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Plant-based Antioxidant Jelly: Formulation and Evaluation of a Nutraceutical from Three Medicinal Fruits: *Carissa carandas, Feronia limonia,* and *Opuntia ficus-indica*

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Abstract

Oxidative stress-related diseases, including cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions, are major contributors to global mortality, as reported by the World Health Organization (WHO). Although synthetic inhibitors such as aminoguanidine and alagebrium, have demonstrated potential in mitigating oxidative stress. However, their clinical efficacy remains limited. In contrast, natural antioxidants derived from medicinal plants, particularly those rich in phenolic and flavonoid compounds, offer a promising alternative. The present study focuses on the formulation and evaluation of a polyherbal jelly made from *Carissa carandas, Feronia limonia, and Opuntia ficus-indica* (CFO), incorporating the fruit pulp of all three plants, which are rich in phenol content and known for their potent antioxidant properties. The jelly formulations (F1 and F2) were assessed for physicochemical parameters, phytochemical composition, antioxidant potential, and nutritional content. F1 and F2 jellies exhibited good clarity, desirable consistency, and a stable pH; however, F2 demonstrated higher viscosity (5891 cP) and F1 was fragmented. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, alkaloids, and glycosides. Quantitative analysis revealed significant total phenolic content (11.31 ± 0.62 milligrams of gallic acid equivalent/gram (mg GAE/g) and total flavonoid content (35.29 ± 0.47 µg quercetin equivalent (QE)/100 mg). Antioxidant activity, measured via 2,2-diphenyl-1-picrylhydrazyl (DPPH), radical scavenging assay, yielded a half maximal inhibitory concentration (IC_{50}) value of 4.13 ± 0.15 mg/mL, indicating substantial free radical neutralizing capacity, comparable to that of ascorbic acid. Nutritional evaluation indicated high carbohydrate content (84.70 g/100 g) with minimal fat, highlighting the jelly's potential as a nutritionally beneficial antioxidant supplement.

Keywords

Functional foods, Antioxidants, Flavonoids, Phenol, *Feronia limonia* Linn., *Carissa carandas, Opuntia ficus-indica,* Nutraceuticals, Bioactive compounds

Introduction

The oxidative stress related diseases such as cancer, diabetes, stroke, heart attack, Alzheimer's, and Parkinson's are among the leading causes of death worldwide, according to the WHO [1]. Antioxidants play a pivotal role in the prevention and treatment of this oxidative stress related disease [2]. Receptors for advanced glycation end-products and advanced glycation end-products inhibitors- such as aminoguanidine and alagebrium- are the synthetic molecules which are under clinical trials to prevent oxidative stress; however, they have shown only limited success in mitigating oxidative damage. Fruits like CFO are known to be rich in phenolic and flavonoids compounds [3]. Although these plants have individually demonstrated significant antioxidant potential, their fruits are sea-

sonal and geographically restricted, which limits year-round availability. Additionally, aqueous nature of their fruits pulps makes them highly perishable and prone to microbial spoilage, thus reducing shelf life and stability. To address this, there is a need to develop a stable, economical, and effective antioxidants formulation using these fruits [4]. Oral jelly formulation is a promising delivery system due to its stability, patient friendliness (especially in pediatric and geriatric populations), cost-effectiveness, ease of manufacturing, and improved patient compliance. Oral jellies are easy to swallow, mask unpleasant tastes, disintegrate rapidly in saliva without the need for water and are portable [5]. Moreover, *C. carandas* and *F. limonia* Linn. both have high natural pectin content 11.94% and 5.30%, respectively making them ideal candidates for jelly formulation [2]. Therefore, the current study aimed to formulate a stable, phytochemical rich, and antioxidant polyherbal jelly by utilizing the fruits pulp of CFO [6].

Materials and Methods

Collection and authentication of plant material

C. carandas (Figure 1) and *O. ficus-indica* (Figure 2) fruits were collected in the month of October 2024 from Medicinal Garden, RK University, Rajkot, Gujarat, India and *F. limonia* Linn. (Figure 3) from near area. Lemon and Rock sugar was purchased from local market. Herbariums for CFO were prepared and authenticated by botanist at School of Science, RK University, with herbarium number SOP/COG/6/2025, SOP/COG/14/2025, and SOP/COG/15/2025, respectively. Herbariums were deposited at herbarium repository at School of Pharmacy, RK University.

