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Impact of Two Different Methods of Extraction on Total Antioxidant Activity and Phenolic Content in an Uncommon Plant (*Sansevieria trifasciata*) and Commonly Consumed Fruits

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ABSTRACT

To determine and compare total antioxidant activity (TAA) and phenolic content (TPC) of *Sansevieria trifasciata* leaves' extract with different fruits' extract (Guava, Indian plum and Indian gooseberry) and fruit juices (Pomelo, Orange, and Beetroot) on the basis of two extraction methods, total phenolic content and antioxidant activity were analysed by Folin-Ciocalteu's and Phosphomolybdate assay, respectively and expressed as equivalent to catechin. The extraction process of selected samples was done by using both decoction extraction method (DEM) and microwave-assisted method (MAE) of water solvent.

Among both techniques of extractions, MAE exhibited significantly higher TPC and TAA than DEM. Compared to *Sansevieria trifasciata* plant extracts, there was a strong positive correlation coefficient (á<0.05) between the total phenolic contents and antioxidant activities of different fruit juices and fruit extracts in both the classical (DEM) and modern (MAE) techniques.

Sansevieria trifasciata possesses a considerable amount of phenolic compounds and antioxidant activity concerning different fruit juices and fruit extracts which directly associated with health-promoting potential and appears as possible drug development for antioxidant. Among all extract and juices, Indian gooseberry possesses the greatest total phenolic content and antioxidant activity due to its higher TPC.

Figures : 07 References : 22 Table : 01

KEY WORDS : Antioxidant activity, Citrus fruits, Decoction extraction method (DEM), Microwave-assisted extraction (MAE), Polyphenols, *Sansevieria trifasciata*.

Introduction

Plants are an unlimited source of natural products. In recent years, there is growing attention for finding antioxidants from natural products. Polyphenols are a class of compounds that largely present in plants as their secondary metabolites and are also known for their antioxidant characteristics.[1] Vegetables and fruits in human diets contain more than 90 percent of vitamin C.[16] "Citrus fruits are not only an important source of vitamin C, but also contain phenolic compounds and have important beneficial properties for human health as an antioxidant, including antioxidant, anti-aging, antibacterial, antiviral, and anti-mutagenic.[19]" The protection given by fruits against some diseases has been attributed to the asserted antioxidant contents. To avoid and restore the stress that occurs during normal cell functions, antioxidants are essential agents. Selected fruits such as pomelo, orange, guava and gooseberry are a rich source of vitamin C.

In particular, in various regions of India, the snake plant is also known as *Sansevieria trifasciata* or mother-in-law's tongue. *Sansevieria trifasciata* is a common perennial that grows wildly and is often found as a decorative plant in suburban areas, parks and woodlands.[17] *Sansevieria trifasciata* is used in the African nation (South Africa) and tropical America for the treatment for the inflammatory condition and sold out on the market as a crude medicine to treat survivors of snake bites.[5] It is commonly used for illustration, for the cure of earache, swelling and fever in India in the therapeutic approaches used for different ailments.[2] As, it is generally acknowledged that the geographical variation,

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TABLE-1 : Sample (Plant/Fruits) with scientific names and used parts

Samples (Plant/fruits)	Common name	Scientific name	Used part
Plant	Snake plant	*Sansevieria trifasciata*	Succulent leaves
Fruits	Indian Gooseberry	*Phyllanthus emblica*	Fruit
	Guava	*Psidium guajava*	Fruit (Pulp)
	Indian Plum	*Ziziphus mauritiana*	Fruit (Pulp)
	Beetroot	*Beta vulgaris*	Fruit juice
	Pomelo	*Citrus maxima*	Fruit juice
	Orange	*Citrus sinensis*	Fruit juice

