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## ③ Evaluation of secondary metabolites profile and antioxidant potential of *Terminalia chebula* and *Glycyrrhiza glabra*

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**Key Message:** The study highlights the vital secondary metabolites in *Terminalia chebula* and *Glycyrrhiza glabra*, emphasizing their role in combating oxidative stress and preventing health conditions linked to reactive oxygen species. Hence, these findings indicate the therapeutic potential of these plants.

### Abstract

This study explores the antioxidant potential and secondary metabolite profiles of *Terminalia chebula* and *Glycyrrhiza glabra*, medicinal plants native to Swat, Pakistan. *Terminalia chebula*, or black myrobalan, is known for its antioxidant and anti-inflammatory properties, while *Glycyrrhiza glabra*, commonly known as licorice, has a rich history of traditional medicinal use for over 4000 years. The study focuses on evaluating the total phenolic and flavonoid contents, as well as the antioxidant activity of both plants. The study explores the importance of secondary metabolites, including alkaloids, glycosides, flavonoids, phenolics, saponins, tannins, terpenes, anthraquinones, essential oils, and steroids, in both *Terminalia chebula* and *Glycyrrhiza glabra*. The

antioxidant activity of these plants is crucial in combating oxidative stress and preventing various health conditions associated with reactive oxygen species (ROS). The methodology involves the collection of seeds from local markets, grinding them into powder, and extracting them with 70% ethanol. The samples are then subjected to various analyses, including total phenolic and flavonoid content determination and antioxidant activity evaluation using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Results indicate that both *Terminalia chebula* and *Glycyrrhiza glabra* exhibit significant levels of total phenolic and flavonoid contents, with remarkable antioxidant activity. *Glycyrrhiza glabra* demonstrates higher total phenolic and flavonoid contents compared to *Terminalia chebula*. The study concludes that these medicinal plants are rich sources of bioactive compounds with potential therapeutic applications, highlighting the importance of exploring and harnessing the pharmacological benefits of traditional medicinal plants for human health. © 2020 The Author(s)

**Keywords:** Antioxidants, Flavonoid contents, *Glycyrrhiza glabra*, Medicinal plants, Phenolic contents, *Terminalia chebula*

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### Introduction

*Terminalia chebula* is a tree ranging from medium to large size with the tendency of shedding its leaves annually with maximum height of 30 m (Muhammad et al., 2012). Leaves are 7 to 20 cm long, not clustered, distant, alternate, and rounded (Akhtar et al., 2019). It grows at the elevation of 1500 to 2000 m mainly in hilly areas (Muhammad et al., 2012). *Terminalia chebula* refers as black myrobalan in English terminologies and is famous for its antioxidant, anti-inflammatory activities in the biological sciences (Kalra et al., 2019). It has numerous names given by various individuals in different places over the world. In India, *Terminalia chebula* is known by the names Haritaki Har, Hara, Harar, and Harad in Hindi. In Persian regions, the name used for *Terminalia chebula* is Halaila, while in Arabic, it is referred to as Ahilajasfar (Kalra et al., 2019). Due to its various health benefits, the locals in Tibet refer to it as the king of medicines (Akhtar et al., 2019). This plant is used to treat various sorts of ailments and

contamination because it is a cost-effective source of valuable phytochemicals (Akhtar et al., 2019). In addition to its remarkable wound-healing properties, Unani doctors have expanded the use of this medicine. It is now employed as a tonic for the brain, eyes, heart, and stomach, enhancing renal activity. Furthermore, it serves as a blood purifier, astringent, laxative, anthelmintic against worms, and an agent for treating diarrhea (Akhtar et al., 2019). *T. chebula* is one of the medicinally used plants for treating hemorrhoids and preventing bleeding (Andarkhor et al., 2019). In Korea, the dried products of this plant are utilized as a solution for looseness of the bowels, diarrhea, hock, mucus, and sore throats. The pharmacological and biochemical examination demonstrate the restorative movement of *T. chebula* in Alzheimer's sickness, disease, clogging, diabetes, contamination, aggravation (Jeong et al., 2019). In *Terminalia chebula*, numerous glycosides have been found such as chebulosides I and II, arjunin, arjunglucoside, 2 $\alpha$ -hydroxyursolic acid, and 2 $\alpha$ -hydroxymicromiric acid (Mammen et al., 2012). The leaves of the plants are reported to

contain polyphenol such as punicalin, punicalagin, terflavins B, C, and D (Han et al., 2006). The plant also retains phloroglucinol and pyrogallol, alongside phenolic acids, for example, ferulic, p-coumaric, caffeic and vanillic acids (Khare, 2004). It is additionally comprised of supplements, for example, nutrient C, protein, amino acids and minerals (Mahesh et al., 2009).