Chemicals and reagents

Gallic acid, quercetin, DPPH, Folin-Ciocalteu reagent, and ascorbic acid were purchased from Sigma Aldrich. All other chemicals and reagents were purchased from S.D. Fine-Chem Ltd. Mumbai, India.

Formulation and evaluation of CFO polyherbal jelly

C. carandas fresh fruits were washed, then pulp was separated from seeds, further blended until smooth pulp appeared. For *O. ficus-indica* pulp preparation, fresh fruits were washed, peeled, and blended until smooth. *F. limonia* Linn. fruit has a very hard, woody shell, by using a hammer, a strong knife, or a machete, then the pulp was scooped out [7]. *F. limonia* Linn. fruit pulp was homogenized in distilled water, followed by the addition of freshly extracted lemon juice to facilitate the extraction of naturally occurring pectin. The resultant mixture was subjected to continuous mechanical stirring in a stainless-steel mixing vessel and heated in a thermostati-

Figure 1: *C. caranda* fruit.

Figure 2: *O. ficus-indica* fruit.

Figure 3: *F. limonia* fruit.

cally controlled water bath maintained at 60 °C for 30 - 40 min to promote pectin solubilization and partial hydrolysis of plant tissues. Post thermal treatment, the mixture was filtered through a sterile muslin cloth or fine mesh to remove coarse particulate matter. The clarified extract was subsequently combined with powdered rock sugar, *C. carandas* fruit pulp, and *O. ficus-indica* fruit pulp under continuous stirring to ensure uniform dispersion of ingredients. The composite mixture was reheated at 60 °C for an additional 30 - 40 min until the desired gel-like consistency was achieved, confirmed by the non-flowability of a drop upon inversion, indicating sufficient gelation. The hot jelly matrix was poured into pre-sterilized molds and allowed to set under ambient conditions. After 6 h, the former jelly was demolded and stored in airtight containers at a controlled temperature of 1 - 7 °C to preserve physicochemical integrity and prevent microbial contamination [8]. F1 and F2 CFO polyherbal jelly were formulated with different proportions of sugar, water, *C. carandas*, and *O. ficus-indica* fruit pulp as presented in table 1.

Table 1: List of ingredients of CFO polyherbal jelly.

Ingredients	F1	F2
F. limonia Linn. fruit pulp	422 g	422 g
Distilled water	700 g	750 g
Lemon juice	10 mL	10 mL
Rock sugar	500 g	536 g
C. carandas pulp	150 g	166 g
O. ficus-indica fruit pulp	70 g	83 g

Evaluation parameters for CFO polyherbal jelly

Physical evaluation

The CFO polyherbal jelly underwent organoleptic and physical evaluation, including assessments of color, odor, clarity, texture, transparency, and consistency [9].

Stickiness and grittiness

The rheological attributes of the CFO polyherbal jelly, specifically its cohesive and particulate characteristics like stickiness and grittiness, were evaluated through light palpation using manual fingertip contact [9].

pH

The pH of the CFO jelly was determined using a calibrated digital pH meter. Approximately 0.5 g of the formulation was accurately dispersed in 50 mL of distilled water, and the pH value was subsequently recorded under controlled conditions [9].

Viscosity

The viscosity of the CFO polyherbal jelly was evaluated using a Brookfield viscometer, employing spindle number 4 to accommodate the formulation's non-Newtonian flow behavior [9].

Physicochemical evaluation of CFO polyherbal jelly

Physicochemical evaluation of CFO polyherbal jelly was evaluated by total ash value and moisture content of polyherbal jelly as per WHO guidelines for quality control methods for medicinal plants [9].

Phytochemical analysis of CFO polyherbal jelly

The preliminary phytochemical investigation was carried out according to the methods described in standard literature [10]. 10 g of jelly were dissolved in 100 mL hot water. The resulting solution was tested for presence of various classes of phytochemicals, including carbohydrates, alkaloids, phytosterols, glycosides, proteins and free amino acids, flavonoids, terpenoids/triterpenoids, phenolic compounds, tannins, saponin glycosides, fixed oils, steroids, anthraquinone glycosides, zinc, calcium, vitamin C, and coumarin glycosides.

