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Shatavari Infused Fruit Drink (Ferti) for the Fertility Enhancement in Women

Anli Dino A[1*], Justin P Jacob S[1], Harnish A K[1], Blessin Ajay S[1], Kripa V Roy[2], Arumugam P[2]

[1]*Department of Biotechnology, St. Joseph's College of Engineering, Chennai-600119, India.*
[2]*ARMATS Biotek Training and Research Institute, Guindy, Chennai, India.*

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ABSTRACT

In recent years, there has been a significant rise in fertility-related issues especially among women, driven by a combination of lifestyle changes, environmental factors, and underlying health conditions. The impact of this issue is widely recognizable nowadays which includes infertility, increased risk of pregnancy, declining birth rates and increasing fertility centers everywhere. The problems associated with women fertility is increasingly rising every day and this has raised the need of a natural product that can improve women fertility and reproductive health. The main aim of this study is to create a nutraceutical health beverage that contains Shatavari (Asparagus racemosus), pomegranate (Punicagranatum), flax seeds (Linum usitatissimum) and honey. The 4 main ingredients for this product were chosen based on their powerful effect on increasing women fertility and to create a potential blend with essential nutrients, vitamins and minerals. Pomegranate is rich in antioxidant, flax seeds having fatty acids and phytoestrogens, honey serves as a natural preservative and immunomodulator, and Shatavari is well known in Ayurveda for its fertility enhancing qualities. Based on the existing studies, we propose that our food product Ferti can improve women fertility, reproductive health and increasing antioxidants level in the body to maintain a good metabolism. Organoleptic studies revealed that this health drink is highly acceptable. Further studies are required to demonstrate the performance of Shatavari-infused fruit drink and their impact on the reproductive health of women at the level of ovary and oocyte. This health beverage aims to give a natural solution to improve female fertility by combining ancient indigenous herb with food technology.

Keywords: Shatavari, pomegranate, flax seeds, honey, female fertility, reproductive health

I. INTRODUCTION

Women's infertility rate is increasing and becoming a more widespread problem worldwide, impacting many women's in their reproductive age (Qiao et al., 2014). Infertility is a serious health issue that accounts large number of couples worldwide especially in older couples due to continuous decline in fertility rate (Dunson et al., 2004). About one-third of infertility cases that affect couples are largely caused by the woman, one-third by the man, one-third by a combination of the two, and 20% are left unexplained (Peterson et al., 2007). Several factors can influence on women's inability to conceive, including age, lifestyle habits, occupational hazards, poor diet, stress, etc. (Bayer, S. R. 2011). There were many studies conducted in the field of reproductive health and methodologies for the increasing incidence of infertility (Greil et al., 2010). Even though in vitro fertilization (IVF) and other assisted reproductive technologies are available options for bearing child for women with fertility issues, they have always been an expensive treatment and not cost effective for everyone (Ombelet et al., 2007). This has led to the increase in the use of functional foods and herbal supplements that promote hormonal balance, reproductive health due to the decline of fertility rate in women after their mid-20's (Reilly, M. 2021). Therefore, the requirement of natural therapy by usage of food and drug supplements (FDS) to replace the traditional treatments has been increased as the modern treatments are not cost-effective (Urman et al., 2014). This research mainly focuses on the usage of natural food-based ingredients such as Shatavari (Asparagus racemosus), pomegranate (Punicagranatum), flax seeds (Linum usitatissimum), and honey for promoting fertility in women.

In Ferti, four different natural ingredients are used such as Shatavari, pomegranate, flax

