based on the analysis, the drink contained a substantial number of phenolic compounds -117.70 ± 3.47 µg of GAE per mg of polyherbal drink as mentioned in table 3 by using calibration curve for gallic acid mentioned. The high level of phenolic compounds suggests a strong potential for antioxidant activity, which was further evaluated through antioxidant assays.

Table 2: Phytochemicals identified in the polyherbal drink.

Phytochemical test	Results
Phenol	+
Flavonoids	+
Protein and amino acids	+
Anthraquinone glycoside	+
Cardiac glycoside	+
Carbohydrates	+
Coumarin	+
Alkaloids	+
Tannin	+
Volatile oil	-
Fixed oils and fat	-
Saponin	-
Phytosterol	+

Note: + indicate positive test and - indicate negative test.

Table 3: Total phenol content of polyherbal drink.

Sample	TPC			Mean ± SEM µg of GAE/mg of drink
	1	L1	1l1	
Polyherbal drink	114.75	121.536	116.84	117.7087 ± 3.475397

Note: Values are expressed as mean (µg mg^{-1} GAE) ± SEM.

In-vitro antioxidant potential of polyherbal drink by DPPH method

At ambient temperature, DPPH remains stable as a free radical and can change into a stable diamagnetic molecule by accepting either an electron or a hydrogen radical. The decrease in the A decrease in the absorbance of the DPPH radical at 517 nm in the presence of antioxidants indicates its reduction capacity. A stable DPPH radical in methanol has an absorbance maximum at 517 nm. As a result of the reaction between antioxidant molecules and the radical, the DPPH radical's absorbance is reduced by antioxidants, causing the radical to be scavenged by hydrogen donation. Since the noticeable shift in color from purple to yellow signifies a decrease in absorbance, DPPH is a frequently used substrate to assess antioxidant activity [38].

Comparing figure 1 and figure 2, the drink effectively scavenged the DPPH free radical in a dose-dependent manner, with an IC_{50} value of 30.85 µg/ml whereas the reference compound ascorbic acid found to have an IC_{50} value 18.59 µg/ml as mentioned table 4.

Formulation and evaluation of effervescent granules from polyherbal drink

Three formulations of effervescent granules were developed as shown in figure 3, each containing different proportion of MCC as mentioned in table 5. It was clear by analyz-

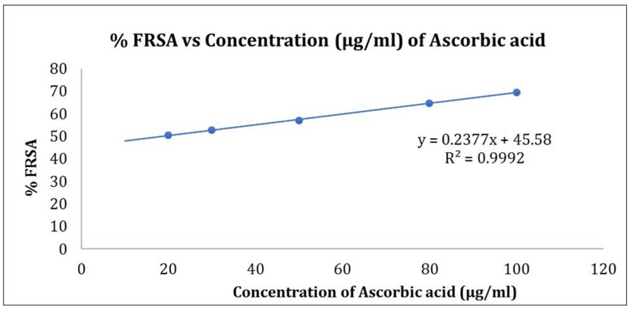

Figure 1: Percentage scavenging of DPPH free radical by ascorbic acid.

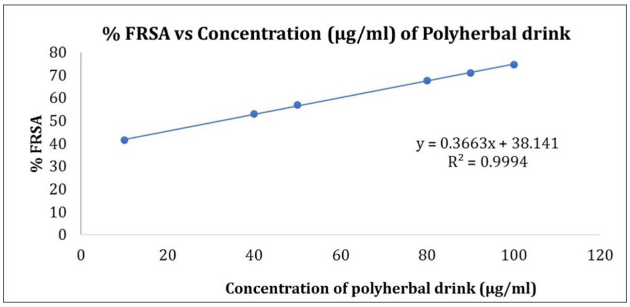

Figure 2: Percentage scavenging of DPPH free radical by polyherbal drink.

ing physicochemical parameters such as bulk density, tapped density, Hausner's ratio, and Carr's index that formulation F3 had superior flow characteristics than F1 and F2, as shown in table 6. This improvement is explained by the higher amount of MCC found in F3. F3 is stable and did not agglomerate or become sticky during storage, whereas F1 and F2 were unstable. The combination of this polyherbal in a particular ratio has been shown to produce a synergistic effect, better therapeutic efficacy and reduced toxicity [39].

In present investigation polyherbal drink have shown presence of all therapeutically important class of phytochemicals like flavonoids, phenols, alkaloids, glycosides, tannins and phytosterols. In present study polyherbal drink from *H. rosa-sinensis* and other medicinally potential herbs were also found rich in total phenol and flavonoid content. Among all phytochemicals, flavonoids, polyphenols and phytosterols/triterpenoids play an important role in providing protection from oxidative stress related diseases because it can not only be scavenging free radicals but also inhibit chain reactions [40]. Oxidative stress related diseases such as diabetes, cancer, stroke, cardiovascular disorders and neurological conditions remain among the leading cause of death globally, according to World Health Organization reports [41]. In present investigation, the antioxidant potential of polyherbal drink was also found comparable to that of the reference standard ascorbic acid and in dose-dependent manner. Antioxidant potential of polyherbal drink might be due to the presence of significant total phenol and flavonoid content. Moreover, based on our literature review of observational studies, it was found that the daily dietary intake of the ingredients used in the polyherbal drink may reduce the risk of various types of cancer [42]. Prepared effervescent granules are more stable due to its solid nature than a polyherbal drink, while polyherbal drink from

Table 4: IC_{50} value of polyherbal drink and ascorbic acid.