Deamination of total phenol content of CFO polyherbal jelly

The phenolic content of the CFO polyherbal jelly were quantified utilizing the Folin–Ciocalteu colorimetric assay [11, 12]. 1 g of jelly was dissolved in distilled water to prepare the sample solution. From this, 1 mL of the prepared test solution was diluted to 10 mL using distilled water. From this, a 500 µL was transferred into a 25 mL volumetric flask. Subsequently, 10 mL of distilled water and 1.5 mL of Folin–Ciocalteu reagent were added to the flask. After allowing the reaction to proceed for 5 min, 4 mL of a 20% sodium carbonate solution was incorporated, and the final volume was adjusted to 25 mL with distilled water. The mixture was kept undisturbed at ambient temperature for 30 min to facilitate chromophore development. Absorbance of the developed blue complex was recorded at 765 nm using a double-beam ultraviolet–visible (UV-Vis) spectrophotometer (Labtronic LT2900). A standard curve was constructed using gallic acid at varying concentrations (µg/mL), and the total phenolic content was extrapolated via linear regression analysis. Results were expressed as mg GAE/g of CFO polyherbal jelly.

Determination of total flavonoids content of CFO polyherbal jelly

According to Chang et al. [13], the aluminum chloride colorimetric method was slightly modified to analyze total flavonoids. Using quercetin, the calibration curve was created. A stock solution of quercetin (10 mg) was prepared in 96% methanol and subsequently diluted to obtain standard concentrations of 2, 4, 6, 8, and 10 µg/mL. For the assay, 1 mL of each standard and sample solution was mixed with 3 mL of 96% methanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water in separate test tubes [14].

The mixture was shaken periodically for 10 min while it was incubated at room temperature. At 376 nm, the absorbance was measured using a double beam UV-Vis spectrophotometer (Labtronic-LT2900) in comparison to a blank that had no aluminum chloride. Mean ± standard error of the mean (SEM) (n = 3) was used to compute total flavonoid, which was then represented as weight of the CFO polyherbal jelly of QE at 100 mg. A standard curve was established by plotting absorbance values against quercetin concentrations (µg/mL) using Microsoft excel 2016. Total flavonoid content in the

CFO polyherbal jelly was quantified through linear regression analysis of the calibration curve and reported as micrograms of QE/100 mg of CFO polyherbal jelly.

Antioxidant potential of CFO jelly by DPPH free radical scavenging assay (FRSA)

The antioxidant efficacy of the CFO polyherbal jelly was assessed using the DPPH radical scavenging assay, as previously described [15, 16]. This method is based on the reduction of the DPPH radical to a non-radical form, indicated by a decrease in absorbance intensity, upon interaction with antioxidant compounds. Ascorbic acid served as the standard reference. A stock solution was prepared by dissolving 10 mg of ascorbic acid in 10 mL of methanol, yielding a concentration of 1 mg/mL. For the test sample, 1 g of the CFO polyherbal jelly was solubilized in 10 mL of hot water to prepare a 100 mg/mL solution. A DPPH working solution was obtained by dissolving 4.3 mg of DPPH in 3.3 mL of methanol and protected from photo-degradation using aluminum foil. For the control, 150 µL of DPPH solution was mixed with 3 mL of methanol, and the absorbance was recorded immediately at 517 nm. For the test, graded volumes (10 - 120 µL) of both the jelly extract and standard were adjusted to 200 µL with methanol, followed by dilution to 3 mL with methanol. Then, 150 µL of DPPH solution was added to each tube. After incubating the reaction mixtures in the dark for 15 min, absorbance was recorded at 517 nm using a double-beam UV-Vis spectrophotometer (Labtronic LT2900), with methanol serving as the blank. The percentage of FRSA was calculated using the formula:

$$FRSA\% = \left(\frac{A_c - A_s}{A_c}\right) \times 100$$

Where, A_c is control absorbance & A_s is sample absorbance.