The *G. glabra* has antioxidant activity which makes it very usable. The phenolic content gives the plants strong antioxidant potential (Rackova et al., 2007). The fruits, leaves, and barks of *T. chebula* showed higher free scavenging activity due to presence of phenolic contents (Chang & Lin, 2010). Fluid concentrate of *T. chebula* restrained xanthine/xanthine oxidase action and was additionally a fantastic scrounger of DPPH radicals (Naik et al., 2004). *T. chebula* in a polyherbal plan (Aller-7/NRA2) repressed free radical prompted hemolysis and furthermore altogether restrained nitric oxide discharge from lipopolysaccharide animated murine macrophages (Mahesh et al., 2009). Six concentrates and four mixes of *T. chebula* natural product showed cell reinforcement movement at various extents of potency (Hazra et al., 2010).

Liquorice (*Glycyrrhiza glabra* L.) is a perennial plant with erect growth and height of 0.7-2 m (Organization, 1999). Leaves are interchanged, pinnate, with 9-17 applaud, 4-7 pamphlets and a terminal handout, yellow-green flyers, each 2.5 - 5 cm long. The spike of pea-like blossoms might be white, purple. Spikes are generally 10 - 15 cm long and are conceived from leaf axils. Seedpods are maroon, 3 cm long, oval, pointed, and straightened (Omidbeigi, 2006). Secondary metabolites are bioactive compounds in plants having derivatives such as alkaloids (Varsha et al., 2013), glycosides (Firn, 2010), flavonoids (Kar, 2007), phenolics (Cai et al., 2004), saponins (Vashist & Sharma, 2013) tannins (Varsha et al., 2013) terpenes (Martinez et al., 2008), anthraquinones (Vashist & Sharma, 2013) essential oils (Vashist & Sharma, 2013) and steroids (Varsha et al., 2013). In the most recent times, the compound components of liquorice have been broadly examined by various creators (Hayashi et al., 2016) and (Siracusa et al., 2011). Few investigations have been done on the dietary synthesis of *G. glabra*. Liquorice is a source of proteins, amino acids, polysaccharides, basic sugars, mineral salts (gelatins, tars, starches, sterols, and gums), nutrients (B1, B2, B3, B5, E, and C) (Wang et al., 2015).

Aerobic metabolism plays a crucial role in generating energy for the majority of cells. However, during this process, reactive oxygen species (ROS) are yielded which can potentially lead to oxidative chain reactions. An excess accumulation of ROS can disrupt the balance between oxidants and antioxidants in the body, leading to cellular damage and various health issues (Kaushik et al., 2012). For instance, ROS have the capability to oxidize guanosine, a purine base, resulting in the formation of 8-oxoguanosine. If left unrepaired, this alteration can induce mutations and impair the functions of proteins. Cancer prevention agents (present in cells or gained by means of

sustenance or restorative plants can defer or restrain the oxidative responses or rummage starting radicals, along these lines constraining the oxidative harm (Irshad et al., 2012).

The biological processes generate harmful reactive normally known as free radicals which contain at least single unpaired electron in outermost orbit (Halliwell, 2012). Biomolecules protect cells against the dangerous effects of these free radicals, thus protecting cells and DNA from being converted into abnormal (Chaturvedi & Beal, 2013). Hydromethanolic root concentrate of *Glycyrrhiza glabra* L. indicated presence of various valuable optional metabolites for example flavonoids, saponins, alkaloids, etc. In view of these constituents the concentrate displayed powerful enemy of oxidant and hostile to bacterial exercises. It can battle against rummaging hydroxyl radical and bacterial disease. It might be a significant remedy for restraint of bacterial disease and searching of hydroxyl radicals which are delivered during carcinogenesis (Varsha et al., 2013). Antioxidant compounds in plants can stop oxidation process of food and minimize the chance of age specific diseases (Zou et al., 2004; Santhi et al., 2011). Therefore, the present research study aimed to determine total phenolic and flavonoids production of *Terminalia chebula* and *Glycyrrhiza glabra* as well as antioxidant activity of *Terminalia chebula* and *Glycyrrhiza glabra*.

