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\* **Corresponding author.**

[nadiminti\\_sekhar@live.com](mailto:nadiminti_sekhar@live.com)

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# Chemistry of Black Tea Extract on Citrus Essential Oils

N. Sekhar<sup>1</sup>, M. Srimannarayana<sup>1\*</sup>, N. Deepika<sup>2</sup>

<sup>1</sup> Department of Chemistry, GITAM School of Science, Hyderabad, Telangana, India

<sup>2</sup> R&D flavours, Mane India Pvt Ltd-Hyderabad, Telangana, India

## Abstract

**Objectives:** To check the chemistry of Ceylon black tea extract on citrus oils. Black tea extract rich in antioxidants aims to investigate their role to protect degradation of citrus oils. GCMS analysis and antioxidant activity helps to interpret the changes and the effect of black tea extract on citrus oils chemical components. **Method:** The black tea extract derived through maceration at different set temperatures ranging from 50 °C, 75 °C, and 100 °C was tested for identification of chemical components by GCMS analysis and its antioxidant activity compared with positive controls like BHA and tocopherol using DPPH assay ( $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl). The decolorization percentage was calculated in the black tea extract (74% - 50 °C, 71% - 75 °C, and 69% - 100 °C) along with positive controls (76.5% - BHA & 76.7% - tocopherol). Based on results the black tea extract collected at 50 °C tested at dosage of 0.1% on citrus oils. **Findings:** Antioxidant activity reveals *C. aurantium* (Narinja-47.8%), *C. hystrix* (Gondhoajlebu-46.1%), *C. limetta* (Mosambi-53.6%) and *C. limon* (Lemon-57.8%), *C. reticulata* Blanco (Nagpur Orange-37.2%) reveals that black tea extract can be used as antioxidant to prevent oxidation of citrus oils. GCMS analysis of citrus oils with black tea extract states that degraded compounds like *p*-cymene, carvone, limonene oxide, peroxides, etc, were not found. **Novelty:** This study is centered on natural extracts instead of synthetic antioxidants. Black tea extract, a natural antioxidant applied in citrus oils helps to create stable and natural citrus flavours especially applicable for tea applications.

**Keywords:** Black tea extract; BHA; Tocopherol; GCMS; Degradation; Citrus Oils; Antioxidant

## 1 Introduction

Tea (*Camellia sinensis*) is second after water in the beverage category. Worldwide, black tea is highly consumed next to oolong, green and white tea<sup>(1)</sup>. Black tea is an excellent option to maintain good health because of its polyphenol content. The main polyphenol content of tea are theaflavins, thearubigins, epigallocatechin gallate, L-theanine, an amino acid, and catechins. They contain flavonoids that protect from chronic diseases caused by the consumption of black tea. These chemical components act as antioxidants and food additives, controlling the release of free radicals in the body when it was consumed and helping to prevent cell damage<sup>(2)</sup>.

Black tea is extracted through maceration using ethanol and water at different set temperatures of 50, 60, 70, 80, and 90 °C for 5 min, and the extracted component is tested for its antioxidant activity<sup>(3)</sup>. Theaflavins exhibited high antioxidant and anti-radical activity during the DPPH assay<sup>(4)</sup>. Estimation of Antioxidant activity can be studied by DPPH ( $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl) assay using tocopherol as a standard. Butyl hydroxytoluene (BHT) and  $\alpha$ -tocopherol were standard for Antioxidant assay<sup>(5)</sup>.

Essential oils undergo several alterations as they age when stored for a long time. The fresh aroma compounds undergo a sequence of degradation and release off odours. Several investigations were done to understand the stability of essential oil under various storage conditions. Essential oils, including citrus oil, are degraded due to isomerization, oxidation, dehydrogenation, thermal degradation, and polymerization. Heat, light, and oxygen are the main factors affecting the degradation process<sup>(6)</sup>. Limonene is the dominant compound among all citrus fruits, and key component is that Citral, is very unstable during storage at low pH, accelerated temperature and when exposed to oxygen. Degradation of Citral and Limonene releases off-odour due to the cyclization and oxidation process<sup>(7)</sup>. Citral degradation forms p-cymene-8-ol and produces aromatic compounds such as p, a-dimethylstyrene, p-cymene, and p-cresol through dehydration. Limonene degradation results in peroxides, perillyl alcohol, perillyl acetate, carveol acetate, limonene oxide, carvone, and carveol<sup>(8)</sup>. There are many synthetic antioxidants like BHA/tocopherol used to improve shelf life of citrus flavours. BHA cannot be used because of its carcinogenic nature in future and tocopherol is expensive. To sustain and protect citrus oil degradation natural antioxidants present in tea extracts can be used. The current research aims for me to experiment and study how black tea extract improves the stability of citrus oils to fulfil the gap by using natural antioxidants. Food flavour can be enhanced by essential oils and if they degrade, they will release undesirable off taste and aroma. Analysts should focus to test the application sample in appropriate way by sensory analysis and interpretation of results help to identify the adulterations or off notes<sup>(9)</sup>. Well trained sensory panel members were selected to describe the attribute and to understand the differences of fresh and degraded off notes<sup>(10)</sup>.

