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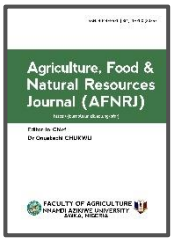

Original Article

Comparative analysis of the phytochemical and physicochemical properties of herbal-infused oils

OPEN ACCESS

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ABSTRACT

Infused oils with plant extracts are well-known for diverse applications, particularly for their therapeutic and cosmetic purposes; herbal oil infusion enhances the bioactive and physicochemical properties of oils, influencing their suitability for cosmetic and dietary applications. This study evaluates the phytochemical and physicochemical properties of herbal infused oils to determine their stability and potential uses. The infused oils were prepared using selected medicinal plants: turmeric, lemon, neem, carrot, and acalypha, with olive oil as the carrier. Phytochemical screening was conducted to identify bioactive compounds, while physicochemical parameters such as acid value (AV), saponification value (SV), iodine value (IV), peroxide value (PV), and ester value (EV) were assessed following standard analytical procedures. Phytochemical screening revealed terpenoids in all infused oils, while alkaloids, anthraquinones, and steroids were present in specific samples. Tannins, flavonoids, glycosides, and phenols were not detected. Physicochemical analysis showed significant ($p<0.05$) alterations in oil properties after herbal infusion. AV ranged from 0.14–11.56 mg KOH/g; SV: 3.99–234.05 mg KOH/g, IV: 5.50–57.77 g/100g, PV: 0.68–2.15 mEq O_2/Kg, and EV: 3.85–222.49 mg KOH/g. Lemon-infused oil exhibited the highest acid, saponification, iodine, and ester values, suggesting increased hydrolysis and ester formation, while peroxide values remained low across all samples, indicating good oxidative stability. These findings suggest that infused herbal oils exhibit enhanced bioactive properties with varying stability profiles. While some, like lemon-infused oil, may require refinement before ingestion, others closely resemble the carrier oil, making them suitable for both cosmetic and dietary applications.

K E Y W O R D S : *Cosmetics, Herbal oils, Oxidative stability, Quality*

INTRODUCTION

Herbal oils have been integral to traditional medicine and cosmetics for millennia (Orchard & van Vuuren, 2019; Vartak *et al*., 2022; Panda *et al*., 2022). Derived from various plant parts, these oils are rich in bioactive compounds such as flavonoids, terpenoids, alkaloids, and tannins that exhibit antimicrobial, anti-inflammatory, and antioxidant properties (Diniz do Nascimento *et al*., 2020; Mohamed and Alotaibi, 2023; Mkaddem, 2024; Qneibi *et al*., 2024). Their therapeutic potential and minimal adverse effects have increased interest in natural alternatives amid concerns over synthetic chemicals in modern cosmetics (Hongratanaworakit *et al*., 2018; Michalak, 2018; Hoang *et al*., 2021; Chandrayan and Hood, 2024).

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In cosmetics, herbal oils offer emollient benefits that protect and moisturize the skin while promoting regeneration and UV defence (Lin *et al*., 2018; Alhasso *et al*., 2022; Ogorzałek *et al*., 2024). Recent reports have indicated the efficacy of plant-based preservatives and bioactive oils in enhancing skin health and improving the stability of cosmetic formulations (Michalak, 2018; Hoang *et al*., 2021; Chandrayan & Hood, 2024). Unlike highly concentrated essential oils, infused herbal oils are safe for direct application (Aichinger & Buchbauer, 2020; Srivastava & Rai, 2023). Their biological activity is attributed to the presence of phytochemicals – bioactive, non-nutritive compounds with protective or disease-preventive characteristics (Mohamed and Alotaibi, 2023; Qneibi *et al*., 2024). Studies have shown that herbal oils exhibit potent antimicrobial and anti-inflammatory activities, making them suitable for therapeutic applications (Aichinger & Buchbauer, 2020; El Omari *et al*., 2022; de Sousa *et al*., 2023). Additionally, these oils have been found to possess antioxidant properties, which help in combating oxidative stress, a key factor in aging and various skin disorders (Tit & Bungau, 2023).

The quality and stability of these oils are governed by physicochemical parameters such as colour, odour, density, and saponification value, which also influence sensory attributes and bioactivity (Michalak, 2018; Barret, 2018; Ahmad & Ahsan, 2020; Yadav, 2022).

Herbal oil infusion involves macerating dried plant material in carrier oils and can be performed via cold-press or hot press methods – the latter enhancing extraction efficiency and reducing processing time (Michalak, 2018; Fakhfakh *et al*., 2019; Krakowska-Sieprawska *et al*., 2022; Masoodi *et al*., 2022). This study aims to prepare herbal-infused oils by infusing selected plant materials into a carrier oil and evaluating their physicochemical and phytochemical properties for cosmetic and dietary applications.