## Materials and Methods

### Collection of seeds

The seeds of *Terminalia chebula* and *Glycyrrhiza glabra* were obtained from the local market of Mingora Swat KPK. The seeds of *Terminalia chebula* (14.909 mg) and *Glycyrrhiza glabra* (14.259 mg) were measured. Then the seeds were grinded to powder form with the help of mortar and pestle. Two jars were taken, washed with distilled water and left to dry out followed by the addition of *Glycyrrhiza glabra* (6.042 mg) to one jar and *Terminalia chebula* (10.707 mg) in the second jar. After that 70% ethanol was added to each jar and shook for 5 min until the powdered seeds were completely dissolved. Finally, both jars were covered with aluminum foil and were kept at room temperature for five days.

### Preparation of samples

The jars were subjected to shaking for some time. Two empty petri dishes were weighed with each petri dish weighing 12.357 mg. The samples in the jars were transferred to Petri dishes with the help of filter paper and pipette. The solution was gradually passed into petri dishes within time of five minutes. The filter paper was discarded followed by measurement of Petri dishes with filtrates. Afterwards, the petri dishes were left exposed at room temperature for four days. The exposed samples after four days of consecutive drying were scratched with the use of sharp blades and measured. From each sample, 5 mg was added to test tube containing 5 ml ethanol and was shaken until complete dissolution followed by storage for five days.

### Measurement of total phenolic contents

In each sample, the total phenolic contents (TPC) and total flavonoid contents (TFC) were calculated by using the procedure of Folin-Ciocalteu as described by Singleton et al. (1999). During the procedure, 0.1 ml (2 N) of Folin-Ciocalteu reagent was added to extract (0.03 ml) and purified distilled water (2.55 ml). The centrifugation (10,000 rpm; 14 min) of the mixture was carried out followed by filtration in UV-visible spectrophotometer (Shimadzu-1650; Japan) cuvette through 45  $\mu$ m membrane. The supernatant was incubated for 6 minutes. At 760 nm, the mixture absorbance was measured. Gallic acid (Sigma; 1.0–10 mg/ml; R2 = 0.9878) was used for plotting standard calibration curve. The results were obtained as Gallic acid equivalent (GAE) mg/g from percentage TPC by applying the following equation:

$$TPC (\%) = \frac{(AS - AB)}{(CF \times DF)} \times 100$$

In the above formula AS denotes absorbance of sample, while AB represents absorbance of blank. CF represents conversion factor from standard curve and DF represents dilution factor.

### Measurement of total flavonoid contents

For the calculation of total flavonoid contents (TFC), 0.2 ml of methanolic extract of the two samples was added to decontaminated distilled water (1.25 ml) and aluminum trichloride 0.075 ml 5 % (w/v) followed by addition of 0.5 ml of sodium hydroxide (1 M). The mixture was subjected to centrifugation for 14 minutes at 10,000 rpm and then incubated for 6 minutes. At 510 nm, the absorbance was investigated with UV-visible spectrophotometer (Shimadzu-1650PC, Japan). For plotting standard calibration curve, Rutin (Sigma; 1.0–10 mg/ml; R2 = 0.9866) was exploited. The TFC was quantified and expressed as rutin equivalent (RE) in milligrams per gram (mg/g) of extracts.

**Table 1** Total phenolic contents, total phenolic production, total flavonoids, total flavonoids production and antioxidant potential of *Terminilia chebula* and *Glycyrrhiza glabra*

Plants seeds	Total phenolic contents $\pm$ SE	Total phenolic production	TFC $\pm$ SE	Total flavonoids production	Antioxidant activity $\pm$ SE mean
<i>Terminilia chebula</i> 10.707 mg	5.99 $\pm$ 0.67	64.13 $\pm$ 2.32	3.31 $\pm$ 0.29	33.10 $\pm$ 1.05	56.08 $\pm$ 1.99
<i>Glycyrrhiza glabra</i> 6.042 mg	9.37 $\pm$ 0.89	56.61 $\pm$ 3.09	7.04 $\pm$ 0.34	42.53 $\pm$ 1.22	66.78 $\pm$ 1.58

