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Phytochemical analysis of *Citrus sinensis* (Orange), *Malus domestica* (Apple)

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Abstract
Phytochemicals can be defined as any compound found in plants (the ancient Greek word *phyton* means plant). Phytochemicals are certain non-nutritive plant chemicals which have some disease preventive properties. However, a diverse range of biologically active compounds found in plants are often described by the term phytochemical. Phytochemicals provide colour, taste and natural pest control to plants.
Numerous epidemiological trials have demonstrated that a rich plant fruit and vegetable has important advantages for human health. These advantages include: i) reduced risk of multiple forms of cancer development, and ii) reduced risk of cardiovascular diseases. The main gain is attributed to the inclusion of phytochemicals in fruits and vegetables. Apples and oranges are widely consumed, rich phytochemical sources and epidemiological studies have combined apple consumption and several health benefits.
The present study was aimed to analyze the phytochemical constituents of *Citrus sinensis* (Orange) and *Malus domestica* (Apple). The quantitative and qualitative analysis was performed to explore the phytochemicals present in the samples of apple and orange. The study confirmed the presence of phytochemicals such as Saponins, Tannins, Flavonoids and Alkaloids in both fruits *Citrus sinensis* (Orange) and *Malus domestica* (Apple). The quantitative analysis also signified that *Malus domestica* (Apple) contains more quantity of phytochemicals constituents present, than *Citrus sinensis* (Orange). *Citrus sinensis* (Orange) and *Malus domestica* (Apple) are widely consumed fruits. The study concluded that both the fruits are rich in phytochemicals and have several health benefits.

Keywords: phytochemicals, *Citrus sinensis, Malus domestica*, aqueous extract

1. Introduction
The regular intake of fruit, vegetable and whole grain decreases the probability of chronic diseases attributable to oxidative harm. Although phytochemicals are not essential to our lives but they are beneficial to our health (Hussain, 2014) [22]. The consumption of fruits and vegetables also seems to protect against coronary heart disease (Joshipura K, 2001) [25]. A survey followed almost 84,000 women for 14 years and 42,000 men for 8 years. They noticed that the incidence of coronary heart attack was 20 percent lower in people who consumed highest amount of fruits and vegetables and lowest risks were seen in people who ate more leafy vegetables, and fruits rich in vitamin C. Not only a diet rich in fruit and vegetable may help prevent heart disease and cancer, but it may also defend against a number of other diseases. For example, a diet rich in fruits and vegetables will help against cataracts, diabetes, Alzheimer's disease and asthma (Woods R, 2003) [48] (W, 2002). Phytochemicals are often applied for diverse uses such as medicinal goods, agrochemicals, flavours, fragrances, colouring agents or biopesticides (Mithen, 2011) [35]. Phytochemicals exhibit antibacterial and antiviral properties against a wide range of pathogenic and non-pathogenic microorganisms (Reichling, 2010) [40]. Phytochemicals are some of the most important natural preservation structures to limit or prevent pathogenic growth and maintain food products' overall consistency. These antimicrobials can protect food products and naturally increase the shelf life (Ibrahim, 2012) [23]. In general, food antimicrobials may be categorised according to their source as natural and synthetic substances. Synthetic antibiotics can exist naturally in fruits, such as benzoic acid in cranberries, tartaric acid in grapes, sorbic acid in rowanberries, malic acid in apples and citric acid in lemons (Negi, 2012) [36].
The beneficial impacts of phytochemicals on human health have been demonstrated by the findings of several studies during the past ten years. Antioxidant use is closely associated with a reduced incidence of cardiovascular, asthma, diabetes and hypertension and other medical conditions (Craig, 1997) [6]. Foods such as wholegrains, rice, berries, vegetables and herbal products, have phytochemicals of nutraceutical importance. These phytochemicals have huge therapeutic ability to treat different diseases, either alone or in combination. Science and ethics, wellness statements, functional foods and such phytochemicals are the foundation for their respective advantages for the wellness. They have unique pharmacologic effects for human wellbeing including anti-inflammation, anti-allergic, anti-bacterial, antifungal, chemo-preventive, hepato-protective, hypolipidemic, hypotensive, antiaging, diabetes, osteoporosis, DNA damage, cancer and heart diseases. They have unique pharmacological effects (Hussain, 2014) [22].