environmental, extraction methods and botanical sections of plants influence the chemical composition of the individual compounds.[13] That chemical composition often relates to the antioxidant properties of phenolic compounds."There are two main forms of extraction methods, including traditional classical methods such as soxhlet extraction, decoction extraction system (DEM) maceration, hydro distillation, infusion, percolation and on the other hand, modern techniques such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), pulsed- electric field (PEF) extraction, supercritical fluid extraction (SFE), enzyme treatment and solid-phase micro-extraction (SPME)".[15] The purpose of this study was to compare total antioxidant activities (TAA) and the total phenolic content (TPC) of *Sansevieria trifasciata* leaves' extract with some selected fruit extracts and fruit juices directly. The extraction of samples was done by using two extraction methods, classical-decoction extraction method (DEM) and modern-microwave-assisted extraction (MAE); which are prevalent methods in the Indian kitchen.

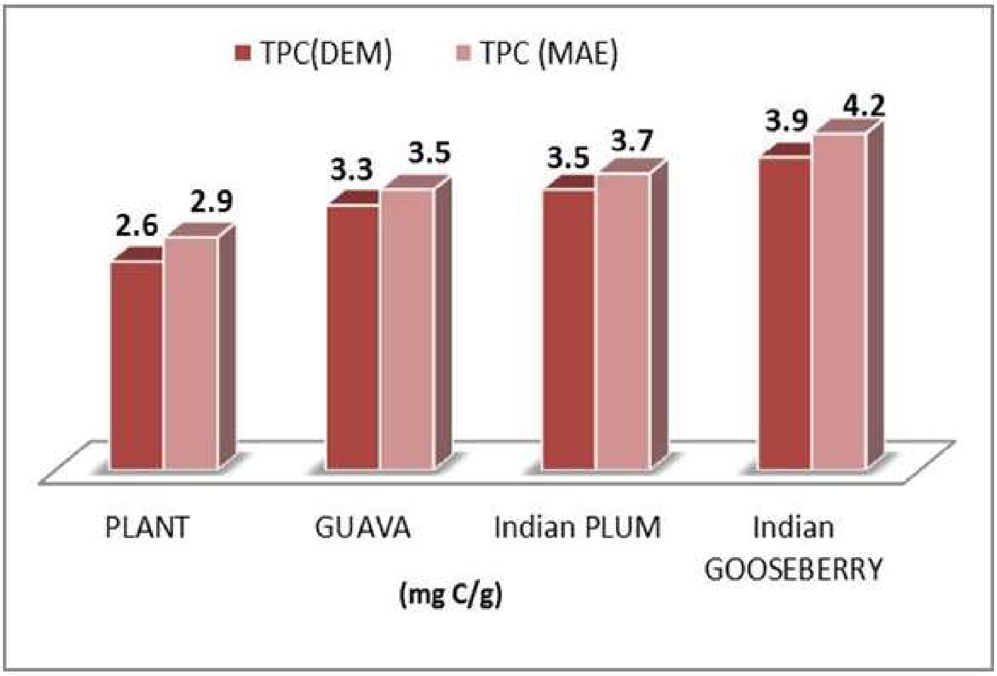

Fig.1 : Total phenolic contents of *Sansevieria trifasciata* leaves extract and fruits (Guava, Indian plum, and Indian gooseberry) extract. Data are represented as the means ± S.D. (n = 3)

Experimental Part

Reagents

Deionized water, sodium bicarbonate ($NaHCO_3$), Folin-Ciocalteu phenol reagent, ammonium molybdate, sodium phosphate, H_2SO_4 (98%), and catechin were purchased from Thermo Fisher Scientific India Pvt. Ltd. All the used reagents and solvents were of analytical grade.

Plant material-

Sansevieria trifasciata belongs to the family "Asparagaceae" were collected from the Jhansi area in Uttar Pradesh, India, and identified by the Department of Botany, Bipin Bihari College, Jhansi (U.P.), and all the fruit samples were purchased from the local market of Jhansi, U.P. (Table-1).