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seeds, and honey to boost female fertility. One of the most widely used herbal remedies for women's reproductive health issues is Shatavari (Asparagus racemosus), which is derived from the Sanskrit word "Ayur," which means "life," and "Ved," which means "knowledge". Shatavari is frequently prescribed to support ovarian health, encourage the synthesis of reproductive hormones, and preserve women's libido (Pandey et al., 2018). In comparison to ovariectomized rats used as a control, it was discovered that a formulation containing asparagus racemosus extract increased uterine weight and uterine glycogen in immature rats without changing serum levels of estrogen and progesterone. According to this study, the phytoestrogen binds to the estrogen receptor directly to carry out its action without raising endogenous estrogen levels. (Gopumadhavan et al., 2005). It is used to treat female reproductive issues such as irregular menstrual cycles, endometriosis, dysmenorrhea, uterine bleeding, amenorrhea, sexual weakness, dysfunction, menopause, and pelvic inflammatory diseases such sexual dysfunction. It has been used as a uterine tonic for PMS since ancient times; as a result, it produces uterine prolapse and strengthens, nourishes, and cleanses the uterus. By harmonizing the hormone levels, it also helps to enhance lactation and avoids miscarriages by eradicating infertility and preparing the uterine wall for contraction during fetal development. It improves folliculogenesis, makes ovaries heavier, and the root extract helps stimulate serum FSH (Somani et al., 2012) (Jagannath et al., 2012) (Yue et al., 2004).

Pomegranates are selected as they are a popular choice for juices, smoothies because of their unique blend of sweet and sour flavors (Mayuoni- Kirshinbaum et al., 2014). Regular consumption of pomegranates is associated with enhanced immune function, a decreased risk of several types of cancer, and better heart health (de Oliveira et al., 2020). According to a new review paper, pomegranates have long been used as a traditional treatment for ailments like intestinal parasites, diabetes, hemorrhoids, sore throats, diarrhea, and vaginal itching (Eghbali et al., 2021). A rich source of many components, pomegranate extract is well-known for its beneficial physiological effects, particularly its anti-inflammatory and antioxidant qualities. Through the actions of certain components, such as antioxidants and ellagic acid, pomegranate extract supports bone health and prevents osteoporosis in perimenopausal and postmenopausal women. Pomegranate extract also lowers blood pressure and cholesterol in perimenopausal and postmenopausal women, which lowers their risk of cardiac conditions including atherosclerosis. According to reports, pomegranate extract affects hormonal balance, especially in women with PCOS by controlling reproductive hormones. Strong anti-inflammatory and antioxidant activities are demonstrated by pomegranate extract, and certain components, such as ellagic acid, have anticancer effects (Jang et al.,2024).

The Linaceae family includes the plant known as flaxseed or linseed (Linum usitatissimum). It has been grown for 5,000 years in China and India and for about 10,000 years in Egypt and Samaria (Saleem et al., 2020). Flaxseed and its active compounds have been shown to have potential applications in the treatment of ovarian cancer and polycystic ovarian syndrome as well as in enhancing the reproductive efficiency of farm animals. Numerous physiological, preventive, and therapeutic benefits of enhancing women's reproductive health can be determined by the biologically active compounds found in flaxseed, which function through a variety of signaling pathways (Sirotkin., 2023). Honey's complex makeup, which includes trace elements like minerals, enzymes, and antioxidants like flavonoids and polyphenols, as well as carbohydrates, is thought to be responsible for its therapeutic qualities. (Ball., 2007) (Santos-Buelga et al., 2017). Because of its high nutritional content and antioxidant qualities, honey is a well-known natural product with therapeutic qualities that can help with a variety of health issues. Honey has several uses in reproductive health, including treating VVC infections, preventing harmful effects, protecting the postmenopausal reproductive system, improving infertility, and maintaining sperm quality by raising testosterone levels (Zaid et al., 2021).

The combination of Shatavari, pomegranate, flax seeds, and honey may give a powerful health drink for boosting fertility for women. Each of these ingredients has a specific property that address different aspects of reproductive health, and when combined, they may work together to enhance hormonal balance, support ovarian functions, improve egg quality. The uniqueness of this product lies in the ingredients I've chosen primarily Shatavari which is infused with pomegranate, flax seeds and honey to develop a ready to serve beverage product. The objective of this research is to assess the combined effects of

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these ingredients in a functional food-based formulation. Although evidences are not available to suggest the impact of these bioactive ingredients together in Ferti for the improvement of reproductive health in women, based on the existing studies we propose that Shatavari-infused fruit drink may improve stress-mediated reproductive health complications due to its antioxidant ability in women.