Sample	IC_{50} value
Standard (ascorbic acid)	18.59487 ±0.24 µg/ml
Polyherbal drink	30.85035 ±0.35 µg/ml

Note: Values are expressed as mean ± SEM, where n = 3.

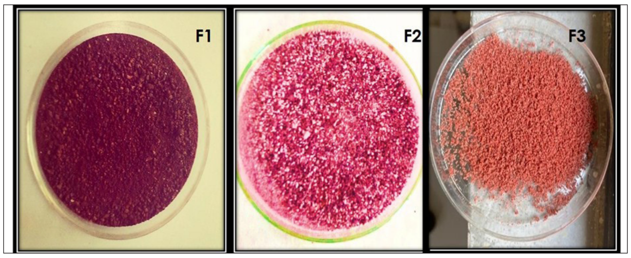

Figure 3: Effervescent granule formulation of polyherbal drink F1, F2, and F3.

Table 5: Composition of polyherbal effervescent granules.

Formulations	Amount of liquid extract	Amount of MCC	Amount of dried extract	Tartaric acid	Citric acid	Sodium bicarbonate
F1	10 ml	0.5 gm	3.0 gm	0.5 gm	0.24 gm	0.84 gm
F2	10 ml	1.0 gm	2.0 gm	0.5 gm	0.24 gm	0.84 gm
F3	10 ml	1.5 gm	1.0 gm	0.5 gm	0.24 gm	0.84 gm

Table 6: Physicochemical evaluation of polyherbal effervescence granules.

S. No.	Parameters	F1	Character	F2	Character	F3	Character
1	Angle of repose	37.43	Fair	33.05	Good	30.02	Excellent
2	Bulk density	0.36	Excellent	0.3	Excellent	0.25	Excellent
3	Tapped density	0.38	Excellent	0.29	Excellent	0.27	Excellent
4	Carr's index	5.26	Excellent	3.33	Excellent	3.23	Excellent
5	Hausner's ratio	1.06	Excellent	1.03	Excellent	1	Excellent
7	Effervescence time	24 s	Excellent	23 s	Excellent	21 s	Excellent
8	Disintegration time	35 s	Fair	40 s	Good	30.20 s	Good
9	Color	-	Purple	-	Purple	-	Purple
10	Odor	-	Aromatic	-	Aromatic	-	Aromatic
11	Taste	-	Sweet	-	Sweet	-	Sweet

H. rosa-sinensis and other herbs is prone to microbial attack due to its aqueous nature. Effervescent granules have several advantages, including enhanced bioavailability and faster drug absorption due to improved solubility [43]. They promote better patient compliance by being easier to swallow and having a pleasant taste [44]. Effervescent granules also improve drug stability, provide accurate dosing, and reduce gastric irritation [45].

Recent studies have demonstrated that nanoformulations of herbal bioactive-such as curcumin, berberine, and *H. sabdariffa* can significantly improve antioxidant activity, bioavailability, and stability, both in preclinical and clinical settings [46]. These advancements highlight the growing interest in developing consumer-friendly herbal formulations with enhanced functionality. While the present study did not involve nanoencapsulation, the formulation of polyherbal effervescent granules offers a promising alternative delivery system, particularly for nutraceutical applications.

However, potential herb–herb interactions were not deeply investigated, and safety assessments such as cytotoxicity or biocompatibility studies were not conducted. Additionally, regulatory aspects including standardization, permissible excipient levels, and stability for scale-up remain to be addressed. Future studies should focus on toxicological evaluation and regulatory compliance to support safe human application.

Conclusion

The present study successfully demonstrated the development of a polyherbal antioxidant drink with significant phenolic content and *in vitro* antioxidant potential, which was further formulated into stable and patient-friendly effervescent granules. These granules offer improved shelf stability, portability, and consumer compliance compared to traditional aqueous formulations. The findings suggest that such polyherbal effervescent granules can serve as a promising, cost-effective nutraceutical intervention for the prevention of oxidative stress-related disorders, including cardiovascular diseases, cancer, and neurodegenerative conditions. This work also provides a scalable and consumer-adaptable format for herbal antioxidants, supporting their use in preventive health. Future research may explore cytotoxicity, bioavailability, and clinical validation to facilitate regulatory approval and commercialization.

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None.

Conflict of Interest

None.

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