

Variation in chemical composition and antioxidant activity of freshly distilled and stored *Ocimum basilicum* L. essential oil

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Article

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Abstract

Essential oils are complex mixtures of volatile phytochemicals whose chemical composition and biological activity can change during storage. The aim of present study was carried out to evaluate the variation in chemical composition and antioxidant activity of freshly distilled and one-year stored *Ocimum basilicum* L. essential oil stored in aluminium metal, transparent glass, and transparent plastic bottles. The essential oil was extracted from aerial parts of basil plants by a Clevenger apparatus using steam hydro-distillation method. Chemical composition was analyzed by GC–MS, and antioxidant activity was determined by DPPH radical scavenging assay using gallic acid as standard.

GC–MS analysis showed that freshly distilled oil contained 19 components with estragole (62.66%), linalyl butyrate (21.12%), and linalyl formate (3.79%) as major chemical components. The quantity of components decreased and major quantitative changes were detected after one year of storage. A number of minor monoterpenes and ester compounds either disappeared or reduced, indicating degradation and transformation during storage, while the amount of estragole increased (79–82%). The DPPH assay revealed that freshly distilled oil exhibited very high antioxidant activity (97.61–91.26%), comparable to gallic acid, whereas the stored oil showed a significant reduction in activity (47.62–0.79%). The results show that prolonged storage leads to loss of bioactive substances responsible for antioxidant activity. Overall, the study suggests that storage conditions have a significant impact on the chemical stability and antioxidant potential of *Ocimum basilicum* essential oil, and freshly distilled oil retains higher quality than stored samples.

1. Introduction

Essential oils (EOs) are complex mixtures of volatile and semi-volatile phytochemicals synthesized through the secondary metabolism of aromatic plants (1). They are widely recognized for their biological activities and have been traditionally utilized in food preservation, herbal medicine, cosmetics, and aromatherapy (2). Nowadays, over 80% of people on the planet use herbal medicines to treat a variety of diseases (3). The chemical composition of an essential oil is strongly influenced by factors such as plant species, environmental conditions, harvesting stage, extraction method, and post-distillation handling (4). Foods derived from plants are becoming more popular due to their high dietary natural antioxidants, such as polyphenols, flavonoids, vitamin C, and vitamin E, are associated to decrease risk of cardiovascular disease, some types of cancer and other chronic diseases (5, 6). Due to their high content of reactive monoterpenes and phenylpropanoids, EOs are highly susceptible to oxidative and thermal degradation during storage, which can alter their functional properties, including antioxidant activity (7).

Ocimum basilicum L. (sweet basil), commonly called as a basil plant, a member of the Lamiaceae family, and one of the most economically important aromatic herbs cultivated worldwide (8). Among more than 150 species in the genus *Ocimum*, basil is the most important essential oil plant that is grown commercially in several countries (9). It's essential oil (EO) is rich in oxygenated monoterpenes and phenylpropanoids such as linalool, methyl chavicol (estragole), and eugenol, which contribute to its

characteristic aroma and numerous pharmacological effects (10). They are mixtures of various chemical compounds based on their chemical composition, with terpenes, aldehydes, alcohols, esters, phenols, ethers, and ketones being the most prevalent. Basil is a great source of calcium, iron, magnesium, and β -carotene (11, 12). They are highly valued in the global market because of their unique characteristics of flavor, scent, and biological activity (13). Consumer curiosity regarding natural food additives, particularly spices and herbs like basil to be utilized as an antioxidant, has increased recently as a result of toxicological research that demonstrated the beneficial impact of synthetic antioxidants in the formation of malignant cells in rats (14). Basil EO has been extensively reported for its antimicrobial, antioxidant, anti-inflammatory, and insecticidal activities, making it valuable in food, pharmaceutical, and cosmetic industries (15). In India, it is used in traditional medicine to cure numerous infectious diseases e.g. skin, liver disorder, fever, malaria, colds and coughs (16).