## 2 Methodology

### 2.1 Materials

1. *C.aurantium* (Narinja), *C. hystrix* (Gondhoaj lebu), *C. limon* (Lemon), *C. limetta* (Mosambi) and *Citrus Reticulata Blanco* (Nagur Orange) were selected from different regions of India; Telangana, Andhra Pradesh, Kolkata and Maharashtra.
2. DPPH ( $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl) from Merck.
3. BHA, Tocopherol of 100% purity from Merck & Ceylon Black tea extract from Mane.

### 2.2 Collection of black tea material and citrus essential oils

We visited different stores to purchase black tea and finally procured black tea leaves Ceylon type from local market Hyderabad. The selected citrus oils extracted from *C. aurantium* (Narinja), *C. hystrix* (Gondhoaj lebu), *C. limon* (Lemon), *C. limetta* (Mosambi) and *Citrus Reticulata Blanco* (Nagur Orange)<sup>(11)</sup>.

### 2.3 Extraction of Black tea extract

Ceylon black tea purchased from the market and 5g of black tea - 3 sets of samples weighed separately, and each sample was dissolved in 50 mL of water and subjected to 50 °C, 75 °C, and 100 °C temperature for 5 min. on a magnetic stirrer with a speed of 250 rpm and after 5 min the contents were filtered using Whatman No. 40 filter paper, Buchner funnel, and a vacuum pump. The filtered contents were stored in the refrigerator for the subsequent experiments.

### 2.4 Antioxidant assay

The Antioxidant activity of black tea extract was studied by DPPH ( $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl) assay. Black tea extract was selected for this experiment since it is rich in antioxidants like theaflavins and catechins and in coordination with citrus antioxidants like vitamin C improves shelf life of citrus oils and slow down degradation process. DPPH is deep blue to purple because it contains free radical organic nitrogen radical and turns pale yellow to colourless if any antioxidant is added. 2.4 mg of DPPH dissolved in 100 mL of Methanol solution (1% in Ethyl alcohol) and 25  $\mu$ l of citrus to be tested were taken, and 975  $\mu$ l of DPPH was added. All the samples were incubated for 30 min., and positive controls like BHA, Tocopherol were selected. Readings were recorded after 30 min. using a spectrophotometer at 525-528 nm. The values are incorporated in the formula below<sup>(12)</sup>.

$$\text{DPPH radical scavenging activity (\%)} = [(\text{OD blank} - \text{OD sample}) / \text{OD blank}] \times 100$$

To check the efficacy of black tea extract as an antioxidant, citrus oils *C. aurantium* (Narinja), *C. hystrix* (Gondhoaj lebu), *C. limon* (Lemon), *C. limetta* (Mosambi) and *Citrus Reticulata Blanco* (Nagur Orange) dosed with black tea extract @0.1% and comparison study made along with BHA and tocopherol. Blank is maintained without any antioxidant or sample to check the efficiency of black tea extract by DPPH assay.

## 2.5 Black tea extract on citrus oils by GCMS analysis

The essential oils extracted from citrus peel were separated into two halves. One set was stored at a refrigerated temperature of 4-8 °C, and another at room temperature for 4 months with black tea extract, BHA & tocopherol, and another set without antioxidants. Gas chromatography Mass Spectroscopy was performed using Agilent instruments for all the samples to understand and identify the degradation compounds released. GC-7890A Gas chromatography was coupled with Mass spectroscopy 5975C-MS and which is connected to the Chem Station data system. The components were identified using the NIST 2014 spectra library and a chromatogram was generated based on the report.