Preparation of plant samples and extraction-

The studied part (succulent leave) of the *Sansevieria trifasciata* plant was cleaned and separated then air-dried at room temperature. A grinder was used to grind plant materials into fine particles. In accordance with the standard protocol, plant leaves and all fruit samples were prepared. In short, samples were collected with 100 ml of deionized water for 50-60 minutes at 70-80°C by both the decoction method and the microwave-assisted method. After that, Extractions were filtered *via* the Whatman No.1 filter paper. The extracts collected were stored and measured within 24hrs. Water has been selected as a solvent for extraction because it is already used in the food industry.

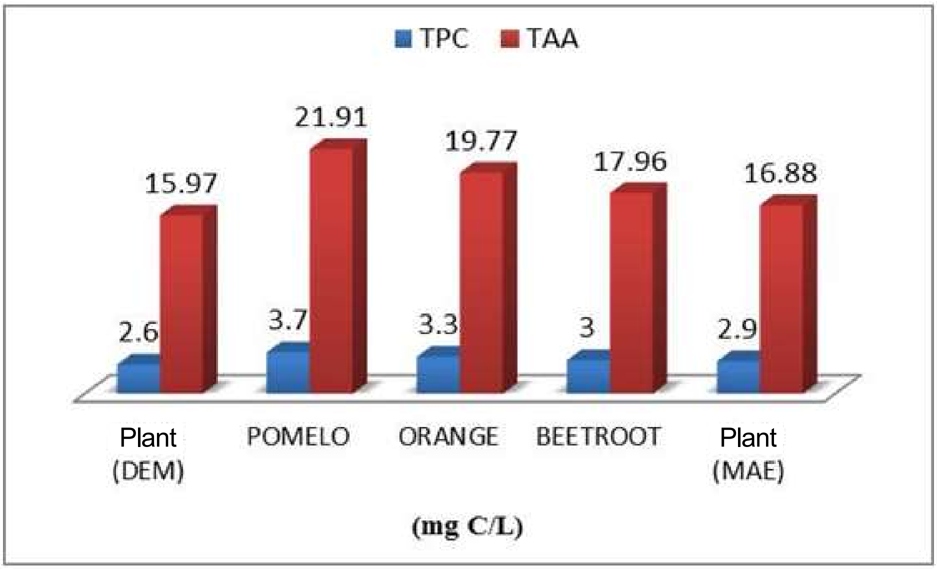

Fig.2. TPC & TAA of *Sansevieria trifasciata* leaves extract (DEM and MAE) and fruit juices (Pomelo, Orange & Beetroot). Data are represented as the means ± S.D. (n = 3)

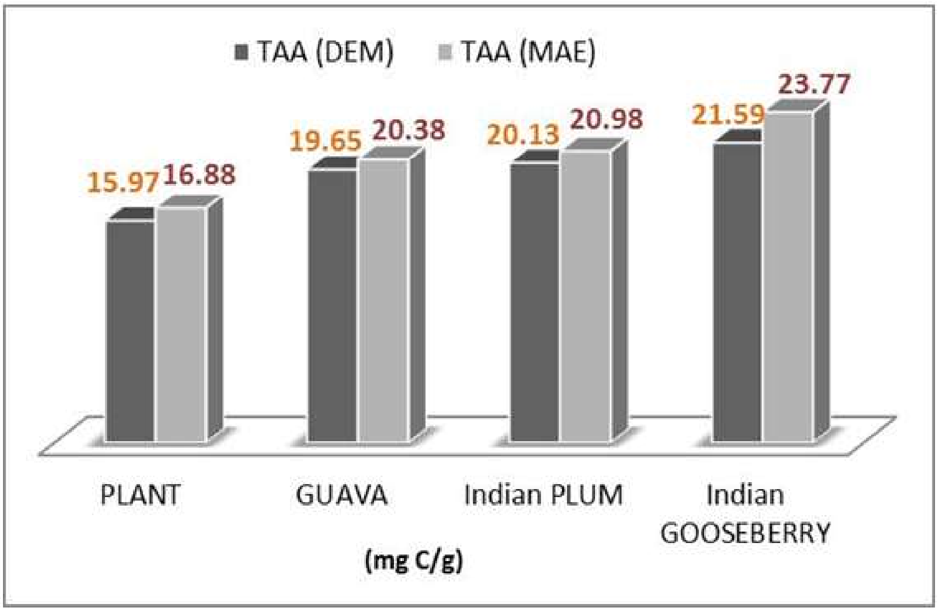

Fig.3. TPC & TAA of *Sansevieria trifasciata* leaves extract (DEM and MAE) and fruit juices (Pomelo, Orange & Beetroot). Data are represented as the means ± S.D. (n = 3)