However, the stability of *Ocimum (O.) basilicum* EO during storage remains a major concern for manufacturers and users (17). Exposure to oxygen, light, temperature fluctuations, and interactions with packaging materials can significantly modify the oil's chemical profile (18). Such transformations may reduce the concentration of key bioactive compounds or generate new degradation products, ultimately affecting antioxidant potential and overall quality (19). Despite the growing market demand for basil essential oil, systematic studies comparing freshly distilled oil with oil stored over extended periods are limited (20).

Therefore, the present study aims to investigate the variation in chemical composition and antioxidant activity of freshly distilled and stored *O. basilicum* EO in aluminium metal, transparent glass and in transparent plastic bottles (containers) with storage duration of one year. By evaluating changes over defined storage durations and conditions, this work provides essential insights into the stability, shelf life, and optimal storage practices required to preserve the therapeutic and commercial value of basil essential oil.

2. Materials and methods

2.1. Collection of Plant material

The freshly cultivated aerial parts of *O. basilicum* L. at the full blooming stage were collected from Barabanki (Uttar Pradesh, India), early in the morning in month of October 2023. The plant materials were thoroughly washed with water to remove dust and contaminants. It was recognized and verified in the herbarium of the botany department at CMP Degree College, Prayagraj, Uttar Pradesh (India), and stored in the laboratory of chemistry at room temperature.

2.2. Extraction of essential oil

The freshly cultivated aerial parts of *O. basilicum* L. were put into the round-bottom flask after that, this flask was kept in a heating mantle and fixed to Clevenger apparatus. The plant materials were allowed to boil at 100°C and then the temperature was lowered to 80°C and left for 4 to 5 hrs.; as a result, essential

oil was extracted using steam hydro-distillation method. The extracted oil was separated from the water solvent, and the moisture was removed from *Ocimum basilicum* EO using anhydrous sodium sulfate and kept fresh sample in the refrigerator, and another sample was stored in aluminium metal, transparent glass and transparent plastic bottles at room temperature for further analysis.

2.3 Chemicals required

Distilled water (Double distillation unit), sodium bisulphate (Loba Chemie Pvt. Ltd., Mumbai, India), *Ocimum basilicum* L. (basil) essential oil (Extracted by Clevenger apparatus) Gallic acid (CDH Pvt. Ltd., New Delhi, India), 1, 1-Diphenyl-2-picrylhydrazyl (DPPH*) (HiMedia Pvt. Ltd., Mumbai, India), Methanol AR (Loba Chemie Pvt. Ltd., Mumbai, India).

2.4. GC-MS analysis of essential oil

The chemical composition of *Ocimum basilicum* L. (basil) essential oil was analyzed by using gas chromatography-mass spectrometry (GC-MS) using a gas chromatograph (Agilent 7890) connected with a mass spectrometer (Accu TOF GCv, Jeol) and an HP-5MS capillary column (30m length, 0.25 µm film thickness, 0.25mm internal diameter). Helium was employed as the carrier gas, with a flow rate of 1.0 mL/min, and n-hexane as the solvent. The injecting volume of the sample was 0.2 microliters. The GC-MS interface temperature was 250°C. The temperature of the oven was initially set to 60°C, then raised to 200°C at a rate of 6°C per minute, 200°C to 275°C at a rate of 8°C per minute, and lastly 275°C to 280°C at a rate of 5°C per minute. With a mass scan range of 35 to 800 atomic mass units, ionization took place at 70 electron volts, and the ionization mode was EI+.

The result was verified by comparing it to retention indices on the exact same column. Using a homologous sequence of n-alkanes (C₈-C₂₀, Sigma-Aldrich), each unique chemical was identified based on retention duration and retention index by comparing its mass spectra to the NIST Mass Spectral Library, co-injection with genuine samples, and literature [21]. Compound percentages in the oil were computed by dividing the proportion of each compound's peak area by the overall peak area calculated according to the gas chromatogram and mass spectrometry findings.

