

Original article

## Antioxidant Activity of Natural Chamomile and Commercial Chamomile in Libya: A Comparative Study

Ashraf Alapid<sup>1\*</sup>, Mohamed Alshintari<sup>2,3</sup>, Asma Alforjani<sup>3</sup>, Ritaj Algriani<sup>3</sup>, Roa Albosefi<sup>3</sup>, Maism Yahmid<sup>3</sup><sup>1</sup>Department of Zoology, Faculty of Science-Alasaba, University of Gharyan, Libya.<sup>2</sup>Libyan Biotechnology Research Center, Tripoli, Libya.<sup>3</sup>Department of Pharmaceutical Science, Tripoli College of Medicinal Science, Tripoli, Libya.**Corresponding Email.** [Asalapid82@gmail.com](mailto:Asalapid82@gmail.com)

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Chamomile (*Matricaria chamomilla* L.) is a medical plant known for its antioxidant properties, which are attributed to bioactive compounds such as flavonoids and terpenoids. The study aimed to compare the antioxidant efficacy of natural chamomile with three commercial brands, A (Italian chamomile (Sonny)), B (Italian chamomile (Restora)), and C (German chamomile (UTZ)) available in Libyan markets. Methanol extracts of all samples were prepared by maceration, and antioxidant activity was evaluated using the DPPH radical scavenging assay. All data were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using one-way ANOVA, with a p-value  $< 0.05$ , which was considered statistically significant. Results revealed significant differences in IC<sub>50</sub> values, with natural chamomile exhibiting the highest potency (IC<sub>50</sub> = 3.35 mg/ml  $\pm$  0.055), followed by Brand A (IC<sub>50</sub> = 3.56 mg/ml  $\pm$  0.04) and Brand C (3.88 mg/ml  $\pm$  0.21). Brand B showed the weakest activity (IC<sub>50</sub> = 5.01 mg/ml  $\pm$  0.04). The statistical analysis confirmed the superiority of natural chamomile over commercial variants. Our findings suggest that processing methods, storage conditions, or potential additives in commercial products may degrade bioactive compounds, reducing antioxidant efficacy. This study highlights the advantage of minimally processed natural chamomile for optimal antioxidant benefits, and it underscores the implications for consumer choice and quality control in the manufacturing of herbal products.

### Introduction

Medicinal plants are considered beneficial for health because they contain bioactive phytochemical compounds responsible for their therapeutic properties (1,2). These plants are highly used to treat many diseases, such as diabetes, hypertension, and cancers, especially in developing countries, as traditional medicines (3,4). One of these plants is the chamomile plant. The chamomile plant, scientifically known as *Matricaria chamomilla* L., belongs to the Asteraceae (*Compositae*) family and is a small, herbaceous annual species distinguished by its bloom. Chamomile has been extensively investigated for its potential therapeutic properties. Its primary medicinal applications stem from its anti-inflammatory, antioxidant, and antimicrobial activities (5,6). Traditional uses include insomnia and the treatment of gastrointestinal disorders (7). Furthermore, chamomile can improve cardiovascular conditions and stimulate the immune system (8). Also, its extracts have effectively reduced anxiety symptoms in some clinical trials (9). The therapeutic effects of chamomile are attributed mainly to its rich phytochemical profile. There are two major classes of bioactive compounds in chamomile, which include the hydrophobic group, such as terpenoids and azulenes, and the second group is hydrophilic, such as flavonoid glycosides and lower amounts of free aglycones (10,11). The essential bioactive phytochemical compounds in the chamomile extract are 36 flavonoids like apigenin, luteolin, and quercetin, followed by 28 terpenoids. In addition, approximately 120 secondary metabolites have been identified in chamomile essential oil (8,12,13).

These phytochemicals' specific composition and concentration can vary depending on the species, growing conditions, extraction methods, and environmental factors such as seasonal variation and water availability, impacting the overall biological activity (14). Libya is characterised by having a good geographical position along the Mediterranean coast. The climate of this area is marked by cold and wet winters and hot and dry summers. This climate allows it to have an interesting diversity of medical plants (15). As mentioned, chamomile has many medicinal properties. In Libya, chamomile flowers are a crude drug, and chamomile tea bags can be found in markets and retail pharmacies.