1.1 Many phytochemicals are available and each works different. These are possible measures

- **Antioxidant:** Most phytochemicals are antioxidants, defend our cells and mitigate the chance of certain forms of cancer. Phytochemicals with antioxidant activity: allyl sulphides (onion, garlic), carotenoids (carrots), flavonoids (fruits and vegetables), polyphenols (grapes, tea).
- **Hormonal action:** Isoflavones, found in soy, help to reduce osteoporosis and lower menopausal symptoms.
- **Stimulation of enzymes:** Indoles, in cabbages, stimulate enzymes that lower estrogen strength, and could reduce the risk of breast cancer. Some phytochemicals interfering with enzymes include protease inhibitors (soy and beans), terpenes (citrus fruits and cherries).
- **Interference with DNA replication:** Saponins that are embedded in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cell. Capsaicin, found in hot peppers, defends DNA from carcinogenic agents.
- **Anti-bacterial effect:** Allicin, found in garlic, has antibacterial properties.
- **Physical action:** Many phytochemicals attach physically to the cell walls thereby avoiding the adhesion of pathogens to the human cell walls. The anti-adhesive property in cranberry is due to the presence of proanthocyanidins. Cranberry use decreases the possibility of infection in the urinary tract and also improves dental hygiene.

Medicines can support our body to recover from illness but in chronic diseases like diabetes, hypertension, cardiovascular diseases, diet plays a very important role and no medicine is the substitute for that. In such diseases phytochemicals can be of great help and they may also help to boost up brain health and immune system. This gives inner strength to our body which is responsible for our work performance (Hussain, 2014) [22].

Researches show that phytochemicals play important role in enhancing the health & nutritional status of children. According to Veugelers P and Heller L "Fruit and vegetable consumption were found to play an important role in children's academic performance" (Heller, 2008) [20]. In the course of this review, a phytochemical research has been carried out on *Citrus sinensis* (Orange), *Malus domestica* (Apple).

Orange (*Citrus sinensis*) a hesperidium of the Rutaceae genus is the most widespread cultivated and marketed citrus plant. Orange is made up of an exterior flavedo (epicarp) and an albedo (mesocarp) sheet (peel), and an internal substance known as an endocarp (pulp), including vesicles.

The by-product of the peeling juice industry is sweet orange oil. It is used for food and beverage as a flavouring agent. Sweet orange oil contains about 90 percent dlimonene, a solvent utilised in a variety of household chemical compounds. Phytochemicals, through the vegetables and fruits, are already a part of our diet. Citrus fruits are found to be rich in phytoconstituents and *Citrus sinensis* (sweet orange) is one of them.

Apple *(Malus domestica)*, fruit of the domesticated tree *Malus domestica* (Family Rosaceae), is one of the most common tree fruits, the apple is a pome (fleshy) fruit, in which the ripened ovary and surrounding tissue both become fleshy and edible. The use of apples has been associated with a number of health benefits, including decreasing incidence of cancer, cardiovascular diseases, asthma and diabetes. In the laboratory, apples have been found to be associated with very broad activity for antioxidants, cancer cell proliferation, lipid oxidation minimisation and lower cholesterol (Liu, 2004) [31]. *Malus domestica* fruits are abundant in phytonutrients. A good nutritional status is associated with the intake of fruits and vegetables which are high in phytochemicals.

Fruit and vegetable intake has been linked with low incidences of cancer, cardiac illness and mortality rates. The diet rich in fruit and vegetable lowers blood pressure, strengthens the immune system, detoxifies and reduces inflammation. The review of literature has signified that apples and oranges are highly consumable fruits. The purpose of this paper is to analyze the phytochemical constituents of *Citrus sinensis* (Orange) and *Malus domestica* (Apple).

2. Material and Methods

2.1 Collection of samples: Fresh orange and apple were collected from the local market of Gohana district Sonipat (Haryana) in the month of May 2019. The samples were washed thoroughly under tap water. The peel was separated then the pulp of Orange and Apple was also separated by cutting them into small pieces, and dried in shade for 3 days at room temperature and then sun-dried for next 3 days. The dried samples were milled into coarse powder by using a mechanical grinder (Harborne J., 1988). The powder was stored in an airtight container and kept in a cool and dry place until further analysis.