MATERIALS AND METHOD

Sample Collection and Preparation

Five different types of plant materials were selected for this experiment: *Daucus carota* (whole carrot), *Citrus limon* (lemon peel), *Azadirachta indica* (neem leaves), *Acalypha wilkesiana* (Copperleaf leaves), and *Curcuma longa* (turmeric rhizomes). The oils were extracted using the hot-press infusion method with a double boiler. In this process, the plant materials were grated/pulverized and immersed in a neutral carrier oil (virgin olive oil), which served as the solvent for extraction. The mixture was gently heated over several hours to facilitate the release of the plant's bioactive compounds into the carrier oil. Following the infusion period, the mixture was strained to separate the plant residue from the infused oil (Zhang *et al*., 2018). The resulting infused oils from carrot, neem, turmeric, *Acalypha wilkesiana*, and lemon were subjected to phytochemical screening and physicochemical analysis to evaluate their bioactive content and physicochemical properties.

Preliminary qualitative phytochemical screening

The infused herbal oils were qualitatively screened for phytochemicals using standard procedures, with results compared against the neutral carrier oil (Sofowora, 1993; Harborne, 1998; Trease & Evans, 2002). Saponins were identified using the frothing test, where persistent froth indicates a positive result. Tannins were detected via the ferric chloride test, characterized by the appearance of a blue-black or greenish-black coloration. Flavonoids were screened using the alkaline reagent test, in which the formation of an intense yellow colour upon adding drops of sodium hydroxide (NaOH) confirms their presence. The Keller-Killiani test was employed to screen for glycosides, with a reddish-brown ring at the interface indicating a positive result. Anthraquinones were detected using the Borntrager's test, where the formation of a pink, red, or violet colour in the ammoniacal layer confirms their presence. The Salkowski test was used to detect terpenoids, with a reddish-brown coloration at the interface signifying a positive result. For steroids, the Liebermann-Burchard test was employed, where the appearance of a blue, green, or bluish-green coloration indicates their presence. The Ferric chloride test was used to screen for phenols, with the formation of a deep blue, green, or purple colour confirming their presence. Lastly, alkaloids were detected using both Mayer's and Dragendorff's tests, where the formation of a creamy precipitate or orange-red precipitate, respectively, indicates a positive result.

Physicochemical Characteristics of Oil

Physicochemical characteristics provide a base line for suitability of oils. The physicochemical properties of the oil (Acid value (AV), Saponification value (SV), Iodine value (IV), Peroxide value (PV) and Ester value (EV)) were determined according to the methods described by the American Oil Chemists' Society (AOCS, 1993).

Determination of Acid Value: The acid value of the herbal infused oils was determined by weighing 2 g of each oil sample into a clean, dry conical flask. To the flask, 25 mL of neutral alcohol (ethanol) was added to dissolve the oil. A few drops of phenolphthalein indicator were then introduced to the solution. The mixture was titrated with a 0.1 M potassium hydroxide (KOH) solution until a pink endpoint was achieved, indicating complete neutralization of the free fatty acids present in the oil. A blank titration was carried out under the same conditions to eliminate any interference from the solvent.

The acid value was calculated using the formula:

$$Acid\ Value = \frac{V \times N \times 56.1}{Weight\ of\ Oil\ (g)} \qquad (1)$$

Where: V = volume (mL) of KOH used in the titration; N = normality of KOH solution (O.1 M).

Determination of Saponification Value: 2 g of each herbal infused oil sample was weighed into a clean, dry conical flask. To the flask, 25 mL of alcoholic potassium hydroxide (KOH) was added, and the mixture was refluxed for 1 hour with periodic shaking until a clear solution was obtained. After cooling, 1 mL of 1% phenolphthalein indicator was added. The excess alkali was titrated with 0.5 M hydrochloric acid (HCl) until a colour change from pink to colourless was observed. A blank titration was performed under identical conditions. The saponification value was calculated using the formula:

$$Saponification\ Value = \frac{(B-S)\times N\times 56.1}{Weight\ of\ Oil\ (g)} \quad (2)$$

Where: B = volume (mL) of HCl in blank; S = volume of HCl in sample; N = normality of HCl (0.5 M).

Determination of Iodine Value: The iodine value indicates the degree of unsaturation in oils. 0.2 g of each oil sample was mixed with 10 mL of carbon tetrachloride and 20 mL of Wijs iodine solution, and the mixture was kept in the dark for 30 minutes at room temperature. After the reaction, 15 mL of 10 % potassium iodide solution and 100 mL of distilled water were added. The excess iodine was titrated with 0.1 M sodium thiosulfate ($Na_2S_2O_3$) using starch as an indicator until the blue-black colour disappeared. A blank titration was performed simultaneously. The iodine value was calculated using the formula:

$$Iodine\ Value = \frac{V_1-V_2\times C\times 12.69}{Weight\ of\ Oil\ (g)} \quad (3)$$

Where: V_1 = volume (mL) of thiosulphate used in the blank; V_2 = volume (mL) of thiosulphate used in the sample; C = concentration of sodium thiosulphate (0.1 M).