Furthermore, most people prefer using commercial chamomile to natural or fresh chamomile. However, is the quality or the benefit of chamomile flowers better in pharmacies, or is it similar to the quality at markets? Due to that, the study was conducted to elucidate the contents of biochemical compounds such as

flavonoids, screen their medicinal properties, such as the antioxidant effects of commercial chamomile, and compare them with natural chamomile by using different extraction methods.

## Materials and methods

### Sample collection

The natural chamomile plant used in this study was obtained from a herbal medicine shop in Libya. In contrast, many types of commercial dried chamomile, which are Brand A (Italian chamomile (Sonny)), Brand B (Italian chamomile (Restora)), and Brand C (German chamomile (UTZ)), have been used. These were purchased from local pharmacies.

### Chamomile Extract Preparation

According to the previously reported procedure, the chamomile extracts were prepared from all samples (16). This procedure involved grinding dried plant material using a laboratory milling machine and sieving through a standardised mesh sieve ( $\leq 1$  mm particle size) to ensure uniformity and remove coarse particulates. For solvent extraction, 27 g of each powdered sample underwent the maceration extraction technique in 100 mL of analytical grade methanol (purity  $\geq 99\%$ ) at room temperature for 72 hours. Methanol, a polar solvent, was chosen for its effectiveness in extracting a wide range of phytochemicals, including phenolic acids and flavonoids. Following maceration, the mixtures were filtered using Whatman No. 1 filter paper to isolate the marc (insoluble residue) from the methanolic extract. The filtrate was then concentrated under reduced pressure (40–60 mbar) at 40°C using a rotary evaporator (Buchi Rotavapor R-300). The resulting crude extracts were stored at  $-20^{\circ}\text{C}$  for later use.

### Yield calculation for chamomile extracts

The percentage yield of all extracts of chamomile was calculated using the following formula:

$$\text{Yield (\%)} = \text{Mass of Extract Obtained (g)} \div \text{Mass of Dry Starting Material (g)} \times 100$$

### Antioxidant Activity Assay (DPPH)

The antioxidant capacity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Different concentrations (0.25–1.00 mg/ml) of each extract were mixed with 0.1 mM DPPH solution in methanol. The mixture was incubated in the dark for 30 minutes, and the absorbance was measured at 517 nm. Ascorbic acid was used as the positive control. The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\text{Scavenging Activity (\%)} = (\text{A control} - \text{A sample} / \text{A control}) \times 100$$

### Statistical Analysis

All experiments were conducted in triplicate, and data are expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using one-way ANOVA, followed by Tukey's post-hoc test to compare group differences. A p-value  $< 0.05$  was considered statistically significant.

## Results

### Extraction Yield of Chamomile Samples

The extraction yields of all chamomile samples varied across commercial and natural sources. As shown in the table.1 The natural chamomile yielded 9.26%, while the German chamomile (UTZ) commercial yielded 7.75%, whereas the Italian chamomile brands (Restora and Sonny) yielded 10%. Based on these results, the Italian commercial samples exhibited slightly higher yields than the natural and German variants.