2.5. Analysis of the antioxidant activity

2.5.1. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH* Assay) [22]

Gallic acid was utilized as the standard and DPPH* as a radical scavenger. Since DPPH* contains an odd electron therefore it shows maximum absorption at 517 nm. The process then exhibits the component's antioxidant activity as the absorption decreases.

$$\% \text{ Inhibition of DPPH* activity} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}$$

Where A_{control} = absorbance of control, A_{sample} = absorbance of sample

2.5.2. DPPH* scavenging assay

Stock solution (1mg/10mL) of *Ocimum basilicum* L. (basil) essential oil was prepared in methanol. The stock solution of DPPH* was also prepared at the concentration of 2mg/100 mL in methanol.

Various concentrations such as 2 mL, 1.5 mL, 1 mL, 0.5 mL, 0.25 mL, 0.125 mL of *Ocimum basilicum* L, was taken out in the test tube separately and made up the volume up to 2 mL by adding methanol respectively. Now fill each test tube with 2 mL of DPPH* solution, increasing the volume upto 4 mL, and left the solutions in the dark for 30 minutes to incubate. After incubation, UV-Visible spectrophotometer 118 was used to record the absorbance of each sample.

3. Results and Discussion

3.1. Chemical composition of *Ocimum basilicum* L. (basil) essential oil with GC-MS

The chemical composition of the freshly distilled and one-year stored *Ocimum basilicum* L. (basil) essential oil was determined by Gas chromatography and Mass spectrometry (GC–MS), and the results are summarized in Table 1. In all samples, the percentage of total identified constituents remained high (99.79% in freshly distilled oil; 99.22%, 99.06%, and 98.99% in aluminium metal, transparent glass, and transparent plastic bottles, respectively), indicating that storage primarily induced compositional changes rather than substantial loss of volatile material.

Table 1

Chemical composition of freshly distilled and one-year stored *Ocimum basilicum* L. (basil) essential oil

Sr. No.	Chemical constituents	% oil			
		Freshly distilled	Stored oil for one year		
Aluminium metal bottle	Transparent glass bottle	Transparent plastic bottle			
1.	α - Pinene	0.52	0.09	0.55	-
2.	Farnesol	2.71	1.10	0.80	1.18
3.	Eucalyptol	1.06	0.17	0.12	0.13
4.	Linalyl formate	3.79	12.44	11.53	12.11
5.	Estragole	62.66	79.93	82.13	81.65
6.	Citral	1.55	1.09	0.98	1.00
7.	Benzyl isobutyrate	1.09	0.67	0.59	0.60
8.	3- Carene	1.5	0.12	-	-
9.	Limonene	0.22	-	-	-
10.	Linalol oxide	0.22	-	-	-
11.	Linalyl butyrate	21.12	0.27	0.22	0.21
12.	Geraniol	0.10	-	-	-
13.	Menthone	0.13	-	-	-
14.	L- Menthol	0.82	0.22	-	0.15
15.	Myrcene	0.22	-	-	-
16.	Citronellol	1.18	0.88	-	-
17.	Valencene	0.69	0.36	0.26	0.16
18.	Methyl Cinnamate	0.18	-	0.06	-
19.	Camphene	0.25	-	-	-
20.	Bisabolene	-	1.80	1.82	1.80
Total identified (%)		99.79	99.22	99.06	98.99

Table 1 and Fig. 2(A) showed total 19 chemical components in freshly distilled oil which was characterized by a predominance of estragole also known as methyl chavicol (Fig-1) (62.66%), followed by linalyl butyrate (21.12%) and linalyl formate (3.79%), confirming an estragole-rich chemotype. After one year of storage, significant quantitative variations were observed. Total 13 chemical components

with a great peak of estragole content increased by 79.93% in aluminium metal bottle (from Table 1 and Fig. 2B), total 11 chemical components with a great peak of estragol content increased by 82.13% in transparent glass bottle (from Table 1 and Fig. 2C) and total 10 chemical components with a great peak of estragole content increased by 81.65% in transparent plastic bottle (from Table 1 and Fig. 2D). Estragole content increased considerably in all stored samples in compared to freshly distilled basil oil. This relative increase can be attributed to the degradation or transformation of other constituents, leading to proportional enrichment of the major compound.