Determination of Peroxide Value: The peroxide value was determined by weighing 2 g of each oil sample into a conical flask, then, 30 mL of acetic acid and chloroform (3:2 v/v) solution was added. A few drops of potassium iodide solution (KI) were introduced, and the mixture was shaken. The flask was then titrated with 0.1 M sodium thiosulfate ($N_{a2}S_2O_3$) solution using starch as an indicator until the yellow colour disappeared. A blank titration was performed under the same conditions. The peroxide value was calculated using the formula:

$$Peroxide\ Value = \frac{V\times N\times 1000}{Weight\ of\ Oil\ (g)} \quad (4)$$

Where: V= volume (mL) of thiosulphate used in the sample; N = normality of sodium thiosulphate solution (0.1 M).

Determination of Ester Value: The ester value is determined by subtracting the acid value from the saponification value. This is the number of mg of KOH required to neutralize the fatty acid obtained solely by hydrolysis of the glycerides contained in 1g of the substance. It reflects the amount of ester present in the oil. The ester value is calculated using the following formula:

$$Ester\ Value = Saponification\ Value - Acid\ Value \quad (5)$$

Data Analysis

The mean values of the physicochemical analysis were presented, data was subjected to a one-way analysis of variance test and Duncan post hoc at $p<0.05$ level of significance.

RESULTS AND DISCUSSION

The phytochemical screening (Table 1) reveals clear differences between the infused herbal oils and the olive oil carrier. For example, turmeric-infused oil contained saponins, anthraquinones, terpenoids, and alkaloids, whereas lemon and carrot contributed anthraquinones and alkaloids; neem provided only alkaloids, and Acalypha added anthraquinones, steroids, and alkaloids to the carrier oil respectively. Terpenoids were consistently present across all samples, suggesting that the base oil may inherently contain these compounds, which are further enhanced by the infusion process.

Terpenoids are well-known for their anti-inflammatory, antimicrobial, and antioxidant properties, and they hold significant economic and cosmetic application value (Câmara *et al*., 2024). The presence of alkaloids in the infused oils indicates an enrichment of bioactive properties, potentially enhancing therapeutic effects such as pain relief and infection prevention. Additionally, the detection of anthraquinones and saponins in certain oils supports reports of anti-malarial and antimicrobial activities, thereby broadening their applicability for both cosmetic and ingestible products.

Conversely, tannins, flavonoids, glycosides, and phenols were not detected in any sample, including the carrier oil. Their absence may be due to their water-soluble nature and possible degradation during heat or prolonged infusion, underscoring the need for process optimization based on the desired phytochemical profile.

Table 1: Result of the Phytochemical Analysis of the Infused Herbal Oils

Test	ON	RT	ME	MIC	LYP	SP
Saponins	-	-	-	+	-	-
Tannins	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-
Anthraquinones	+	+	-	+	+	-
Terpenoids	+	+	+	+	+	+
Steroids	-	-	-	-	+	-
Phenols	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	-

Key: ON: Lemon oil, RT: Carrot oil, ME: Neem oil, MIC: Turmeric oil, LYP: Acalypha oil, SP: Carrier oil; +: Detected, -: Not detected.

The physicochemical parameters of the sample oils were measured to assess their current condition and quality. As summarized in Table 2, the physicochemical properties of the

infused herbal oils exhibited significant variations across the samples. The acid value ranged from 0.14 mg KOH/g in turmeric-infused oil to 11.56 mg KOH/g in lemon-infused oil. The saponification value ranged from 3.99 mg KOH/g in turmeric oil to 234.05 mg KOH/g in lemon oil. Similarly, the iodine value ranged from 5.50 g/100g in turmeric oil to 57.77 g/100g in lemon oil. The peroxide value varied from 0.68 mEq O_2/Kg in carrot-infused oil to 2.15 mEq O_2/Kg in turmeric-infused oil. The ester value ranged from 3.85 mg KOH/g in turmeric-infused oil to 222.49 mg KOH/g in lemon-infused oil. These results indicate that the infusion of herbal materials significantly altered the physicochemical properties of the oils ($p > 0.05$).