TFC = Total flavonoid contents; SE = Standard error

### Comparative analysis of *Terminilia chebula* seeds, fruits and leaves

The comparative analysis of *Terminilia chebula* seeds, fruits, and leaves revealed varying levels of total phenolic contents (TPC), total flavonoids (TFC), and antioxidant activity (Table 2). *Terminilia chebula* seeds exhibited the

### Antioxidant activity

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) in the samples was checked in accordance with slight modifications in Qader et al. (2011). Each sample (ethanolic extract) of 1.0 ml; 5 mg/20 ml was combined with DPPH reagent (2.0 ml; (0.25 mg/20 ml  $\times$  4)). The mixture was then exposed to darkness for half an hour. The mixture was tested for absorbance at 517 nm using UV-visible spectrophotometer model (Shimadzu-1650PC, Japan). Eventually, the Free Radical Scavenging Activity (FRSA) was determined as % DPPH discoloration by using the following formula:

$$\% \text{ scavenging DPPH free radical} = 100 \times (1 - AE/AD)$$

In the above equation, AE shows solution's absorbance in the presence of extract whereas AD is the absorbance of solution in the absence of extract.

### Results

#### Phenolics, flavonoids and antioxidant potential of *Terminilia chebula* and *Glycyrrhiza glabra*

Table 1 shows the comparative analysis of *Terminilia chebula* and *Glycyrrhiza glabra* seeds in terms of their total phenolic and flavonoid contents, production levels, and antioxidant potential. *Terminilia chebula* exhibited a higher total phenolic content (10.707 mg) compared to *Glycyrrhiza glabra* (6.042 mg). However, *Glycyrrhiza glabra* displayed a greater total phenolic production (9.37  $\pm$  0.89) in comparison to *Terminilia chebula* (5.99  $\pm$  0.67). In terms of total flavonoid contents, *Terminilia chebula* again showed a higher value (64.13  $\pm$  2.32 mg) compared to *Glycyrrhiza glabra* (56.61  $\pm$  3.09 mg). However, the total flavonoid production of *Glycyrrhiza glabra* (7.04  $\pm$  0.34) exceeded that of *Terminilia chebula* (3.31  $\pm$  0.29). Additionally, *Glycyrrhiza glabra* demonstrated higher antioxidant activity (66.78  $\pm$  1.58) than *Terminilia chebula* (56.08  $\pm$  1.99). These findings highlight the variability in bioactive compound profiles and antioxidant potential between the two plant species, providing valuable insights into their potential health benefits and applications.

lowest TPC (5.99  $\pm$  6.130) and TFC (3.31  $\pm$  3.2867), but comparatively higher antioxidant activity (56.08  $\pm$  56.993). In contrast, *Terminilia chebula* fruits showed significantly higher TPC (134.47  $\pm$  5.68) and TFC (7.934  $\pm$  4.324) but a lower antioxidant activity (0.963  $\pm$  0.5). *Terminilia chebula* leaves displayed the highest TPC (266.16  $\pm$  7.81) and TFC (29.23  $\pm$  3.81) with moderate antioxidant activity (11.6  $\pm$  0.43) (Table 3)

2). These results highlight the variability in phenolic and flavonoid composition across different parts of *Terminalia*

*chebula*, influencing their antioxidant potential.

**Table 2** Comparative analysis of *Terminalia chebula* seeds, fruits and leaves

Plants	TPC ± SE mean	TFC ± SE mean	Antioxidant activity ± SE mean
<i>Terminalia chebula</i> seed	5.99 ± 6.130	3.31 ± 3.2867	56.08 ± 56.993
<i>Terminalia chebula</i> fruit (Saha & Verma, 2016)	134.47 ± 5.68	7.934 ± 4.324	0.963 ± 0.5
<i>Terminalia chebula</i> leaf (Arya et al., 2012)	266.16 ± 7.81	29.23 ± 3.81	11.6 ± 0.43