A substantial reduction in linalyl butyrate was evident, decreasing from 21.12% in the fresh oil to trace levels (0.27%, 0.22%, and 0.21%) in aluminium metal, transparent glass, and transparent plastic bottles, respectively. Such decline suggests instability of ester compounds during prolonged storage, probably due to hydrolysis or oxidative processes. On the other hand, linalyl formate showed a significant rise (about 11–12%), suggesting that esterified terpenoids may interconvert or have varying levels of stability. After being stored, a number of minor monoterpenes and oxygenated compounds, such as α -pinene, 3-carene, limonene, myrcene, geraniol, menthone, and camphene, were either greatly decreased or disappeared, which is in line with their high volatility and decay susceptibility. In contrast, bisabolene, which was not present in freshly distilled oil, was present in all kept samples (~ 1.8%), indicating subsequent transformation reactions.

Variations between storage bottles were also detected. Major components were somewhat better preserved in glass bottle, but some minor components were lost more readily in transparent plastic bottle. Overall, the results demonstrate that long-term storage significantly modifies the chemical profile of *O. basilicum* essential oil, which may consequently affect its functional properties.

3.2. Antioxidant activity of *Ocimum basilicum* L. (basil) essential oil was evaluated by DPPH* assay

From Table 2 and Fig. 2, the antioxidant activity of *Ocimum basilicum* L. essential oil was evaluated at different concentrations for freshly distilled oil (Sample A) and oil stored for one year in an aluminium metal bottle (Sample B), with gallic acid used as the standard reference.

The freshly distilled essential oil (Sample A) exhibited consistently high antioxidant activity across all measured concentrations even more than standard gallic acid. The percentage inhibition ranged from 97.61% at the highest concentration (2.0) to 91.26% at the lowest concentration (0.125). As the concentration decreased, there was only a slight reduction in activity, suggesting that the freshly distilled oil has a major ability to scavenge radicals even at reduced concentrations. This behavior indicates the presence of stable and potent antioxidant components within the essential oil.

Table 2

Variations in % antioxidant activity of *Ocimum basilicum* L. (basil) essential oil for sample (A) freshly distilled and sample (B) one year stored in aluminium metal bottle with standard gallic acid

Sr. No.	Conc. (mg/10 mL)	% Antioxidant activity		
		Activity (%) – A (Fresh)	Activity (%) – B (Stored, 1 year)	Gallic Acid (%)
1	2.0	97.61	47.62	94.78
2	1.5	96.03	34.92	93.35
3	1.0	95.53	34.12	92.20
4	0.5	94.44	11.90	90
5	0.25	92.06	9.52	88.50
6	0.125	91.26	0.79	87.82

On the other hand, the stored essential oil (Sample B) showed considerably lower antioxidant activity. The percentage of inhibition was 47.62% at the maximum concentration (2.0), which is significantly less than freshly distilled oil (Sample A). As concentration dropped, activity sharply decreased and reached 0.79% at 0.125 concentration, suggesting an important impact loss of antioxidant activity after storage. The decline strongly indicates that bioactive substances were degraded or changed during storage.

Gallic acid which was used as the positive control, showed high antioxidant activity throughout the concentration range (94.78%–87.82%), confirming the reliability of the assay. When compared to the stored oil sample, the standard values stayed comparatively constant.

It is evident from the comparative investigation that storage significantly affects antioxidant activity. Freshly distilled oil performs effectively because important antioxidant molecules are preserved, while prolonged storage probably caused the active ingredients to oxidize, volatilize, or undergo chemical changes. It is well known that essential oils are highly sensitive to environmental impacts like light, air, and container interactions, which may explain the observed reduction in one year stored oil in aluminium metal bottle.

From Fig. 3, it can be easily understood that freshly distilled basil essential oil has highest antioxidant activity than one year stored basil essential oil and even standard gallic acid at all concentrations. One year stored basil essential oil has least antioxidant activity across all concentrations.