Evaluation of Total Phenolic Content

Folin-Ciocalteau reagent with minor modification was used to determine the concentration of phenolics in prepared water extracts and fruit juices[8]. By mixing 0.5 ml of sample extract/standard, 2.5 ml of 10 % Folin-Ciocalteu reagent dissolved in distilled water and after some time, add 2.5 ml of 7.5 % $NaHCO_3$, the reaction mixture was prepared. Also, Blank was prepared simultaneously. Using an UV spectrophotometer at max = 765mn after 1 hour of incubation against a blank, the absorbance was measured. The Phenolic content in extracts and juices was measured in terms of catechin equivalent (mg C/g of extract) and (mg C/L) based on the measured absorbance.

Evaluation of Antioxidant Activity (Phosphomolybdate assay)

By using the phosphomolybdenum assay, the total antioxidant potential of the samples was calculated.[7] 0.3 ml aliquot of sample extracts and standard solutions with different concentrations were prepared with 3 ml of reagent solution

(0.6M sulphuric acid, 28mM sodium phosphate, and 4mM ammonium molybdate). The test tubes were coated and incubated in a water bath at 95°C for 90min. The mixture's absorbance was measured at 695nm using a UV spectrophotometer against a blank after the sample was cooled. Catechin was used as a standard.

Results and Discussion

Total Phenolic Contents (TPC)

Using Folin-Ciocalteu's reagent, the total phenolic content was measured and stated as catechin equivalent (mg C/ g) using the standard catechin curve. All the extracts contain a significant amount of phenolic content varies from 2.6±1.73 to 3.9±1.20 mg C/g of extract in the decoction extraction method (DEM) and 2.9±1.7 to 4.2±1.9 mg C/g of extract in the microwave-assisted method (MAE) (figure 1). In both DEM and MAE methods, Indian gooseberry possesses the highest total phenolics. The total phenolic content of *Sansevieria trifasciata* is also approaching the total phenolic content of Indian plum and guava in both extraction methods.