Statistical analysis

Dose-dependent DPPH radical neutralization by ascorbic acid (µg/mL) and CFO polyherbal jelly (µg/mL) graph was plotted using Microsoft's excel 2016. IC_{50} values were calculated from graph using linear regression.

Nutritional profile of CFO polyherbal jelly

Nutritional profile of CFO polyherbal jelly was evaluated by measuring total carbohydrate, protein, fat, and crude fiber by using the Association of Official Analytical Chemists standard procedures [17-19].

Results and Discussion

Formulation and organoleptic evaluation of CFO polyherbal jelly

Two CFO polyherbal jelly F1 and F2, incorporating the fruit pulp of CFO, were developed and assessed for key quality parameters. Both F1 and F2 demonstrated acceptable clarity, non-sticky texture, and a pleasant fruity odor and absence of syneresis, confirming adequate gel formation and shelf stability (Table 2). Notably, F2 showed improved consistency and a significantly higher viscosity (5891 cP) compared to F1 (4037 cP), indicating enhanced gel strength and superior mouthfeel. While, F1 showed a disintegrated semi-solid structure, and F2 maintained an intact solid structure, as illustrated in figure 4 and figure 5. The pH of both F1 and F2 was approximately 6.0, which is within the optimal range for oral delivery, ensuring product stability and consumer safety.

Table 2: Evaluation parameter of CFO polyherbal jelly.

S. No.	Test parameter	F1	F2
1	Color	Red	Red
2	Clarity	Transparent	Transparent
3	Consistency	Fluid	Acceptable
4	Texture	Non sticky	Non sticky and non-gritty
5	Odor	Pleasant and fruity	Pleasant and fruity
6	pH	6	6
7	Viscosity	4037 cP	5891 cP

Phytochemical screening of CFO polyherbal jelly

Preliminary phytochemical analysis confirmed the occurrence of key phytoconstituents including flavonoid com-

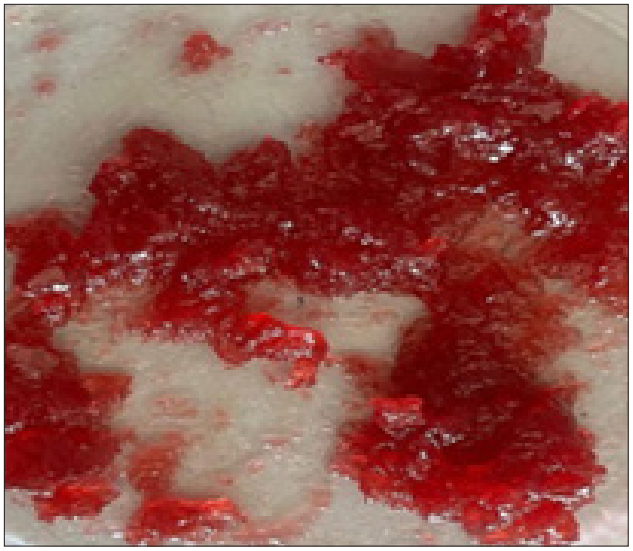

Figure 4: F1 of CFO polyherbal jelly.

Figure 5: F2 of CFO polyherbal jelly.

pounds, phenolic derivatives, and alkaloid-based metabolites, tannins, proteins, glycosides, and essential minerals such as calcium and zinc, all of which enhance the nutraceutical value of CFO polyherbal jelly. The results are summarized in table 3. These phytoconstituents are well-documented for their pharmacological properties, particularly in combating oxidative stress [20-22]. The absence of iron and fixed oils may further contribute to the product's stability and reduce susceptibility to oxidation-related spoilage [23, 24].

Physicochemical properties of CFO polyherbal jelly

The physicochemical investigation was evaluated in accordance with WHO guidelines [9], focusing on total ash and moisture content. As presented in table 4, the CFO jelly exhibited a total ash of 0.77 ± 0.53%, indicating minimal inorganic contamination. The moisture content was 29.21 ± 0.32%, contributing to favorable texture and palatability while also influencing shelf life. These values are within acceptable limits for semisolid oral formulations, suggesting good quality and storage potential.