2.2 Preparation of extracts

2.2.1 Aqueous extract: 25 gm of both samples, were suspended in 200 ml of distilled water. Extraction was done at 70 °C for 30 minutes, followed by filtering of the extracts using Whatman filter paper No.1. Extracts were then evaporated at 45 °C for 72 hours to form a paste, and further transferred into sterile bottles and refrigerated until use.

2.2.2 Ethanolic extract: 95 percent ethanol was added to 25 gm of sample. Extraction was allowed to stand for 72 hours at 27 °C and then filtered with Whatman filter paper No.1. Extracts were evaporated at 45 °C for 72 hours to form a paste, then transferred into sterile bottles and refrigerated until use (Sofowara, 1993) [42].

2.3 Phytochemical analysis

Quantitative and Qualitative Analysis of the phytochemicals in different samples was performed and the observations were recorded.

a. Saponin (C. M. Ejikeme, 2014) [2]
b. Flavonoids (Sofowara, 1993) [42] (Harborne J. B., 1973)
c. Alkaloids (H. Hikino, 1984) [17]
d. Tannins (C. M. Ejikeme, 2014) [2]

3. Results and Discussion

Phytochemicals analysis was performed on samples of *Citrus sinensis* (Orange) and *Malus domestica* (Apple) in both extracts. The study found the following results:

3.1 Phytochemicals in *Citrus sinensis* Orange (Pulp)

Table 1: Phytochemicals in Orange

Qualitative Analysis		
Constituents	**Aqueous**	**Ethanolic**
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	+
Quantitative Analysis		
Constituents	Ethanolic Extract (g/100g)	
Saponins	0.05 ± 0.01	
Tannins	0.21 ± 0.03	
Flavonoids	0.32 ± 0.01	
Alkaloids	0.40 ± 0.08	

In the present study, the phytochemicals such as saponins, tannins, flavonoids and alkaloids were found in both aqueous and ethanolic extracts of orange.

A pulp and its seeds lead to the bulk of fruit weight comprising about 46% and 44% respectively while peel is approximately 10%. The orange is very nutritious and rich in protein and minerals (Ibrahim, 2012) [23]. Orange juice is a rich source of vitamin C and an essential water-soluble antioxidant. The key role of vitamin C is prevention of scurvy.

The results are consistent with the findings of a study by Chede *et al*., (2013) [4]. Chede *et al*., (2013) [4] has performed the phytochemical analysis of *Citrus Sinesis* (Orange). The study was performed using aqueous as well as ethanolic extracts of the pulp. The study revealed the presence of carbohydrates, alkaloids, tannins, fixed oils and lipids, steroids, amino acids whereas the terpenoids are present only in ethanolic pulp extract (Table 2) (Chede P.S., 2013) [4].

Table 2: Phytochemical analysis of *Citrus Sinesis*

Phytochemicals	**Extract**	
	Aqueous	**Ethanolic**
Carbohydrates	+	+
Sugar	+	+
Protein	+	+
Tannins	+	+
Steroids	+	+
Amino acids	+	+
Terpenoids	-	+
Anthraquinones	-	-
Alkaloids	+	+
Chalcones	-	-
Saponins	-	-
Fixed oils and lipids	+	+
Note: + indicates ‘Present’; - indicates ‘Absent’		

Another study performed by Arora, M., and Kaur, P. (2013), to explore the phytochemicals in *Citrus Sinesis* (Orange). The results of the study are summarised in table 3 (Mamta Arora, 2013) [34]. The results of this study are also found to be consistent with the results of present study. However, this study was failed to found the alkaloids in both extracts.

Table 3: Phytochemical analysis of *Citrus Sinesis*

Phytochemicals	**Extract**	
	Aqueous	**Methanol**
Alkaloids	-	-
Amino acids	-	-
Tannin	+	+
Anthraquinones	-	-
Saponins	+	+
Proteins	-	-
Terpenoids	+	+
Cardiac glycosides	-	+

3.2 Phytochemicals in *Malus domestica* (Apple)

Table 4: Phytochemicals in Apple

Qualitative Analysis		
Constituents	**Aqueous**	**Ethanolic**
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Alkaloids	+	+
Quantitative Analysis		
Constituents	Ethanolic Extract (g/100g)	
Saponins	0.21 ± 0.01	
Tannins	0.86 ± 0.02	
Flavonoids	5.78 ± 0.08	
Alkaloids	1.34 ± 0.03	

The present study has shown the presence of phytochemicals such as saponins, tannins, flavonoids and alkaloids in both aqueous and ethanolic extracts (Table 4).