4. Conclusion

The current study showed that the chemical components and antioxidant activity of *Ocimum basilicum* L. essential oil are significantly affected by storage conditions. The estragole-rich chemotype of basil essential oil was confirmed by GC-MS analysis, which reported that the freshly distilled oil showed a

greater number of chemical constituents (19 compounds), with estragole (62.66%), linalyl butyrate (21.12%), and linalyl formate (3.79%) as the major components. The number of identified constituents reduced and significant quantitative changes were seen after one year of storage in aluminum metal, transparent glass, and transparent plastic bottles. While a number of minor monoterpenes and ester compounds, including linalyl butyrate, limonene, myrcene, and camphene, decreased or disappeared completely, suggesting degradation, oxidation, or transformation processes during storage, the relative percentage of estragole increased in all stored samples. The appearance of bisabolene in stored samples further suggests the occurrence of secondary chemical changes over time.

The antioxidant activity evaluated by the DPPH radical scavenging assay showed that the freshly distilled essential oil possessed very strong antioxidant activity, even comparable to or higher than the standard gallic acid at all tested concentrations. On the other hand, the oil that was kept in an aluminum metal bottle for one year showed a significant reduction in antioxidant activity, with inhibition values decreasing rapidly as concentration decreased. This decrease clearly indicates that prolonged storage leads to the degradation of bioactive compounds responsible for radical scavenging activity.

Overall, the study suggests that prolonged storage adversely impacts *Ocimum basilicum* essential oil's chemical stability and biological activity. The highest antioxidant activity of freshly distilled oil is retained, but storage for one-year results in compositional changes and significant reduction of activity.

Declarations

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Author Contribution

Ashok Kumar Ranjan and vimal kumar wrote the main manuscript text and Sunil yadav and Pratima Bharti prepared figures and aswani did analysis of antioxidant activity.