II. MATERIALS AND METHODOLOGY

2.1. Sample Collection and Formulation

The four main ingredients used for formulating this drink is Shatavari, pomegranate, flax seeds and honey. Shatavari powder(250g) was sourced from the local country medicine shop in Perungudi, Chennai. Pomegranate was purchased from local fruits shop. Flax seeds (200g) and honey (500ml) was purchased from local daily essentials stores. The product Ferti was prepared by first peeling off the pomegranate skin and the seeds were extracted, washed and weighed. Shatavari powder and flax seeds were ideally weighed. The pomegranate seeds were put in a grinder and grinded till they obtain a juicy state after which the juice was filtered separately leaving the semi-solid residue behind. The pomegranate juice extracted was ideally used for preparing the product. The juice was again put in grinder after which weighed Shatavari powder and flax seeds were added to the grinder. Now, all of them were grinded together till they were dissolved completely. Now, the juice was filtered again using tea strainer to remove the flax seeds residue. Finally, 2 tablespoons of honey were added to enhance the flavor of sweetness and taste. Now, the product Ferti is ready-to-serve. Table 1 depicts the different trials of ingredients we used for Ferti for finding the best combination. The formulation was inspired from the Shatavari, Aloe and Mango Ginger Blended Nectar Beverage where different proportions of all ingredients were chosen for obtaining best results (Ravindra et al., 2012).

POMEGRANATE	100g	150g	200g	150g	144g
FLAX SEEDS	2g	1.5g	0.5g	1.5g	1.1g
SHATAVARI	2g	1.5g	0.5g	0.5g	0.4g
HONEY	2 tbsp	1 tbsp	1 tbsp	2 tbsp	2 tbsp

Table 1. Different trials of ingredients

2.2. Shelf-Life test for Shatavari Infused Fruit Drink

Freshly made Ferti was kept in food-grade sterile bottles and labelled with date and kept in refrigerator for analysis. The sample was evaluated on day 1, day 7 and day 15 and parameters like color, odor and total plate count, zone formation and count were performed. Take the sample from cold storage and dissolve it in 99ml of distilled water, place it in the shaker for 15 minutes. Take the sample from shaker then add 1 ml in first testube mix it well, then add 1 ml from this Testube to subsequent test tubes, repeat the process. Then added 100µl of sample from each test tubes to separate Petri plates. Leave it undisturbed for overnight and counted the number of colonies present. Another innovative product called Herbal apple rabri was made using brahmi and shatavari powder, with a shelf life of 15 to 16 days in refrigerated storage where they followed similar procedure and counted colonies (Lata et al., 2023).

2.3. 2,2-Diphenyl-1-1-Picrylhydrazyl Assay for Antioxidant activity

To assess "Ferti's" antioxidant capacity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used. 1ml of Ferti was centrifuged for 10 minutes at 5000rpm and supernatant was filtered using Whatman No.1 filter paper. The extract was then diluted with methanol. 0.1 mM DPPH solution was used. 1ml of 0.1mM DPPH solution was mixed with 1ml of sample extract. The blank was made with methanol and control sample was made with 1ml DPPH solution along. 7 test tubes were taken, 1 was kept as control, different concentrations of sample (20ul-120ul) were added respectively in each test tubes. 1ml of 0.1mM DPPH solution was added to all test tubes. All the test tubes were kept in test tube stand and incubated at room temperature in dark for 30 minutes. Absorbance was measured at 520nm in a UV-Vis spectrophotometer and percentage of DPPH radical inhibition was found for all different concentrations. The antioxidant capacity of the Ferti was assessed using the DPPH (2, 2-diphenyl-1-picryl hydrazyl-hydrate) inhibition method, as

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described by (Mimica-Dukic et al., 2003) with minor modifications

2.4. Total Phenol Content Analysis for Shatavari Infused Fruit Drink

The phenolic compounds amount is found to discover the antioxidant potential of the product Ferti. For Total Phenol Content (TPC) analysis, a gallic acid stock solution was prepared in 1mg/ml ratio in methanol and various concentrations were prepared by diluting 1ml of Ferti was centrifuged for 10 minutes at 5000 rpm and supernatant was filtered using Whatman No.1 filter paper. 100ul of sample extract was combined in a test tube and 500ul of Folin-Ciocalteu reagent was added. 400ul of 7.5% sodium carbonate was added and the mixture was vortexed. The vortexed mixture was let to sit at room temperature in the dark for 30 minutes. Finally, the absorbance was measured at 765nm with a UV-Vis spectrophotometer (Zhang et al.,2006).