The concentration of flavonoids in Apple as well as a number of other phytochemicals may depend on various factors such as cultivar of apple, harvesting, storage and processing of apple. Phytochemical concentrations often differ considerably from apple to apple. In the present study, the concentration of flavonoids in ethanolic extract was found to be 5.78 g/100 g (Table 4).

The average amounts of major phenolic compounds in six apple cultivars have been investigated by researchers. They found that the average phenolic concentrations among the six cultivars were: quercetin glycosides, 13.2 mg/100 g of fruit, vitamin C, 12.8 mg/100 g fruit, procyanidine B, fruit 9.35 mg/100 g, chlorogenic acid, fruit 9.02 mg/100 g, epicatechin, fruit 8.65 mg/100 g and phloretin glycosides, fruit 5.59 mg/100 g (Lee K, 2003).

Procyanidins, catechins, epicatechins, chlorogenic acid, phloridzine and the conjugates of quercetin are the most common compounds present in apple peels. In apples, the content of catechin, procyanidine, epicatechin and phloridzine is slightly less than that of the peels. Quercetin conjugates are found exclusively in the apple peel. Chlorogenic acid tends to be higher in the flesh than in the peel (Escarpa A, 1998) [16]. Apples with peels are more effective in inhibiting cancer cell proliferation when compared to apples without peels (Eberhardt M, 2000) [13]. Many of these apple phytochemicals have been thoroughly studied and many potential health benefits have been attributed to these particular phytochemicals. The strong antioxidant activity of procyanidins, catechin and epicatechin inhibit LDL oxidation in vitro (da SIlva Porto P, 2003) [9].

A study by Doss *et al.*, (2012) [1] performed the phytochemical analysis of *Malus domestica* (Apple). The study was carried out using aqueous as well as methanolic extract of the apples. The analysis revealed that the saponins and steroids were present in both aqueous and methanol extracts while alkaloids, phenolic compounds, flavonoids, carbohydrate & glycosides, protein & amino acids were present only in methanol extracts (Table 5) (A. Doss, 2012) [1].

Table 5: Phytochemical analysis of *Malus domestica*

Phytochemicals	**Extract**	
	Aqueous	**Methanol**
Alkaloids	-	+
Saponins	+	+
Steroids	+	+
Phenolic compounds	-	+
Tannins	-	-
Flavonoids	-	+
Carbohydrates & Glycosides	-	+
Protein & Amino acids	-	+

The results of this study (A. Doss, 2012) [1] are found to be consistent with the results of the present study. However, the study has not found alkaloids, flavonoids and tannins in aqueous extract of apple.

On comparing the quantitative analysis of both samples, the study found that the phytochemical constituents were found in more amount in apple than the orange ethanolic extract (refer table 1 & table 4).

4. Conclusions

The present study has explored the phytochemical analysis of *Citrus sinensis* (Orange) and *Malus domestica* (Apple). The quantitative and qualitative analysis was performed to explore the phytochemicals present in the samples of apples and oranges. The study confirmed the presence of phytochemicals such as Saponins, Tannins, Flavonoids and Alkaloids in both fruits *Malus domestica* and *Citrus sinensis*. The quantitative analysis also signified that *Malus domestica* (Apple) contains more quantity of phytochemicals constituents present, than *Citrus sinensis* (Orange). *Citrus sinensis* (Orange) and *Malus domestica* (Apple) are widely consumed fruits. The study concluded that both the fruits are rich in phytochemicals and have several health benefits.

In numerous epidemiological studies, the consumption of apples and oranges have been found to be associated with

the decreased incidence of chronic diseases such as coronary disease, cancer or asthma. In vitro and animal studies have demonstrated that apples have high antioxidant activity, may inhibit proliferation of cancer cells, decrease lipid oxidation and lower cholesterol, potentially explaining their role in minimising risk of chronic diseases. *Malus domestica* and *Citrus sinensis* contain a wide variety of phytochemicals, many of them have a strong antioxidant and anticancer activity. However, the study signified that more study is required to further explain the phytochemical analysis of apples and oranges.

The potential health benefits of *Malus domestica* and *Citrus sinensis* are numerous. Regular consumption of fruits and vegetables, including apples and oranges, as part of a healthy diet may aid in the prevention of chronic disease and maintenance of good health.

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