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Phytochemical, Antioxidant and Antibacterial Activities of Golden Berry (*Physalis Peruviana* L.) Extract and Its Effects on the Storage Stability of Tomato Paste

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Abstract: This study focused on the phytochemical, antioxidant, and antimicrobial activities of the ethanol extracts of golden berry calyx and fruits. The phytochemical analysis of calyx and fruit extracts revealed high total phenols and flavonoids. High antioxidant capacity was recorded for fruit extracts. Fruit extracts also gave the highest antibacterial activity against *Pseudomonas fluorescens*, *Escherichia coli*, and *Salmonella typhimurium*. Furthermore, tomato paste was prepared using sodium benzoate and golden berry extract as food preservatives. The incorporation of golden berry extract and chemical preservatives maintained the paste quality indicators over the control samples. A rapid decline in total antioxidant capacity was also observed in control samples (21.6%) as compared with the treated samples (15.21% and 15.41% for sodium benzoate and golden berry extract, respectively) after 30 days of storage. This study demonstrated that dried fruit extract with high phytochemical and antimicrobial properties could be used as a natural food preservative.

1 Introduction

In recent years, the growing frequency of new foodborne illness outbreaks caused by pathogenic microorganisms has increased food safety concerns. Therefore, managing microbial contamination in food and its products, minimizing the demand for antibiotics, prolonging the shelf-life to eradicate undesired microorganisms and postponing food spoiling are priorities for food safety (El-Chaghaby et al 2014). The side effects linked to available antibiotics are terrifying. Thus, it is important to find natural alternatives to these chemical antibiotics. Plant extracts are being investigated as natural antimicrobial sources against many bacterial species (Kushwaha et al 2012). Medicinal plants are recognized for their effective antioxidant properties as they contain bioactive substances such as benzoic acid, carotenoids, cinnamic acid, folic acid, phenolic compounds, and flavonoids (Tungmunnithum et al 2018).

Natural compounds found in fruits and vegetables, such as antioxidants, have health benefits and nutritional value. The Physalis genus has received a considerable amount of attention as a possible source for novel food crops **(**Abd-ELmageed et al 2019). Golden berry (*Physalis peruviana)* is a medicinal plant that is frequently used in folk medicine to treat some illnesses. It belongs to the family *Solanaceae*. Golden berry can be cultivated as either an annual or a perennial crop. It has since been widely planted in many subtropical places. Owing to its numerous advantages, it

may also be cultivated in temperate zones in greenhouses or similar facilities (Rop et al 2012). Its medicinal benefits are attributed to its antioxidant capacity of polyphenol content (Ertürk et al 2017). Golden berries have high nutritional value owing to their many phytochemicals, such as minerals, vitamins, and antioxidants. They also have antibacterial and antiinflammatory activities (Corrales-Bernal et al 2015, Çakir et al 2014).

Golden berry extracts contain significant chemical components, including saponins, annelids, Peruvian, kaempferol, Iranians, quercetin, and diglycosides and triglycosides (Nocetti et al 2020). Different parts of golden berry were proven against several microbial species, such as *Pseudomonas aeruginosa*, *Enterobacter aerogens*, *Bacillus megaterium*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Klebsiella pneumoniae*, *Trichophyton sp.*, *Proteus vulgaris*, *Epidermophyton* sp., and *Escherichia coli* (Singh et al 2019, Osho et al 2010). The nutritional profile of *Physalis Peruvian* is primarily responsible for the health advantages associated with its ingestion. Berries are rich in many biochemical constituents, such as carotene; vitamins B, C, E and K; also, phytosterols, as well as vital minerals, such as iron and levels of polyunsaturated fatty acids (Ali et al 2019).

Tomatoes (*Solanum lycopersicum*) are one of the most frequently produced fruits on the planet. They are susceptible to fungal degradation due to their high-water content and fragile endocarp. Tomatoes are commonly processed into juice, ketchup, puree, and paste, all of which are sold to customers (Basak 2018). To extend the shelf-life of tomato paste, producers use additional preservatives, such as benzoates, nitroso/nitrites, and sulfite. Chemical preservatives are essential to keep food fresh and economical; however, their detrimental influence on global health has been a concern for consumers (Jafari et al 2020, George-Okafor et al 2020). Thus, it is important to find natural additives for tomato paste preservation. For this reason, this study aimed to (1) to evaluate the antioxidant and antimicrobial activities of the different parts of golden berry and (2) evaluate the impact of adding golden berry extracts to the nutritive and microbiological quality of tomato paste during preservation.