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Figures

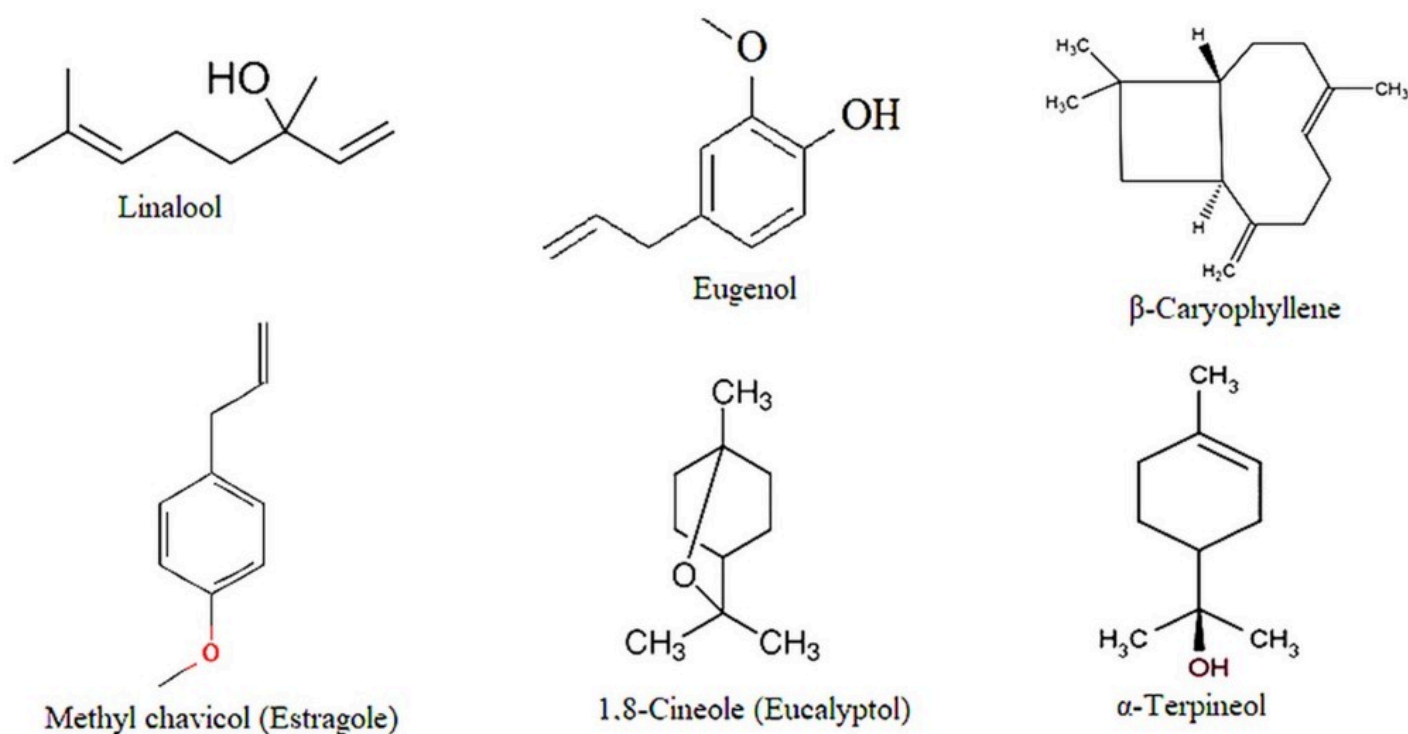


Figure 1

Structures of major components of basil essential oil

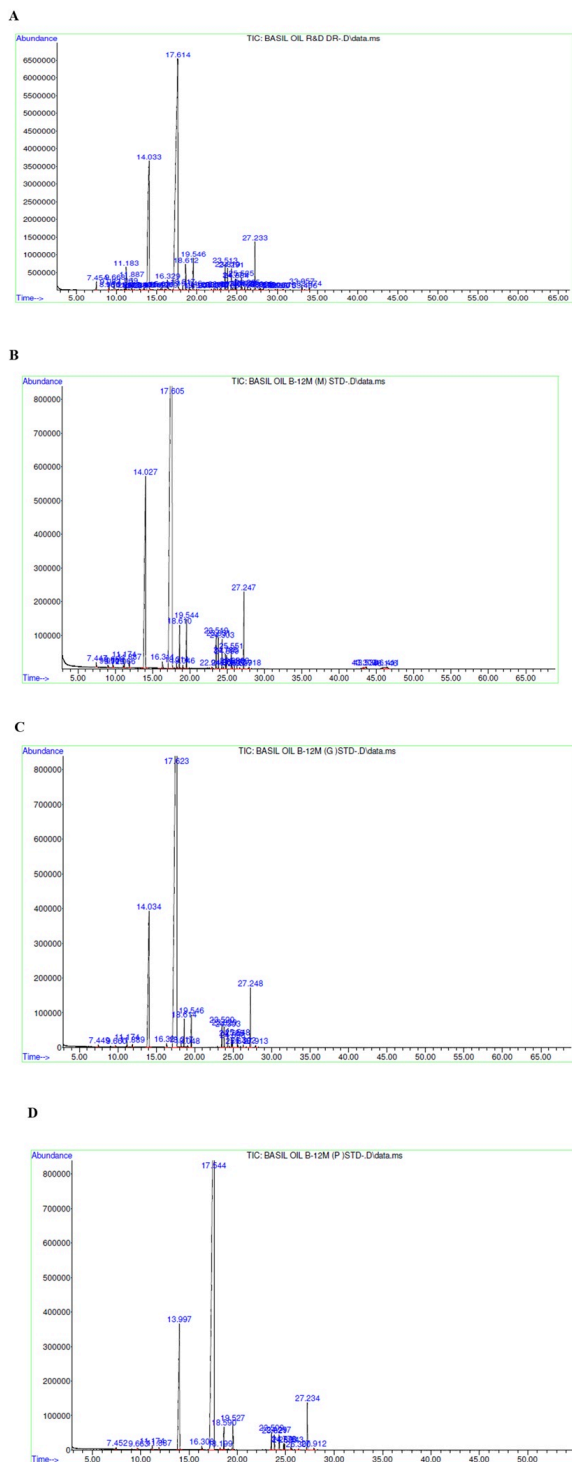


Figure 2

Essential oil chromatograms of *Ocimum basilicum* L. for (A) freshly distilled, (B) Stored in metal bottle, (C) Stored in glass bottle, and (D) Stored in plastic bottle