## 2.6 Stability studies

Stability studies were performed by descriptive sensory analysis recording attributes of the samples using Hedonic scaling (1-9; 1 - Dislike extremely, 2 - Dislike very much, 3 - Dislike moderately, 4 - Dislike slightly, 5 - Neither like nor dislike, 6 - Like slightly, 7 - Like moderately, 8 - Like very much, and 9 - Like extremely) to identify the difference between degraded components-off notes and undegraded or stable fresh components. For brewing 2g of flavoured (citrus oils) tea and plain tea base was taken in beaker and 80 ml of boiling water was added. Brew the contents for 5min (please don't disturb or stir the contents while brewing). After 5min of brewing the contents were mixed once (single stir) and filtered using tea strainer and proceeded for tasting and the difference were noted through sensory (trained) panel members<sup>(13)</sup>.

## 3 Results & Discussions

### 3.1 Antioxidant activity

A Most popular, quick, and simple method of antioxidant activity is the DPPH assay (Diphenyl-1-picrylhydrazyl) to measure the antioxidant properties of a selected testing compound<sup>(14)</sup>. Black tea extract derived was 0.5-0.8 g at each set temperature of 50 °C, 75 °C, and 100 °C. The black extract at 50 °C was stable hence tested for antioxidant activity compared to 75 °C, and 100 °C. The efficiency of black tea extract antioxidant activity was tested through a DPPH assay, and positive controls BHA, tocopherol was selected for comparison. There is an immediate colour change from violet to colourless in BHA, tocopherol, and black tea. Based on test results, Black tea extract was tested on selected citrus oils at 0.1% dosage. There is a slight change in colour in all citrus oils after 30 min. of incubation, but not completely. Figure 1 states that BHA, tocopherol has 76-77% antioxidant activity, followed by Black tea extract 74%, Orange 24%, Gondhoraj lebu showed 23.4%, Narinja 13.2%, Mosambi 4.4% and Lemon 2.2%.