Table 2: Physicochemical Properties of Infused Herbal Oils

Test	MIC	RT	ON	ME	LYP	SP
Acid Value (mg KOH/g oil)	0.14^{a}	0.36^{a}	11.56^{b}	0.44^{a}	0.66^{a}	0.19^{a}
Saponification (mg KOH/g oil)	3.99^{a}	18.6^{c}	234.05^{d}	11.3^{b}	11.3^{b}	12.63^{b}
Iodine Value (g/100g oil)	5.50^{a}	10.78^{a}	57.77^{b}	10.82^{a}	51.56^{b}	9.54^{a}
Peroxide Value (mEq O_2/Kg)	2.15^{c}	0.68^{a}	1.47^{bc}	1.47^{bc}	1.17^{b}	1.17^{b}
Ester Value (mg KOH/g oil)	3.85^{a}	18.24^{c}	222.49^{d}	10.86^{b}	10.64^{b}	12.44^{b}

Key: MIC: Turmeric oil, RT: Carrot oil, ON: Lemon oil, ME: Neem oil, LYP: Acalypha oil, SP: Carrier oil. Values with same alphabet in the same row are not significantly different ($p<0.05$)

The physicochemical parameters provide insights into the freshness, quality, and potential applications of the oils. The acid value (AV) reflects free fatty acid content and freshness, with a permissible limit of 0.6 mg KOH/g oil. In this study, turmeric, carrot, neem, and Acalypha infused oils exhibited AVs similar to the carrier oil and within this limit, whereas the lemon-infused oil showed a significantly elevated AV ($p > 0.05$), suggesting greater hydrolysis or a higher inherent free fatty acid content. Elevated AV may reduce suitability for consumption but favour industrial uses such as in paints, soaps, and shampoos (Aremu *et al*., 2006).

The saponification value (SV) measures oxidation and deterioration, with higher values indicating increased volatility. Lemon infusion significantly increased the SV, which can be advantageous for soap making but might necessitate further refinement for safe ingestion and could influence the sensory attributes of cosmetic formulations. Similarly, the ester value was exceptionally high in the lemon-infused oil (222.49 mg KOH/g oil). This suggests that lemon infusion substantially increases esterified fatty acids, potentially enhancing skin absorption in cosmetics, though it may raise concerns about oxidative stability in edible oils (Ahmad & Ahsan, 2020).

Iodine value (IV) quantifies unsaturation and thus the oil's susceptibility to oxidation. While IVs were generally low, lemon and Acalypha oils significantly elevated the IV of the carrier oils, indicating higher levels of essential unsaturated fatty acids. Although nutritionally beneficial, such unsaturation demands the use of antioxidants or appropriate storage to prevent rancidity (Aremu *et al.*, 2006).

Peroxide value (PV) is a key indicator of lipid oxidation; the low PVs observed across the samples suggest minimal oxidative rancidity, which is critical for ensuring both the safety of ingestion and the preservation of cosmetic qualities. The infusion of bioactive compounds is one of several methods available to enhance the oxidative stability of oils (Madhujith & Sivakanthan, 2018; Garg *et al*., 2025).

In summary, these physicochemical measurements collectively demonstrate that the infusion process alters the oil profiles. For cosmetic applications, low AV and PV, along with balanced SV and ester values, are desirable for reducing irritation, extending shelf life, and enhancing skin absorption. For edible oils, maintaining low free fatty acid and oxidation levels is essential for safety and palatability. The distinct profile of the lemon-infused oil characterized by elevated AV, SV, IV, and ester value indicates that, despite its potential bioactive benefits, further processing may be needed before it is suitable for consumption. Conversely, the other herbal oils maintain properties closer to the carrier oil, supporting their dual use in cosmetic and nutritional applications.

Thus, the phytochemical and physicochemical results emphasized the importance of comprehensive quality assessment for herbal oils. Such analyses not only guide the optimal application whether for topical cosmetic formulations or dietary supplements, but also help in predicting shelf life and ensuring consumer safety.

CONCLUSION AND RECOMMENDATION

The study has shown that infusion of herbal extracts into suitable carrier oil significantly modified its phytochemical and physicochemical profiles. Phytochemical screening revealed a diverse range of bioactive compounds including terpenoids, alkaloids, anthraquinones, saponins, and steroids unique to each herbal infusion, while the carrier oil exhibited a more limited profile. Physicochemical analyses demonstrated that, although most infused oils maintained acceptable acid, peroxide, and iodine values, the lemon-infused oil showed markedly elevated acid, saponification, and ester values, indicating greater hydrolysis and unsaturation. These alterations suggest that while herbal infusion can enhance bioactivity and potentially improve cosmetic attributes such as skin absorption and texture, further refinement may be necessary to ensure safety for ingestion. Based on our findings, we recommend further refining the infusion process – particularly for lemon-infused

oil – to optimize its physicochemical properties and enhance its safety for both cosmetic and dietary applications.

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Authors' Contributions

ILS and YOB conceptualized the study, collected the samples and prepared the herbal oil infusion, POO and EAO carried out the chemical analysis of sample, data analysis and wrote the first draft, AAA and EPC managed the literature search and reviewed the manuscript. All authors approved the final manuscript.

Ethical Statement

Not applicable

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