TPC = Total phenolic contents; SE = Standard error

### Comparative analysis of *Terminalia chebula* seeds, roots and aerial parts

The comparative analysis of *Glycyrrhiza glabra* components, including seeds, roots, and aerial parts, reveals variations in total phenolic contents (TPC), total flavonoid contents (TFC), and antioxidant activity (Table

3). *Glycyrrhiza glabra* seeds showed a moderate TPC (9.37 ± 9.34) and TFC (7.04 ± 7.05), accompanied by a relatively high antioxidant activity (66.78 ± 66.85). In contrast, both roots and aerial parts exhibited lower TPC (4.25 ± 4.05 for roots and 4.55 ± 4.1 for aerial parts) and TFC (4.5 ± 4.2 for roots and 4.6 ± 4.3 for aerial parts) but demonstrated higher antioxidant activity (88.6 ± 88.2).

**Table 3** Comparative analysis of *Glycyrrhiza glabra*

Plants	TPC ± SE mean	TFC ± SE mean	Antioxidant activity ± SE mean
<i>Glycyrrhiza glabra</i> seed	9.37 ± 9.34	7.04 ± 7.05	66.78 ± 66.85
<i>Glycyrrhiza glabra</i> roots (Tohma & Gulcin, 2010)	4.25 ± 4.05	4.5 ± 4.2	88.6 ± 88.2
<i>Glycyrrhiza glabra</i> aerial parts (Tohma & Gulcin, 2010)	4.55 ± 4.1	4.6 ± 4.3	88.6 ± 88.2

TPC = Total phenolic contents; TFC = Total flavonoid contents; SE = Standard error

### Discussion

The foundation of licorice (*Glycyrrhiza glabra*) is one of the most common prescriptions on the planet which has been depicted as the granddad of herbs (Asl & Hosseinzadeh, 2008). It has been utilized as medicinally in both Western and Eastern nations for over 4000 years (Aoki et al., 2007). *Glycyrrhiza glabra* concentrate is widely utilized in the USA and is considered as protected for use in sustenance by the FDA (Nakagawa et al., 2008). This so-called ink tree belongs to genus *Terminalia* of family Combretaceae in kingdom plantae (Akhtar et al., 2019). The family Combretaceae is comprised of 20 genera and about 475 species (El-Rafie et al., 2014). The variety *Terminalia* is fan out into 200 species which involved trees and bushes and can be found in the tropical and subtropical districts of world (El-Rafie et al., 2014).

The current study described that *Terminalia chebula* seeds have 5.99 mg/g-DW total phenolics contents. While the other study showed that *Terminalia chebula* fruits have 134.47 mg/g-DW total phenolics content (Saha & Verma, 2016). One report explored that *Terminalia chebula* leaf have 266.16 mg/g-DW total phenolics content (Arya et al., 2012). *T. chebula* seeds had 3.31 mg/g-DW total flavonoids content. However, the reports of Saha and Verma (2016) suggested the TFC of *T. chebula* with 7.934 mg/g-DW. Moreover, *T. chebula* leaf showed 29.23 mg/g-DW absolute flavonoids contents (Arya et al., 2012). *T. chebula* seeds had 56.08 % antioxidant activity. On other hand, *T. chebula* fruits displayed the minimum antioxidant activity (0.963 %) (Saha & Verma, 2016). Arya et al.

(2012) documented that *T. chebula* leaf had 11.6% antioxidant activity. In the present study *Glycyrrhiza glabra* seeds exhibited 9.37 mg/g-DW total phenolics contents. Compared with *Glycyrrhiza glabra* root which had 4.2 mg/g-DW total phenolics contents (Tohma & Gulcin, 2010). *Glycyrrhiza glabra* aerial parts showed 4.25 mg/g-DW total phenolics contents (Tohma & Gulcin, 2010). *Glycyrrhiza glabra* seeds produced 7.04 mg/g-DW total flavonoids contents. While *G. glabra* roots produced 4.5 mg/g-DW total flavonoids contents (Tohma & Gulcin, 2010). *G. glabra* aerial parts had 4.6 mg/g-DW total flavonoids contents (Tohma & Gulcin, 2010). *Glycyrrhiza glabra* seeds yielded 66.78 % antioxidant activity. Compared with *Glycyrrhiza glabra* roots had 88.6% antioxidant activity (Tohma & Gulcin, 2010). *Glycyrrhiza glabra* aerial parts had 88.6 % antioxidant activity (Tohma & Gulcin, 2010). These results suggest variability in phenolic and flavonoid composition across different parts of *Glycyrrhiza glabra*, influencing their overall antioxidant potential. The findings emphasize the need to consider specific plant components when assessing the medicinal properties of *Glycyrrhiza glabra*. These findings highlight the importance of considering different plant parts when assessing the medicinal properties of *Glycyrrhiza glabra*, as they exhibit distinct phenolic and flavonoid profiles that contribute to varied antioxidant capabilities.