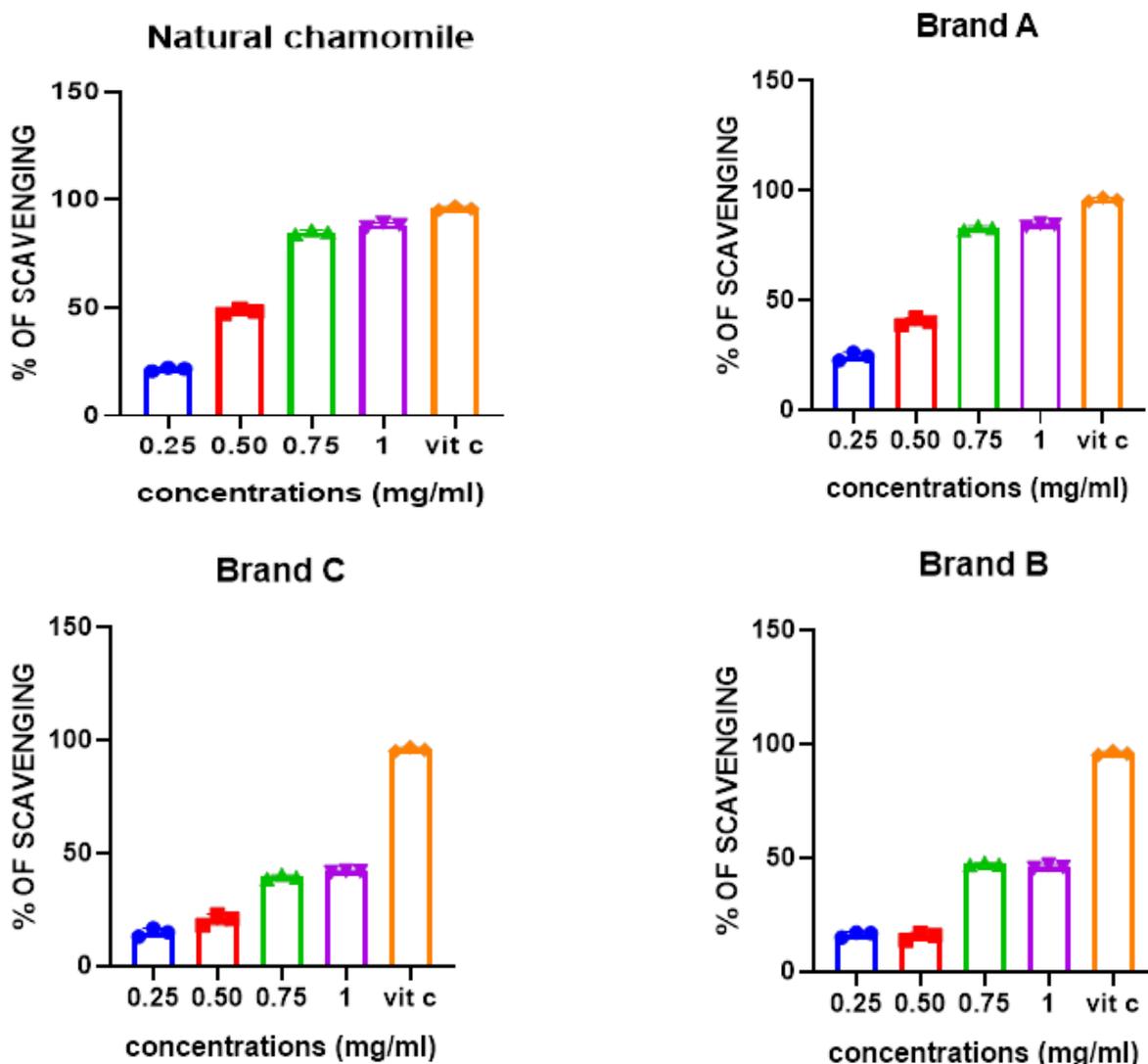
**Table 1. The extraction yield of the chamomile samples**

Chamomile Samples	% of yield
Italian chamomile (Sonny) Brand A	10%
Italian chamomile (Restora) Brand B	10%
German chamomile (UTZ) Brand C	7.75%
Natural chamomile	9.26%

### Antioxidant Activity of Chamomile Extracts

The results of the antioxidant activity of all chamomile samples are illustrated in Figure 1. The DPPH radical scavenging assay revealed significant differences in antioxidant efficiency. Natural chamomile demonstrated the lowest  $\text{IC}_{50}$  value ( $3.35 \text{ mg/ml} \pm 0.055$ ) compared to all samples Figure 1 A. Indicating the highest antioxidant potency of natural chamomile, followed by the chamomile Brand A, showed moderate activity  $\text{IC}_{50}$  ( $3.56 \text{ mg/ml} \pm 0.04$ ), Figure 1 B. After that, chamomile Brand C show high activity ( $\text{IC}_{50}$ :  $3.88 \pm 0.21$ )

Figure 1 C. In contrast, chamomile Brand B demonstrated the highest  $IC_{50}$  value ( $5.01 \text{ mg/ml} \pm 0.04$ ) Figure 1 D., indicating the lowest antioxidant efficacy compared to other samples. The results showed that significant differences between the natural and commercial groups were found by statistical analysis (one-way ANOVA,  $p < 0.05$ ), highlighting the greater antioxidant activity of natural chamomile compared to the commercial.