2 Materials and Methods

2.1 Golden berry collection

Healthy mature berries were purchased from a commercial market in Egypt. The berries were separated from their calyx; and washed with running tap water, oven-dried until constant weight was reached, and finally crushed into powder using an electric grinder. The plant calyx and fruits were stored for further utilization.

2.2 Preparation of golden berry calyx and fruit extracts

The ultrasound-assisted extraction method was employed. Powdered sample (5 g) was mixed with 50 ml aqueous ethanol (80%). Extraction was performed in a sanitary water bath with a fixed frequency of 40 kHz for 2 h. After extraction, the extracts were immediately filtered through Whatman paper No. 1. The solvent was evaporated using a rotary evaporator and the obtained extracts were stored at 4 °C until further use.

2.3 Phytochemical screening

Golden berry calyx and fruit extracts were subjected to phytochemical screening tests for plant secondary metabolites, phenolic compounds (ferric chloride test), cardiac glycosides (Keller–Kiliani test), tannins (lead acetate test), $FeCl_3$ test, steroids (Salkowski's test), glycosides were conducted following Harborne (1973)**,** flavonoids, saponins and phlobatannins were conducted according to method described by Evans (1996).

2.4 Determination of TAC

The total antioxidant capacity **(**TAC) of golden berry calyx and fruit extracts was analyzed using the standard phosphomolybdenum method (Prieto et al 1999). Antioxidant activity was expressed as milligram ascorbic acid equivalent per gram (mg AAE/g).

2.5 Determination of total phenol content (TPC)

The TPC of the extracts was determined using the Folin–Ciocalteu method (Agbor et al 2014). Using a gallic acid standard curve, absorbance was converted to milligram gallic acid equivalent per kilogram of dry material (g GAE/kg).

2.6 Determination of total flavonoid content (TFC)

The TFC was measured using a colorimetric technique with the aluminum chloride method as adapted by El Ouadi et al (2017). The results were expressed as milligram quercetin equivalent per kilogram of dry material (g QE/kg) using quercetin as the reference material.

2.7 Antimicrobial activity of golden berry calyx and fruit extracts

Three gram-negative pathogenic bacteria were used in this study, (*Pseudomonas fluorescens*, *E. coli* O157: H7, and *Salmonella typhimurium*). The isolates were maintained through monthly transfer onto nutrient agar and stored at 4°C. Standard inocula were made by inoculating a conical flask (100-mL capacity) with 50-mL buffered peptone water (pH 7.2) for 24 h at 37°C with a loop of *P. fluorescens*. Another flask with an *E. coli* loop and another with a *S. typhimurium* loop yielded viable cells. For each strain, counts were measured *via* serial dilution and subsequent enumeration on a specified selective agar.

The plate agar diffusion technique was employed to assess the antibacterial activity of *P. peruviana* ethanol extracts (fresh juice, dried berries, and calyx) against the chosen**.** In sterile Petri dishes, 20-mL nutrient agar was seeded with 0.2-mL broth culture of the test organisms. Slow rotation of Petri plates was adopted to guarantee a consistent dispersion of bacteria. The nutrient agar was left in the dish to harden. In the nutrient agar, holes of 8.0 mm diameter were created using a sterilized cork borer. The extracts were suspended in 1 mL dimethyl sulfoxide before inoculation into the cups using a micropipette (at a ratio of 100:1 of the extract). The plates were left at room temperature for 30 min to allow an optimum dispersion of the extract. The plates were incubated for 24 h at 37 °C. At the end of the incubation period, inhibition zones formed on the medium were measured in millimeters.

2.8 Tomato paste preparation

Fresh tomatoes were brought at commercial maturity from a local market; they were free from mechanical damage and insect injury. The tomatoes were washed with flowing water after treatment with 200-ppm sodium hypochlorite for 30 s (Basak 2018). To obtain tomato juice, the washed tomatoes were diced and ground into pulp using a mixer grinder and sieved to remove the skin and seeds. Evaporation at high temperatures (80°C) was employed to concentrate the juice (Ajayi 2013). The paste was allowed to cool and divided into three portions of 1 kg each. One portion of the prepared sauce served as the control without any additives. Sodium benzoate and golden berry extract were added at a 1-g/kg concentration for the second and third portions of tomato paste, respectively. All samples were kept refrigerated at a temperature of 4°C.