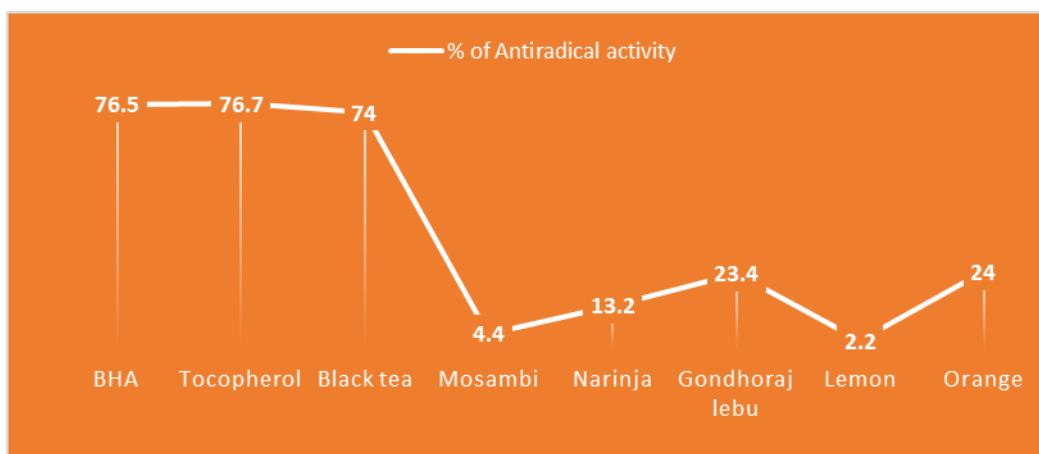


Fig 1. Antioxidant activity

Antioxidant activity was performed for all the selected Citrus oils with 0.1% of selected antioxidants, separately BHA, Tocopherol & Black tea extract. Based on the results Citrus oils with BHA & Tocopherol (positive controls) are performed extremely well in antioxidant activity compared to Black tea extract (Figure 2). Though the activity of black tea extract is low, it can be used as a natural antioxidant to improve the shelf life of citrus oils and protect them from degradation. These results are compared with oil and extracts of *Citrus aurantifolia*. DPPH - antioxidant activity of essential and extract reveals extract of *Citrus aurantifolia* has high antioxidant activity ( $0.66 \pm 0.009$  and  $0.92 \pm 0.012$  mg/mL) compared to oil ( $3.03 \pm 0.019$  and  $4.27 \pm 0.023$  mg/mL)<sup>(15)</sup>. DPPH is pink or violet in colour, has an absorbance value of 517nm and in the presence of *Citrus reticulata* and [(*Citrus unshiu*  $\times$  *Citrus sinensis*)  $\times$  *Citrus reticulata*]  $\times$  *Citrus reticulata*, there is a reduction process exhibiting antioxidant activity changed to yellow diphenyl picrylhydrazine<sup>(16)</sup>. Citrus essential oils extracted from *Citrus sinensis* (32.0  $\mu$ g/ml), *Citrus limon* (27.29  $\mu$ g/ml), *Citrus reticulata* (33.0  $\mu$ g/ml) and *Citrus auratifolia* (28.67  $\mu$ g/ml) subjected to DPPH assay has potential antioxidant activity. The same colour change noticed in current research for selected citrus oils and antioxidants<sup>(17)</sup>. Ascorbic acid, BHA, BHT and tocopherol was used as positive control in all the literature studies. Above comparative research states citrus fruits have antioxidants and can be assessed by DPPH assay.

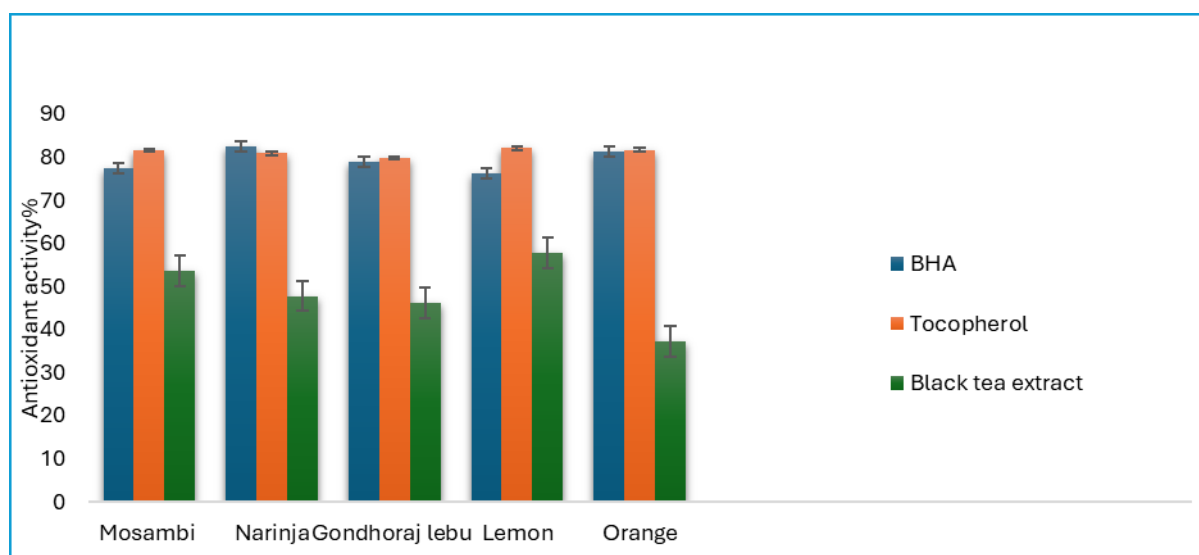


Fig 2. Antioxidant activity of citrus oils with antioxidants

### 3.2 GCMS analysis to identify degraded compounds

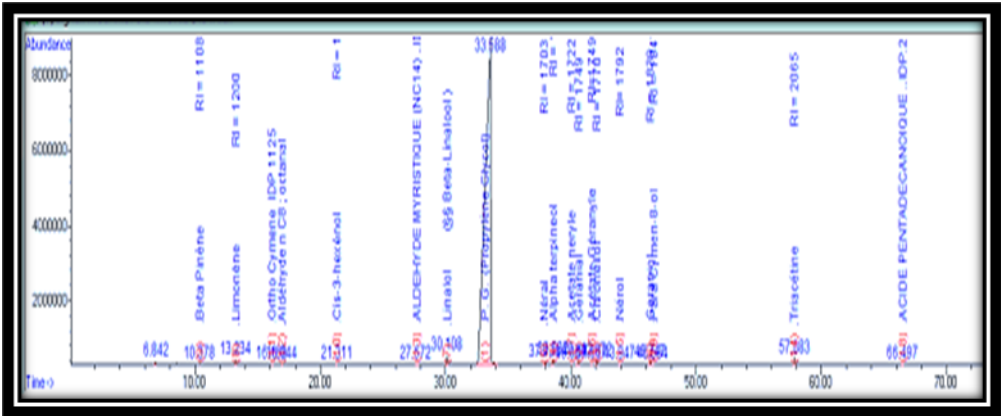
GCMS analysis of all fresh citrus oils containing high amounts of Limonene (95-99%) and Citral (3-4%) and GCMS analysis results for the samples kept at room temperature and refrigerated condition with and without black tea extract. Citrus oils without black tea extract consist of degraded compounds of both Citral and Limonene for the citrus oils kept at room temperature. Citral degradation results in the formation of isomerization of geranial to neral and then forms alcohols, p-menthadien-8-ol and p-menthadien-4-ol, which in turn can oxidize to form p-cymene-8-ol and through a dehydration reaction produces aromatic compounds such as p, a-dimethyl styrene, p-cymene, and p-cresol. p-Cresol and p-methyl acetophenone are two of the most potent Citral degradation products. Cresol is not observed in present reports. Limonene degradation results in peroxides, perillyl alcohol, perillyl acetate, carveol acetate, limonene oxide, carvone, and carveol (Table 1).