**Figure 1.** The comparative bar graphs of the  $IC_{50}$  values (inhibitory concentration) for different chamomile extracts. (A) represents neutral chamomile  $IC_{50}$ :  $3.35 \pm 0.05$  (B) represents brand A  $IC_{50}$ :  $3.56 \pm 0.04$ , (C) represents brand C  $IC_{50}$ :  $5.01 \pm 0.04$ , (D) represents brand B  $IC_{50}$ :  $3.88 \pm 0.21$ . Data are expressed as mean  $\pm$  standard deviation (SD).

## Discussion

The main aim of this study was to compare the antioxidant effect between natural chamomile and different brands of chamomile bags collected from the Libyan market. To achieve this aim, all samples were macerated in methanol to extract the active compounds, which have potential antioxidant effects. The data presented in Table 1 shows the extraction yield of different chamomile samples. There is no significant difference in the extraction yield. The extraction yield (percentage of extracted material relative to the starting dry weight) is an important factor in assessing the efficiency of the extraction process and the potential concentration of bioactive compounds (17–19). The close values of % of yield could be because all sample extraction procedures are similar (20).

Chamomile (*Matricaria chamomilla* L.) is widely known for its effective antioxidant properties, primarily attributed to its rich content of phenolic compounds, flavonoids (e.g., apigenin, quercetin), and terpenoids. These bioactive components efficiently scavenge reactive oxygen species (ROS) and inhibit oxidative stress, which is linked to chronic diseases such as inflammation, cancer, and neurodegenerative disorders

(2,21,22). The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method to estimate antioxidant capacity by measuring the ability of compounds to scavenge free radicals. A lower IC<sub>50</sub> value indicates higher antioxidant activity, as less extract is needed to neutralise 50% of DPPH radicals. The study's results (Figure 1) showed that the highest antioxidant effect was from natural chamomile (IC<sub>50</sub> = 3.35 mg/ml). This value suggests that the minimal processing preserves bioactive compounds (e.g., flavonoids, terpenoids) responsible for radical scavenging. Commercial Brands C (3.88 mg/ml) and Brand A (3.56 mg/ml) exhibited adequate and comparable activity, slightly weaker than natural chamomile. Differences may arise from blending with other herbs or additives, which affect the concentration of active compounds. Meanwhile, Brand B (5.01 mg/ml) had the weakest activity, possibly due to longer storage time leading to antioxidant degradation, lower-quality raw materials, or exposure to heat/light during production. For Ascorbic acid (IC<sub>50</sub> = 4.86 mg/ml), unexpectedly, all chamomile samples (Except Brand B) outperformed pure Ascorbic acid in DPPH scavenging. This highlights that chamomile's antioxidants (e.g., apigenin, quercetin) may have synergistic effects, making them more efficient than isolated Ascorbic acid in this assay (23). In more detail, chamomile (*Matricaria chamomilla*) contains bioactive compounds like apigenin and quercetin, which show strong antioxidant effects (24). Research highlights that these polyphenols work synergistically as complex mixtures, potentially outperforming single antioxidants like ascorbic acid in certain contexts (25,26). Generally, natural chamomile may retain a higher quantity of polyphenols, which are key to antioxidant effects. Whereas some commercial brands might contain contaminants or lower-grade chamomile, reducing potency.

## Conclusion

The DPPH radical scavenging assay showed important differences in antioxidant efficiency among chamomile samples. Natural chamomile displayed the highest antioxidant influence, as evidenced by the lowest IC<sub>50</sub> value (3.36 mg/ml ± 0.055), followed by Brand A (IC<sub>50</sub>: 3.56 mg/ml ± 0.04) and Brand C (IC<sub>50</sub>: 3.89 mg/ml ± 0.21). In contrast, Brand B showed the weakest antioxidant activity (IC<sub>50</sub>: 5.02 mg/ml ± 0.04). Statistical analysis using one-way ANOVA confirmed these significant differences between natural and commercial chamomile, highlighting the superior antioxidant capacity of natural chamomile over processed commercial brands. These findings suggest that commercial product processing methods and storage conditions may reduce antioxidant efficiency, emphasising the potential advantages of using fresh or minimally processed chamomile for optimal bioactive benefits.

## Conflicts of Interest

There are no conflicts of interest to declare.

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