2.8.1 Tomato paste storage experiment

Tomato paste samples containing sodium benzoate, golden berry extract, and control were stored in the refrigerator at 4°C for 30 days. On the 1st, 15th, and 30th days, the samples were analyzed to determine their antioxidant activity and microbiological quality. The TAC of the tomato paste was determined using the standard phosphomolybdenum method (Prieto et al 1999)**,** as previously mentioned. Total bacterial count, total yeasts and molds were counted in the previous samples.

2.9 Statistical analysis

Analysis of variance was (ANOVA) conducted using CoStat version 6.4 (USA). The significance of the difference between sample means was calculated using Duncan's multiple range tests at a significance level of 0.05. The results were expressed as mean ± standard deviation (SD).

3 Results and Discussion

3.1 Phytochemical screening

Preliminary phytochemical analysis of golden berry calyx and fruit extracts **(Table 1)** revealed the presence of flavonoids, steroids, phenols, tannins, phlobatannins, and saponins. These secondary metabolites play an important role in the biological actions of therapeutic herbs, such as antioxidant, antibacterial, and anti-inflammatory activities, among others (He et al 2021)**.** Flavonoids are called "nature's biological response modifiers" owing to their intrinsic capacity to adjust the body's response to allergens and viruses; moreover, they have anti-inflammatory, antiallergic, antimicrobial, and anticancer properties (Mansuri et al 2014)**.** Steroids are well known for their cardiotonic characteristics and insecticidal and antibacterial capabilities. They are also used in food, herbal medicine, and cosmetics. Tannins have antiviral, antibacte-

rial, and antitumor properties. Furthermore, specific tannins might selectively decrease HIV replication and could be used as a diuretic. Saponin is used to treat hypercholesterolemia and hyperglycemia, as an antioxidant, anticancer, and anti-inflammatory agent, and for weight reduction, among others. Saponin also has antifungal effects (Butt et al 2021).

Table 1. Phytochemical screening of golden berry calyx and fruit extracts

Phytochemicals	Calyx extract	Fruit extract
Steroids	+	+
Phenols	+	+
Flavonoids	+	+
Tannins	+	+
Phlobatannins	+	+
Saponins	+	+
Glycosides	+	+

+, Present ; - , Absent

3.2 Total antioxidant activity (TAA) of golden berry extracts

The total antioxidant activity for the different extracts of golden berry parts is presented in **Table 2**. Antioxidant capacity was significantly higher in the fruit extract (12.92 mg AAE /g) than in the calyx extract (8.143 mg AAE /g). These results indicated that the extracts exhibited an electron-donating activity, suggesting that they may function as radical chain terminators, converting reactive free radical species into more stable non-reactive products (Shahidi and Zhong 2015).

3.3 TPC of golden berry extracts

The TPC of golden berry is presented in **Table 2**. The high values reported for the TPC of both calyx and fruit extracts were comparable with little difference (3043.0 and 3320.5 mg GAE /kg, respectively) and higher than those determined by Shenstone et al (2020). There was a strong association between antioxidant activity and TPC although other chemicals, such as vitamins E and C, could increase antioxidant activity. The capacity of phenolics to chelate metals, inhibit lipoxygenase, and scavenge free radicals may be connected to their bioactivity (Barros et al 2007).

3.4 TFC of golden berry extracts

Table 2 also presents the TFC of golden berry. The most frequent polyphenolic chemicals in plant extracts are phenolic acids, flavonoids, and tannins. Flavonoids are polyphenols widely dispersed in plant flora. These chemicals, along with other phenolic structures of plant origin, have been described as reactive oxygen species scavengers and are viewed as possible therapeutic medicines for free radical diseases (Kumar and Pandy 2013). The flavonoid contents in the calyx extract were the highest value (1430.5-mg QE/kg), followed by dried fruit extract (495.3-mg QE/kg). The results also agreed with previous studies that these compounds could be found not only in the edible part of golden berry but also in the non-edible portions (Shah and Bora 2019, Ertürk et al 2017).

Table 2. Antioxidant properties of golden berry ethanolic extracts

Phytochemicals	Calyx extract	Fruit extract
TFC (ppm)	1430.5 ± 0.87 ^{a}	495.3 ± 7.93 ^{b}
TPC (ppm)	3320.5 ± 0.11^{a}	3043.0 ± 1.90 ^{b}
TAA(mgAAE/g)	8.143 ± 0.87 ^{b}	12.92 ± 0.20 ^{a}

TFC: total flavonoids content; TPC: total phenols content; TAA: total antioxidant activity. Values are expressed as mean± SD (n=3); significant differences within the same row.