Total phenolic and flavonoid content of CFO polyherbal jelly

The total phenolic and flavonoid contents were quantified using the Folin–Ciocalteu assay and the aluminum chloride colorimetric method, respectively [12-14]. As shown in table 5 and table 6, CFO polyherbal jelly contained 11.31 ± 0.62 mg GAE/g (n = 3) of phenolic compounds and 35.28 ± 0.47 µg QE/100 mg of flavonoids. Phenols and flavonoid groups of phytochemicals are well-documented for their antioxidant, anti-inflammatory, and anti-carcinogenic properties. The high flavonoid content is particularly notable, as flavonoids effectively scavenge reactive oxygen and nitrogen species [25-28].

Antioxidant potential of CFO polyherbal jelly

The antioxidant potential of the CFO polyherbal jelly was evaluated using the DPPH radical scavenging assay [15, 16]. As depicted in table 7, figure 6, and figure 7, the jelly exhibited an IC_{50} value of 4.13 ± 0.15 mg/mL, compared to 81.83 ± 1.15 µg/mL for the standard antioxidant ascorbic acid. The CFO jelly demonstrated significant antioxidant potential in a dose dependent manner.

Oxidative stress is major contributing factor to the development of chronic diseases, such as cardiovascular disorders, neurodegenerative conditions (including Alzheimer's and Parkinson's), diabetes, cancer, and other inflammation-related ailments [29]. These diseases are also among the leading causes of death according to WHO [1]. In recent years, antioxidants have emerged as one of the most important classes of nutraceuticals due to their potential to counteract oxidative damage. Nutraceuticals with high antioxidant potential typically contain phytochemicals like polyphenols, flavonoids, and vitamins C and E, which help neutralize free radicals, disrupt oxidative pathways, and enhance endogenous antioxidant enzymes such as super oxide dismutase, catalase, and glutathione peroxidase [30, 31]. In the present investigation, CFO jelly was found to contain a significant amount of phenolic and flavonoid phytochemicals and exhibited antioxidants potential comparable to that of ascorbic acid. Hence, regular dietary intake of this phytochemical-rich CFO jelly could help prevent oxidative damage and reduce the risk of oxidative stress-related chronic diseases [32].

Table 3: Phytochemical analysis of CFO polyherbal jelly.

Class of phytochemical	Polyherbal jelly
Carbohydrates	+
Alkaloids	+
Phytosterols	+
Proteins and free amino acid	+
Flavonoids	+
Terpenoids	+
Phenolic	+
Tannins	+
Saponins glycoside	+
Fixed oil	-
Coumarin glycoside	+
Anthraquinone glycosides	+
Zinc	+
Calcium	+
Iron	-

Note: + indicate present and - indicate absence.

Table 4: Physicochemical properties of CFO polyherbal jelly.

S. No.	Physicochemical parameter	Value in % (Mean ± SEM (g/100 g))
1	Total ash	0.77 ± 0.53
2	Total moisture	29.21 ± 0.32

Note: Values are expressed as % mean ± SEM (n = 3).

Table 5: Total phenol content of CFO polyherbal jelly.

Sample	Total phenol in mg/g of GAE
CFO polyherbal jelly	11.31724 ± 0.62069

Note: Values are expressed as mean ± SEM (n = 3). Total phenolic content is expressed in mg GAE/g.

Table 6: Total flavonoids content of CFO polyherbal jelly.

Sample	Total flavonoid in µg of QE/100 mg
CFO polyherbal jelly	35.2856 ± 0.474

Note: Values are expressed as mean ± SEM (n = 3). Total flavonoid content is expressed in µg QE/100 mg.

Table 7: IC_{50} value of ascorbic acid and CFO polyherbal jelly.

Sample	IC_{50} value
Ascorbic acid	81.83 ± 1.15 µg/ml
CFO polyherbal jelly	4.134 ± 0.15 mg/ml

Note: Values are expressed as mean ± SEM (n = 3).