2.5. Antimicrobial Activity Test for Shatavari Infused Fruit Drink

To ascertain the drink's possible preservative and therapeutic advantages, it was tested against certain microbial strains utilizing the agar well diffusion or disc diffusion procedures. For antimicrobial test, Escherichia coli strain was used and for antifungal test, Candida albicans strain was used. Nutrient agar was prepared and filled in sterile petri dish for bacterial culture and Potato dextrose agar was prepared and filled in sterile petri dish for fungal culture. A cotton swab was taken and dipped in microbial culture and the plates were swabbed thoroughly with bacteria and fungi for nutrient agar and potato dextrose agar respectively. Wells were punched into the agar using a well cutter and the plates were labelled accordingly. The wells were filled with 25ul-75ul of sample into every well. A positive control of tetracycline antibiotic was added in the center and the plates were incubated for 24 hours. After incubation, the zone formation was observed, and zone of inhibition was measured (Kumaran et al.,2015) (Nayak et al.,).

2.6. Nutritional Analysis for Shatavari Infused Fruit Drink

A thorough nutritional analysis was conducted to ascertain the macronutrient and micronutrient makeup of "Ferti." The energy, fat, carbohydrates and protein content in the sample were identified using nutritive analysis by a series of chemical analysis (AC, et al., 2018). Nutritional analysis of Ferti helps to reveal the nutrient composition of the product. The product aims to focus more on energy and protein content and less fat content to get a wide appeal everywhere. Freshly prepared sample was sent to nearby diagnostic laboratory for finding out the composition of Ferti.

III. RESULTS AND DISCUSSION

3.1 Formulation of Shatavari Infused Fruit Drink

For formulation, several trials were carried out for different compositions of ingredients to find out the best blend which had great taste, odor and flavor. Our first trial had the following composition: Pomegranate was weighed 100g, Flax seeds was weighed 2g, Shatavari was weighed 2g, 2 tablespoons of honey. After various trials, we found that this composition has great taste and organoleptic properties (Table 2) in which Pomegranate was weighed 144g, Flax seeds was weighed 1.1g, Shatavari was weighed 0.4g, 2 tablespoons of honey. The products were evaluated for acceptability using organoleptic score (i.e., color, flavor, mouth feel, taste and overall acceptability) by using a nine-point hedonic scale (Amerine et al., 1965). Optimization of process variables for the selection of products was performed based on organoleptic attributes.

Trial no.	Color & Appearance	Body & Texture	Flavor	Mouth feel	Overall acceptability
1	7	8	7	8.5	8
2	8	9	8	9	8.5
3	8.5	7	8	9	9
4	7.7	6.5	7.5	8	7.5
5	7	8	8.5	7.5	8

Table 2. Testing different trials for their organoleptic attributes

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3.2 Shelf-life test for Shatavari Infused Fruit Drink

The shelf life was evaluated based on the physicochemical, antioxidant, phenolics, and microbial content like total plate count and yeast and molds count. The number of colonies formed were counted on day 1, day 7 and day 15. Figure 1 depicts the Ferti drink made in day 1 which is in ready-to-serve state. Maximum number of colonies were found to be present in day 15, so we concluded that our product Ferti possesses a shelf life of 7 days. Ferti can withstand and remain consumable up to 7 days which is 168 hours. The shelf-life of Herbal apple rabri made using Brahmi and Shatavari was found to be 15-16 days in refrigerated storage (Lata et al.,2023) whereas pomegranate juice can withstand a shelf-life of 35 days in refrigerated condition (Varela-Santos et al.,2012). Flax seeds which are milled have a shelf-life of 128 days (Malcolmson et al., 2000). Ferti stands out as a competition to other similar natural beverage products which have a shelf life of 1-weeks. Ferti in normal room temperature itself possessed a shelf-life of 15 days.