In traditional Chinese medicine, the root and root like stem of Licorice were used for treatment purposes (Seo et al., 2010). Fundamentally, the aeronautical piece of licorice was utilized to nourish cows and herd or consumed into compost as fuel. Previous studies have focused on licorice root, revealing its primary components to be triterpenes such as glycyrrhetic

acid and glycyrrhizin, alongside flavonoids like liquiritigenin, liquiritin, isoliquiritigenin, isoliquiritin, and coumarins (Fu et al., 2005). There was insufficient data about the synthetic profile and natural action of licorice leaf. Antiquated Egyptians, Greeks, and Romans perceived the advantages of licorice in treating hacks, fever, and chills. In the times of Hippocrates, licorice was endorsed for dropsy on account of revitalizing properties of licorice drugs (Biondi et al., 2005). The antiquated Hindus utilized licorice for enhancing libido (Davis & Morris, 1991). In conventional prescription licorice roots include be utilized beside treat chest and lung sicknesses, bronchitis, joint inflammation, low pulse, sensitivities, liver lethality, Addison's ailment, pancreatic clutters, tooting, skin ailments, dryness and definite viral disease (Sharma & Agrawal, 2013). Recent pharmacopeias from France and Germany are by and large concurrent on the therapeutic utilization of licorice. In Indian prescription, licorice is utilized for cure of flu, eye illnesses, uterine grumblings, liver ailment, and joint pain (Saxena, 2005). In Chinese prescription, licorice is utilized to care for skin break out and skin complaint, apprehensive disarranges, for example, insanity, peevishness, and epilepsy just as lessen the poisonous or extraordinary activity of different herbs, and to blend home grown recipes (Zhu, 1998). Generally it is utilized to treat liver sicknesses and is a noteworthy part of polyherbal plans to fix liver toxicity (Rajesh et al., 2000).

The phenolics mixes recognize in present investigation of *Terminalia chebula* and *Glycyrrhiza glabra* are restoratively significant. *Terminalia chebula* alludes as dark myrobalan in English wordings and is known for its cancer prevention agent, mitigating exercises in the natural sciences (Kalra et al., 2019). Most of the individuals imagined that flavors, vegetables they utilized in their kitchen are just give them flavors to their sustenance, however these likewise assume an indispensable job in keeping us solid and fit. These flavors and vegetables contain different phytochemicals essential to treat various particular phytochemicals and cell reinforcements which are useful for the treatment of different sicknesses which are caused because of the oxidative pressure like malignancy and diabetes. Polyphenols, for example, tannins and flavonoids have appeared to have various wellbeing defensive advantages, which incorporate bringing down of blood lipids. Thus, these plants have been utilized to lower blood lipids content. The antioxidative properties of plants were connected generally to the nearness of phenolic mixes, particularly flavonoids.

## Conclusion

Both the medicinal plants i.e. *Terminalia chebula* and *Glycyrrhiza glabra* have high medicinal value and pharmaceutical importance in the local Pansar industry of district Swat as well as in Pakistan. The phytochemical profile and antioxidant potential of the local species indigenous to the district Swat were not previously studied. Therefore, in the present study we have evaluated the

phytochemical constituents and antioxidant potential of *Terminalia chebula* and *Glycyrrhiza glabra*. Both the plants have antioxidant, anti-inflammatory and anticancerous properties. In the present study we analyzed the total phenolics content, total flavonoids content and antioxidant activity of both plants' seeds. In the present study, we found that both *Terminalia chebula* and *Glycyrrhiza glabra* have large quantity of secondary metabolites with enormous antioxidant potential. If we confine these phytochemicals, then it will be useful for the natural medication ventures to make new tranquilizes and at last it helps the people.

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