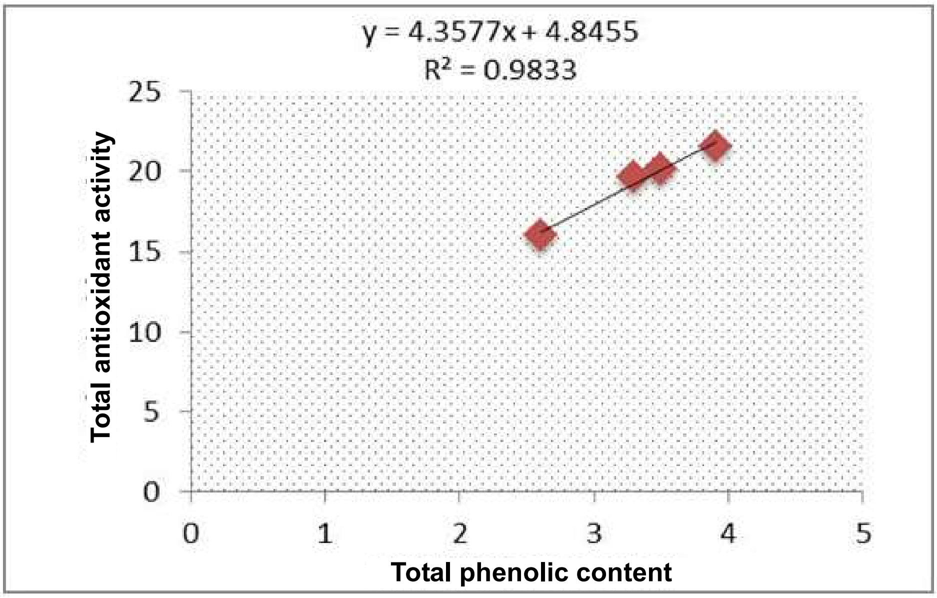

Fig. 4 : Correlation between TPC & TAA of fruits with *Sansevieria trifasciata* plant in the DEM. P<0.05 values were taken to be significant in the experiment.

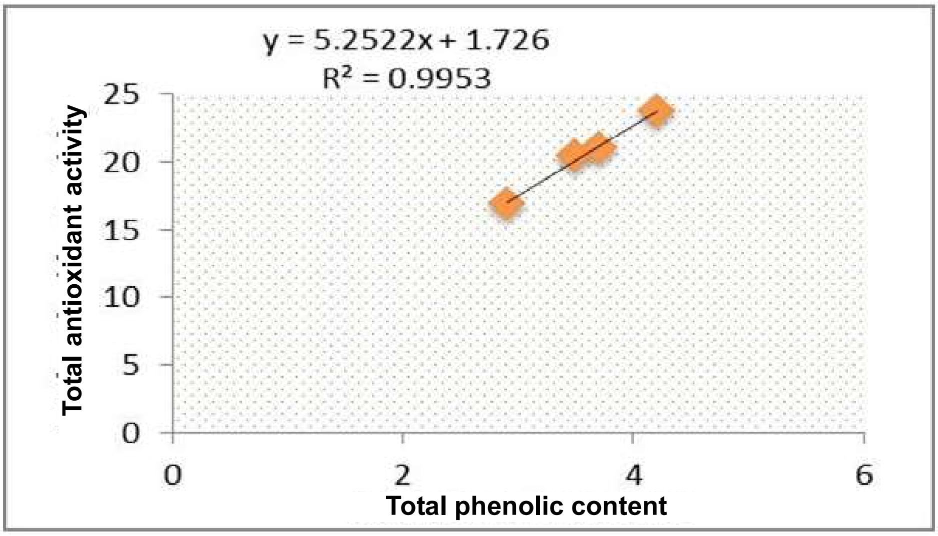

Fig. 5 : Correlation between TPC & TAA of fruits with *Sansevieria trifasciata* plant in the MAE. P<0.05 values were taken to be significant in the experiments.

In fruit juices, Pomelo exhibited the highest total phenolic content (3.7±1.5), followed by orange and beetroot (Fig. 2). Though, *Sansevieria trifasciata* is a comparatively good source of TPC.

Total Antioxidant Activity (TAA)

The cumulative activity of antioxidants in *Sansevieria trifasciata* extracts and some commonly consumed fruits and fruit juices were tested using a 695nm absorption phosphomolybdate assay in both the DEM and MAE methods and expressed as equivalent to catechin. In both DEM and MAE methods, Indian gooseberry showed the maximum total antioxidant activity followed by Indian plum, guava, and *Sansevieria trifasciata* (fig. 3). The values of TAA ranged from 15.97±0.58 to 21.59±1.20 mg C/g of extract in the decoction extraction method (DEM) and 16.88±1.28 to 23.77±1.10 mg C/g of extract in the microwave-assisted method (MAE).

In fruit juices, Pomelo showed the highest total antioxidant activity (21.91±1.9). Moreover, *Sansevieria trifasciata* (both DME & MAE extract) was quite equivalent to orange and beetroot. (Fig. 2).