Figure 1. Formulated Shatavari Infused Fruit Drink

3.3 2,2-diphenyl-1-1-picrylhydrazyl Assay-Antioxidant activity

The 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) Assay for Antioxidant activity results reveal the absorbance value at 520nm.The maximum DPPH radical scavenging activity was 65.73% at 60 mg/ml concentration and minimum DPPH radical scavenging activity was 0.56% at 20 mg/ml in methanol extract. The higher DPPH inhibition activity in the optimized sample is due to the presence of herbs (Shatavari). The DPPH inhibition activity study of herbal apple rabri revealed that the optimized sample had 58.41 ± 0.03% DPPH inhibition activity (Lata at al., 2023) which is lesser than Ferti's DPPH inhibition activity of 65.73% at a concentration of 60 mg/ml which is higher than the previous product compared. Higher antioxidant potential of Ferti makes it a valuable product in the existing beverage market. Table 3 below shows the respective percentage of inhibition and absorbance at 520nm for their respective concentrations of Ferti (20-120mg/ml) and Figure 2 depicts the line graph between concentration (x-axis) and percentage of inhibition (y-axis).

S.NO	Concentration mg/ml	OD at 520 nm	Percentage of inhibition
1	control	0.178	-
2	20	0.177	0.56
3	40	0.170	4.49
4	60	0.061	65.73
5	80	0.144	19.10
6	100	0.161	9.55
7	120	0.165	6.17

Table 3. Table depicting absorbance value at 520nm and Percentage of inhibition for different concentrations of sample

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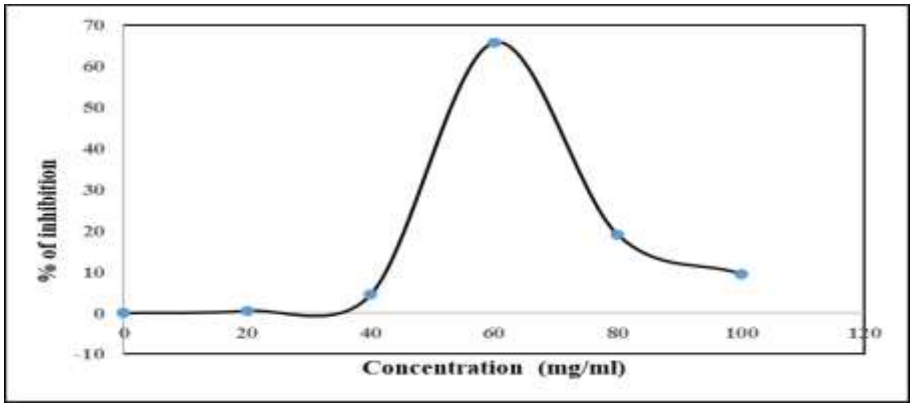

Figure 2. DPPH Assay for Ferti with concentration (x-axis) vs % of inhibition(y-axis)

3.4 Total Phenol Test

Total Phenol test studies were done for concordant values and absorbance was measured at 765nm after taking the sample from incubation in dark. The OD values were found to be 1.303 and 1.321. From this we infer Ferti has high number of phenolic compounds and rich in antioxidant activity. The effect of pomegranate on foods and the biological benefits were associated with phenolic compounds, especially anthocyanins and hydrolyzable tannins (de Oliveira et al., 2020). Aril color and peel of fruits were responsible for the majority of the total phenolic content of pomegranate. Cultivars with a dark red color peel and aril displayed a higher phenolic content (Gözlekçi et al., 2011). The phenolic content of Shatavari supplemented milk was 0.691±0.0004 mg/ml (VEENA ., 2010). Ferti has comparatively higher phenol content due to the presence of Shatavari, pomegranate, flax seeds and honey thereby Ferti is rich in anti-oxidants.

3.5 Anti-Microbial activity test

The product Ferti showed strong zone formation for Escherichia coli sample with positive control of tetracycline which proves that Ferti has strong antimicrobial action. The methanol extract of Asparagus racemosusroots have shown strong antibacterial activity against Vibrio cholerae, Shigelladysenteriae, Pseudomonas putida Staphylococcus aureus, Shigella flexneri, Escherichia coli, Salmonella typhi, Salmonella typhimurium, Shigellasonnei, and Bacillus subtilis (Mandal et al., 2000) (Patel et al., 2013). It was seen that tetracycline standard showed higher activity than ampicillin standard against tested microorganisms. This makes Ferti a strong anti-bacterial beverage product.