3.5 Anti-bacterial activity of golden berry extracts

Table 3 demonstrates that all ethanolic extracts exhibited antibacterial effects with different inhibition zone diameters varying from 14 to 26 mm. Dried fruit extract had the highest inhibition zone diameters, followed by calyx extract, against the three tested bacteria (*P. fluorescens*, *E. coli*, and *S. typhimurium*).

These results agreed with those of Bayas-Morejon et al (2020), who demonstrated that using *P. peruviana* extracts as a pathogen-fighting agent might be a realistic option, and it could be a component of naturally produced antimicrobials. There were no previous data on the antigrowth activity of *P. peruviana* against *E. coli*; however, this study confirmed the antibacterial

effects of *P. peruviana* against *E. coli* by an inhibition zone of about 22 mm. The presence of bioactive plant components, such as tannins, phenolic compounds, polyphenols, and flavonoids, may be responsible for the antibacterial action of plant extracts. Among these bioactive molecules, phenols had the most effective antibacterial substances (El-Chaghaby et al 2014). Based on previous results, the most effective part of golden berry (which is dried berry extract) was selected for use as a preservative for tomato paste and to study its effects on the shelf life of tomato paste during different storage periods.

Table 3. Antibacterial activity of the ethanolic extracts of *P. Peruviana*

Microorganisms	Inhibition zone diameter in mm (inhibition %)	
	Fruit extract	Calyx extract
P. fluorescens	16	14
E. coli O157: H7	22	21
S. typhimurium.	26	23

3.6 TAC of tomato paste through storage period

Tomato is a very nutritious food item widely utilized in food preparation worldwide, and its processing into various products, such as puree, ketchup, and juice, is well documented.

Fig 1 demonstrates that the incorporation of golden berry extract and commercial preservatives kept the antioxidant activity of tomato paste better than in control samples. The TAC of the control paste was significantly reduced from 3967.8 to 3109.4 ppm after 30 days of storage, whereas the TAC of tomato paste + golden berry extract and tomato paste + benzoate showed a reduction from 4113.9 to 3479.9 and from 4262.3 to 3617.5 ppm, respectively. Prolonged storage at relatively high humidity increased oxygen availability within the packing material, which is responsible for the oxidation of naturally existing antioxidants in tomatoes (Basak 2018).

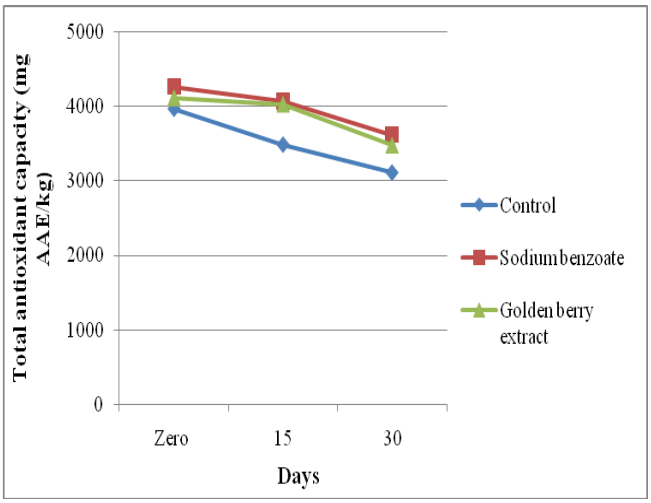


Fig 1. Influence of golden berry extract on the antioxidant capacity (ppm AAE) of tomato paste during storage

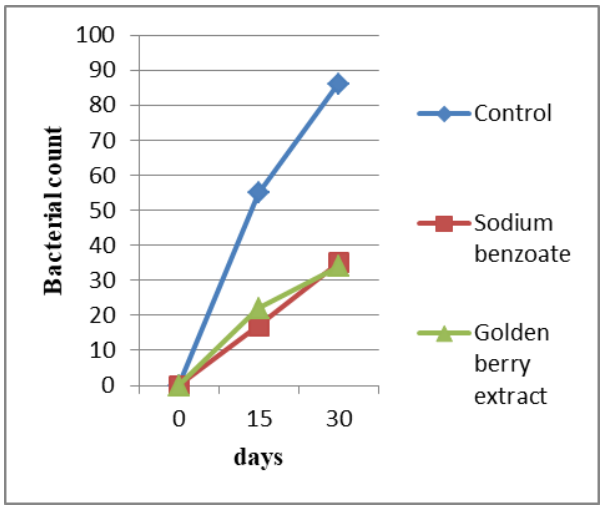
The reduction of antioxidant activity was more rapid in control samples (21.6%) than in treated samples (15.21% and 15.41% for sodium benzoate and golden berry extract, respectively). This might be attributed to increased water mobility at high relative humidity, which accelerated the reaction rate, resulting in the breakdown of phenolic components and TAA in the control paste samples (Xu et al 2016). Thus, the natural compounds of the fruit extract with antioxidant capacity and the average pH or acidity of golden berry can be sufficient to preserve the paste and increase the time for phenol decomposition.