Antioxidants are found not only in polyphenols, but also in a widely diversified class of amino acids, phenylamine and tyrosine, including, simple phenolics, hydroxybenzoic acid, tannins and flavonoids among others. Since these compounds exist as aglycones and glycosides, as monomers or strongly polymerized and show greater diversity of structure.[9,10,12] Their stability varies significantly, making it difficult to extract them. According to our study, total phenolic contents and antioxidant activity in the modern extraction technique (MAE) are better as compared to the classical method (DEM). The effectiveness of microwave energy depends heavily on the solvent mixture's dielectric constant. "Solvents with a high dielectric constant and strongly absorb microwave radiation, such as ethanol, methanol and water, are therefore sufficiently polar for microwave energy to be heated."[11] The heating causes plant cell evaporation to lose moisture; the steam produced swells and eventually splits the cells, emancipating their active components. Polar solvents have a dielectric constant that is much greater and can absorb more microwave energy than non-polar solvents, which can result in a higher phenolic yield.[22]

In our research, Indian gooseberry extract showed higher TPC and TAC than guava, also found earlier. [21] There was a good TAC value by the DPPH method

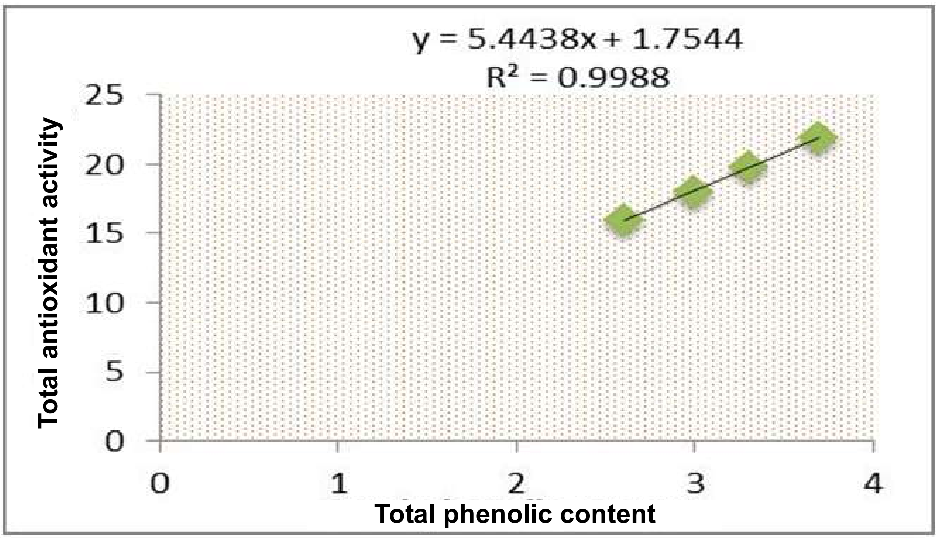

Fig.6 : Correlation between TPC & TAA of fruit juices with *Sansevieria trifasciata* leaves in DEM. $P<0.05$ values were taken to be significant in the experiments.

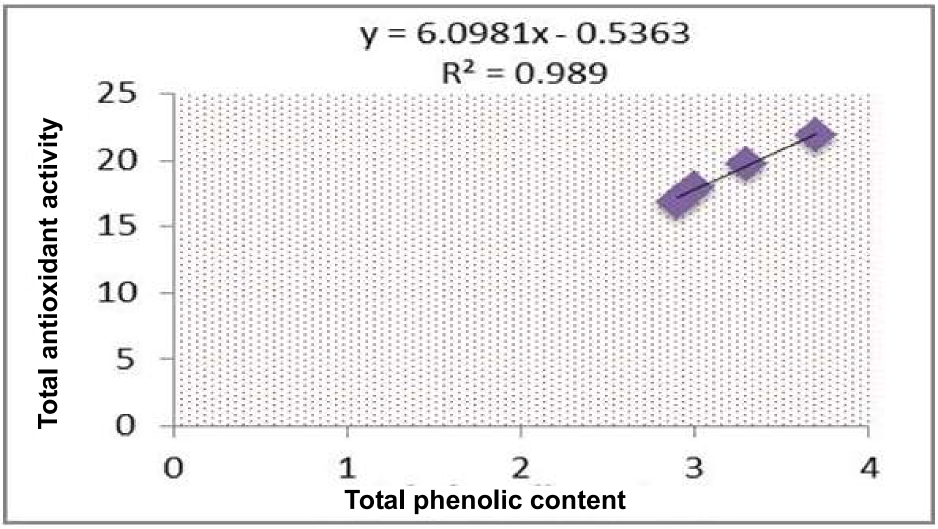

Fig.7 : Correlation between TPC & TAA of fruit juices with *Sansevieria trifasciata* leaves in MAE. $P<0.05$ values were taken to be significant in the experiments.