For anti-fungal test, Ferti showed strong zone formation for Candida albicans sample with positive control of tetracycline which proves that Ferti has strong antifungal action too. The root extract of Shatavari offers strong anti-fungal activity against Candida, Malassezia furfur andM.globosa (Uma et al., 2009) (Onlom et al., 2014). The presence of Shatavari in Ferti substantially increases anti-fungal activity against Rhizopus oryzae, Candida albicans (Nayak et al.,). Shatavari has been extensively reported for its strong anti-microbial activity which makes Ferti rich in anti-microbial activities. Figure 3 below represents the plates showing antibacterial and antifungal activity of Ferti respectively.

Figure 3. Antifungal andAntibacterial activity result of Ferti

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3.6 Nutritive Test

From the results received from the testing lab, The nutritive test report revealed the contents of Ferti, and energy was found to be high amounting to 73.13 Kcal/100g. Fat was found to be low accounting to Below detectable limit (BDL). Carbohydrates content was decent accounting to 17.69 g/100g. The protein content was found to be 0.57 g/100g. From the given composition, Ferti is found to be an overall nutraceutical health drink which is both healthy and nutritious. The nutritional composition of Shatavari supplemented milk was composed of 4.36% of fat, 3.38% of protein, 4.88% lactose, 11.6% of total solids, 0.19% of ash (VEENA ., 2010). On comparing with Ferti, Ferti is found to have high energy, less fat, more carbohydrates and sufficient protein in it making Ferti an innovative and all-rounder product. Table 4 shows the parameters, test method and result for Ferti's nutritional analysis.

S.NO	PARAMETERS	TEST METHOD	RESULT
1	Energy	ALPL/FD/SOP/06	73.13 Kcal/100g
2	Fat	ALPL/FD/SOP/02	BDL(DL:0.1) g/100g
3	Carbohydrate	ALPL/FD/SOP/065	17.69 g/100 g
4	Protein	IS:7219 : 1973	0.57 g/100 g

Table 4. Result of nutritional analysis of Ferti for different parameters

IV. CONCLUSION

The creation of the fruit drink "Ferti," which contains shatavari, offers a practical and natural way to improve women's fertility and reproductive health. This beverage uses nutrients like pomegranate, flax seeds, honey, and Shatavari that have been clinically proven to assist hormonal balance, enhance ovarian function, and enhance general well-being. "Ferti" stands out as a practical, wholesome, and all-encompassing substitute for traditional fertility supplements considering the growing demand for nutraceutical and herbal-based health treatments. Its composition is intended to be both tasty and advantageous, guaranteeing convenience of use while supplying vital nutrients. Further research, including sensory evaluation, consumer acceptance studies, and potential clinical validation, can strengthen the product's credibility and marketability. Overall, "Ferti" has the potential to be a valuable addition to the functional beverage industry, offering a natural means to support women's reproductive health in a safe and effective way. The potential of "Ferti" extends beyond its current formulation. Future research and developments can focus on Clinical Validation: Conducting clinical trials to scientifically validate the drink's efficacy in improving female fertility, Nutritional Optimization: Enhancing the formulation by incorporating additional fertility-boosting ingredients or fortifying it with essential vitamins and minerals, Product Variants: Developing different flavors and formulations to cater to varying consumer preferences, including sugar-free and probiotic-enriched versions, Shelf Life & Packaging Innovation: Studying ways to extend the product's shelf life while maintaining its nutritional value and ensuring sustainable packaging solutions, Market Expansion: Exploring commercialization opportunities through collaborations with healthcare professionals, wellness brands, and online retail platforms to reach a broader consumer base. With further research, validation, and strategic market expansion, "Ferti" has the potential to become a widely accepted functional beverage in the nutraceutical and wellness industry, offering a safe and natural means to support women's reproductive health.

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