It is worth noting that samples containing golden berry extract produced the closest results to benzoate-treated samples, indicating that the treatment has a great potential to give some natural options for replacing chemical preservatives. Benzoic acid and its salt are commonly used preservatives in large-scale foods and beverages as antifungal and antibacterial agents. However, deleterious effects of benzoates have been documented, including non immunological activity in sensitive individuals and hyperactivity in youngsters (Piper and Piper 2017). Aside from the health advantages of antioxidants, it is also important to evaluate their influence on the shelf life, storability, and resilience of food and food products. In this regard, golden berry is a potential fruit species (Rop et al 2012).

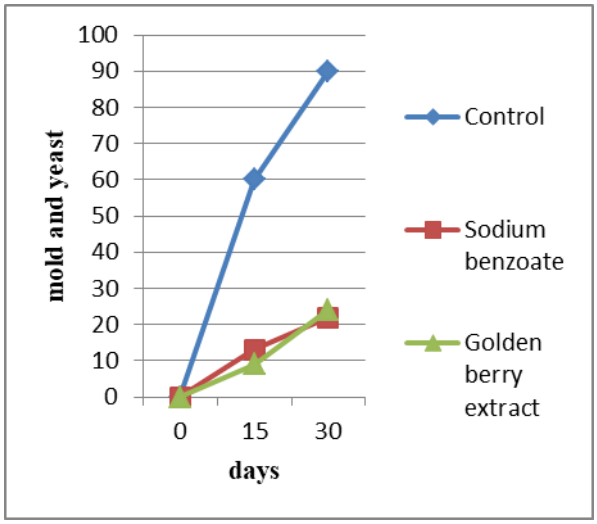
3.7 Microbiological analysis of tomato paste

Bacteria and mold infiltrate living plant tissue *via* many mechanisms, promoting the formation of heat-resistant spores of microorganisms in processed tomato paste. To lessen the microbial burden, tomatoes

were washed with chlorinated water, jars were sterilized, and filling and packaging were performed aseptically. The heating treatment of tomato products can successfully inactivate bacteria that cause food deterioration (Devseren et al 2021). **Fig 2** demonstrates that the highest counts of total bacterial and total molds and yeasts were observed in control samples. Contrarily, counts of bacteria, yeasts, and molds were slightly low in samples preserved with sodium benzoate and golden berry extract. The effects of golden berry extract were so close to sodium benzoate.



(a)



(b)

Fig 2. Microbial parameter changes in tomato paste as affected by storage time: (a=total bacterial count (cfu/mL) and (b= mold and yeast (cfu/g).

Microbial evaluation results were compatible with the antioxidant content in tomato paste samples. According to Ertürk et al (2017) and Basak (2018), the antioxidant, phenolic, and flavonoid contents were strongly associated with the antimicrobial activity of the golden berry extract. Sodium benzoate is a common preservative and antibacterial agent used in various foods and soft drinks. Although this molecule is widely accepted as a safe food additive, data suggest that increased sodium benzoate consumption may be linked to attention-deficit hyperactivity disorder in children (Khoshnoud et al 2018). Under acidic pH, heating, and storage circumstances, sodium benzoate might generate genotoxic and cell-transforming agents (Chaleshtori et al 2018).

4 Conclusion

Golden berry calyx and fruit extracts have potential antioxidant and antimicrobial activities and known nutritive value, making them excellent candidates as food additives. This study highlighted the possible addition of berry extract as an alternative to chemical preservatives for tomato paste storage. The addition of berry extract to tomato paste led to low changes in antioxidant capacity and its microbial loading during storage, which was close to the chemical preservation of sodium benzoate. More research is needed to evaluate the antibacterial property of golden berry extract using different extraction methods, at various concentrations, and against a broader range of microorganisms, allowing for a more precise dosage determination and clinically detectable effects in animal models and humans.

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