To protect them from degradation, antioxidants like BHA, tocopherol and black tea extract were used and kept for stability in the refrigerator and at room temperature. Where we found that the citrus oils were stable without degradation in refrigerated conditions and minimal degradation at room temperature conditions in a four-month study (Figure 3 & 4).

The results were compared with research work done earlier, and it was noticed that Limonene was a monoterpene hydrocarbon present in lime oil decomposed due to pyrolysis. Sequences of reactions take place due to dehydrogenation and oxidation, which release aromatic rings like p-cymene and p-cymene. Degradation studies on citrus oils study reveal the formation of  $\beta$ -myrcene and ocimene isomers due to the decomposition of linalyl acetate. Bergamot oil has lower levels

**Table 1.** Citrus oil Degradation compounds after four months with & without black tea extract

S. No	RT	Without black tea extract	With black tea extract
1	8.37	Gamma terpene	Gamma terpene
2	10.42	Beta pinene	-
3	10.7	Beta phellandrene	-
4	11.79	Myrcene	Myrcene
5	13.77	Limonene	Limonene
6	15.17	Cis ocimene	-
7	16.98	aldehyde C08	-
8	25.02	Limonene oxide	-
9	25.68	Limonene oxide	-
10	26.04	Delta Elemene	-
11	27	-	Citronellal
12	30.13	Linalool	Linalool
13	30.65	Alcohol C08	-
14	30.77	Linalyl acetate	-
15	31.18	Limonene oxide	-
16	33.16	Caryophyllene	-
17	34.77	Cis paramenthadien-2-8-ol	-
18	36.29	1-Nonene	-
19	37.54	Decyl Acetate	-
20	38	-	Citral
21	40.06	Neryl acetate	-
22	40.81	Carvone	-
23	41.83	Alcohol C10	-
24	43.86	Para Menthadiene	-
25	43	-	Caryophyllene
26	46.34	Geraniol	-
27	47.25	2Dodecenal	-
28	47.38	Peryllic alcohol	-
29	56.01	Nerolidol	-
30	56.78	Caprylic acid	-
31	66.01	Spathulenol	-
32	66.51	Acid C10 capric	-



**Fig 3.** Identification of degradation molecules of citrus oils without black tea by GCMS analysis

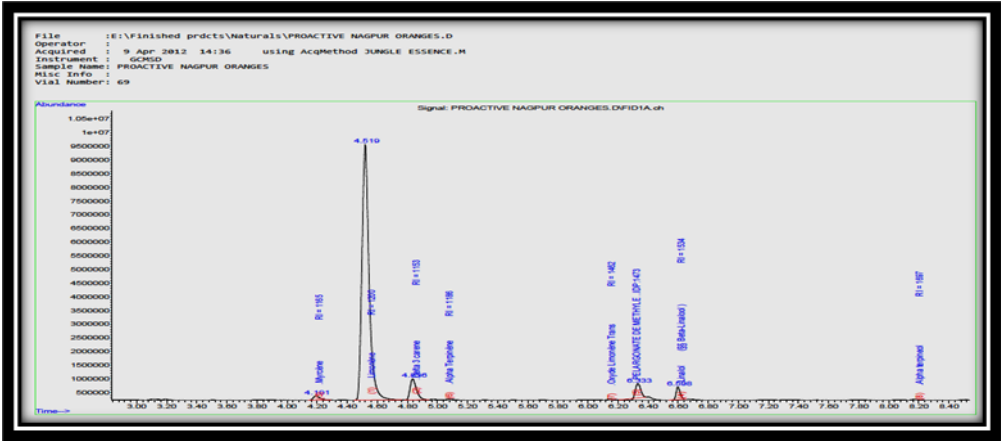


Fig 4. Identification of degradation molecules of citrus oils with black tea by GCMS analysis