in *Sansevieria cylindrical.*[20] *Sansevieria trifasciata* leaves' crude extract has antidiabetic potential.[3] The DPPH test, a standardized test to determine the free radical scavenging effect of plant extracts, is based on a reduction in antioxidants in the solution of methanol DPPH, resulting in the formation by reaction of non-radical DPPH-H. However, DPPH and some other methods in previous studies have already recorded all the selected fruits in this study. But here in this paper, we analyzed total phenolic and antioxidant of fruits by phosphomolybdate method using two extraction methods as antioxidant activity and phenolic content depend on the extraction method and geographical region. The most abundant antioxidants present in most plants are phenolics and the Folin-Ciocalteu assay offers a strong ballpark for its determination. Although the F-C assay is used not only as a measure of total phenolics but also as a method of antioxidant potential involving transfer of a single electrons (SET) yet phenolic substances act differently towards the F-C reagent. The phenolic quantification method is considered to depend on electrons' transfer in an alkaline medium (pH of $NaHCO_3$ is 8.4), whereas the phosphomolybdate assay is based on an acid medium (pH of 98% H_2SO_4 is 0.1) for the determination of antioxidants. "Therefore, the reactivity of the F-C reagent depends on the pH and it is assumed that a single electron, presumably a phenol, is transferred from the substrate to the complex Mo (V) in the reagent, since Mo (VI) is reduced to Mo (V)[14]." A major antioxidant capacity assay is divided into a reaction-dependent assay based on the chemical reaction involved in the transfer of hydrogen atoms (HAT) and single electron (SET). In this study, we used a phosphomolybdate assay for antioxidant determination based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex."

Correlation between total phenolic contents and antioxidant activities

The secondary metabolites and the principal contributor to the antioxidant are phenolic compounds as they are directly associated with their antioxidant. However, the antioxidant activities of fruits/ fruits juices cannot be entirely predicted based on their phenolic content, as vitamin C and carotenoids in fruits also partially contribute to antioxidant activities.[4] But sometimes, samples with low phenolic content might show high antioxidant activity and vice-versa because of the presence of other non-phenolic reducing agents such as organic acid, sugar and amino acid nitrogenous compounds.[6]

Linear regression curves were stated as figures and the Pearson correlation coefficient was obtained between total phenolic content and antioxidant activity. According to our findings, the total phenolic content was directly associated with the overall antioxidant activities of fruit extracts in both extraction methods (Figs. 4 & 5) and fruit juices (Figs. 6 & 7), with *Sansevieria trifasciata*. The results indicated that the total phenolic content in fruit & *Sansevieria trifasciata* extract was positively and strongly associated with antioxidants in the DEM (r=0.983) (fig 4) and MAE techniques (r=0.995) (Fig-5).

There have been a lot of studies on the correlation between TPC and TAC. The quality of ascorbic acid in citrus fruits and commercial orange juices, on the other hand, contributed significantly to antioxidant activity.[18]

Conclusion-

According to our results, *Sansevieria trifasciata* possesses a significant amount of phenolics and antioxidants which is directly associated with health-promoting potential and appears as possible drug development for antioxidant. It is also concluded that Indian gooseberry has the maximum total phenolic content and antioxidant activity among all extract and juices. Although numerous previous fruit studies present, we are again monitoring TPC and TAA of fruit extracts and juices by a different method. We suggest that their consumption during this COVID pandemic period is helpful to improve immunity.

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