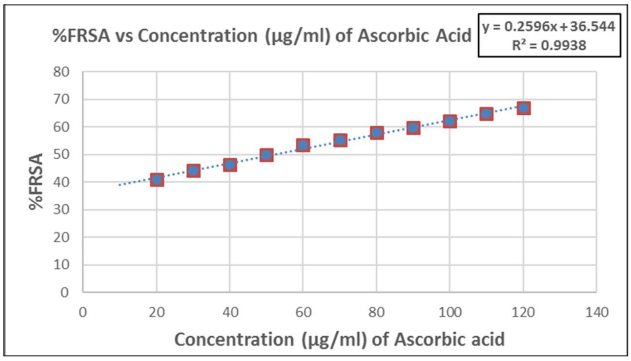

Figure 6: FRSA% by ascorbic acid at different concentrations.

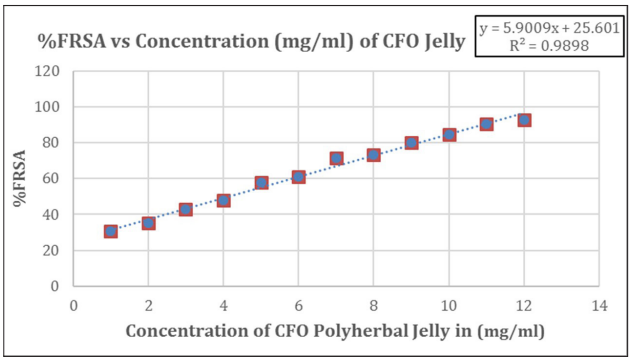

Figure 7: FRSA% by CFO polyherbal jelly at different concentration.

Nutritional evaluation of CFO polyherbal jelly

The nutritional analysis revealed that CFO polyherbal jelly has a high carbohydrate content (84.70 g/100 g), moderate protein levels (1.17 g/100 g), and negligible fat (0.03 g/100 g), including no detectable trans-fat. These characteristics make the formulation suitable for health-conscious consumers and individuals following low-fat diets. Additionally, the low fiber content (0.02 g/100 g) is appropriate for a jelly-based product, ensuring a smooth and palatable texture (Table 8).

A key limitation of the present investigation is the high carbohydrate content which could pose concerns for certain populations. Additionally, the aqueous nature of the formulation may increase risk of microbial contamination during long-term storage. Further investigations should include detailed stability testing, herb interactions studies, extended shelf-life analysis, and patient acceptability evaluations.

Conclusion

The present investigation involved the successful formulation and comprehensive evaluation of a CFO polyherbal jelly utilizing the fruit pulps of CFO botanical species traditionally documented for their rich phytochemical profiles and significant antioxidant efficacy. Among the two formulations, F2 was superior in terms of viscosity, consistency, and overall physical stability. The CFO polyherbal jelly demonstrated excellent organoleptic and physicochemical properties, making it suitable for oral consumption. Phytochemical profiling revealed a broad spectrum of secondary metabolites, while quantitative assessments of total phenolic and flavonoids demonstrated a high antioxidant capacity, indicative of significant free radical scavenging potential. The pronounced FRSA of the CFO polyherbal jelly highlights its therapeutic potential as a functional food or nutraceutical candidate for the mitigation of oxidative stress-related disorders. Furthermore, its high carbohydrate content and minimal fat profile enhance its appeal as a low-fat, energy-rich supplement. Future work should involve *in vivo* studies and clinical trials to confirm its efficacy and safety profile in human populations, as well as optimization of shelf-life parameters and patient acceptance studies. Additionally, the formulation's reliance on locally available fruits and simple preparation techniques makes it feasible for scale-up by small and medium food enterprises. With appropriate compliance with food safety regulations (e.g., Food Safety and Standards Authority of India), this jelly could be positioned as a preventive dietary supplement. Its integration into public health nutrition programs may offer a cost-effective strategy for combating oxidative stress-linked non-communicable diseases.

Table 8: Nutritional profile of CFO polyherbal jelly.

S. No.	Parameter	Result (g/100 g)
1	Carbohydrate	84.70
2	Protein	1.17
3	Fat	0.03
4	Saturated fat	0.01
5	Trans fat	0.00
6	Fiber	0.02

Acknowledgements

None.

Conflict of Interest

None.

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