of bicyclic compounds and higher levels of esters and alcohols. Pyrolysis of esters in Bergamot generates geranyl and Neryl acetate<sup>(18)</sup>. Factors like water and light affect the stability of citrus oil and lead to degradation, such as alcohols and ketones. We even observe colour change with off notes. There was a decrease in the number of terpenes and oxidation of Limonene, resulting in the formation of Limonene oxide, carveol and p-cymene<sup>(19)</sup>.

Oxidation plays a significant role in the degradation of essential oils. There are two types of oxidation methods: 1. Atmospheric oxygen and 2. Singlet oxygen. Sometimes autooxidation takes place in the essential oils stored at room temperature, releasing alkyl groups and a radical that interacts with oxygen and progresses to the degradation process. Oxidation or degradation of terpenes occurs based on the temperature conditions. Storage temperature played a critical role and interlinked with oxidation. Terpenes are sensitive to acid, oxygen and storage temperature. P-cymene is formed due to the oxidation of gamma terpene. Citral, a mixture of neral and geranial, degrades and releases p-cymene, predictable by-products like p-cresol, p-methyl acetophenone and intermediate compound p-cymenols were released during degradation of citrus oils<sup>(20)</sup>.

3.3 Sensory analysis

Sensory attributes of fresh and degraded components of citrus oils with and without black tea were recorded as mentioned in Table 2 (1-dislike extremely to 9 like extremely) and data is represented in spider map (Figure 5). During evaluation of black tea application sample freshness is reduced and peely, pithy notes with oxidized off notes are noticed in samples without black tea extract compared to samples with black tea extract. This indicates black tea extract protects the citrus oils degradation process.

Table 2. Sensory descriptors of orange and lemon oil using hedonic scale-9 = like extremely to 1 = dislike

S. no	Attribute	Without black tea extract	With black tea extract
1	Fresh	3	8
2	Juicy	3	7
3	Sour	4	7
4	Sweet	6	8
5	Zest	3	7
6	Off note/oxidized	3	-
7	Peely	4	8
8	Pithy	3	7

The above sensory data compared with other research work and captured attributes of the samples done through sensory analysis which aims to interpret, evaluate and prove the results by describing the profile. Different odour attributes were noticed from peel extracts of pomelo fruit and the data was captured using 9 scale where 0 is unperceivable attribute and 9 is meant for strong intensity attribute. Sensory is subjective and depends on the surroundings. Overall flavour perception, sweetness, sourness, and off odours were recorded by the panel members to understand the acceptance of the flavour<sup>(21)</sup>.





Fig 5. Sensory analysis of citrus oils with and without black tea extract

## 4 Conclusion

In present world there is a vast change in society to use natural solutions to consume food and flavours to sustain and maintain good health. This study reveals the usage of black tea extract a novel approach in citrus oils to protect degradation as well as to avoid synthetic antioxidants like BHA/tocopherol. Above study declares black tea extract collected through maceration has excellent antioxidant activity on citrus oils *C. aurantium* (Narinja), *C. hystrix* (Gondhoaj lebu), *C. limon* (Lemon), *C. limetta* (Mosambi) and *Citrus Reticulata Blanco* (Nagur Orange) by DPPH assay. GCMS analysis reports significantly states that the main components of the citrus essential oils without black tea extract experienced degradation releasing off notes/chemical components like p-cresol and p-methyl acetophenone, peroxides, perillyl alcohol, perillyl acetate, carveol acetate, limonene oxide, carvone whereas these compounds are absent in citrus oils with black tea. Further to reconfirm sensory analysis asserts there is difference in juicy fresh, peely, pithy notes of citrus oils and noticed oxidized off notes are more perceivable in samples without black tea extract. Further research is in progress to confirm citrus oils will be used only for tea category or for other beverage categories by LCMS analysis. Hence the role of natural antioxidants like black tea extract can be used to protect and safeguard citrus oils from degradation.

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