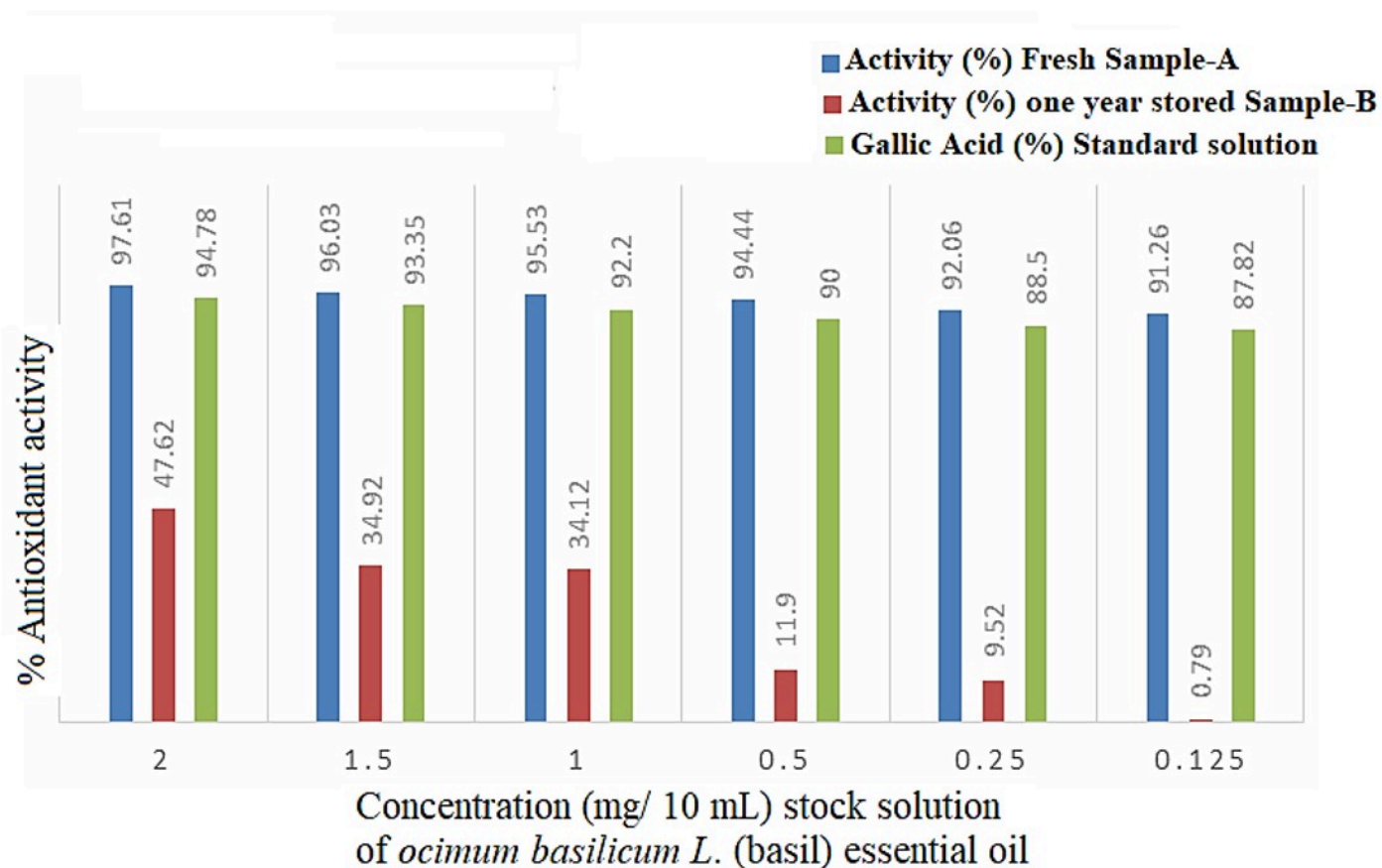


Figure 3

Freshly distilled *Ocimum basilicum* L. (basil) essential oil and stored in aluminium metal bottle with standard gallic acid at different concentrations