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# *EXPOSE THE BIOACTIVE PROPERTIES OF PICRORHIZA KURROA ROOT EXTRACT OIL (PKEO): PHYTOCHEMICAL COMPOSITION AND THERAPEUTIC ACTIVITIES*

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***ABSTRACT***

**Background:** The present study aims to explore the bioactive properties of essential Oil (PKEO) derived from *Picrorhiza kurroa* (commonly known as kutki), a medicinal plant known for its therapeutic potential. *Picrorhiza kurroa* essential oil has a distinct chemical profile, which sets it apart from other essential oils. The bioactive compounds present in *Picrorhiza kurroa* essential oil may lead to the development of new drugs, particularly for treating inflammatory and oxidative stress-related disorders. The research aims to study the extraction, phytochemical composition, and various biological activities of PKEO. **Methodology:** Oil obtained through hydro-distillation contains various phytochemical compounds, including steroids, triterpenoids, alkaloids, phenols, proteins, flavonoids, and tannins. Its bioactivity and aroma are attributed to its phenolic and sesquiterpene esters. **Results and Discussion:** The total phenolic content is 250.47 µg GAE/g, and the total flavonoid content is 245.26 µg QE/g. UV-visible and IR spectroscopic analyses confirm the presence of phenolic and terpenoid ester functional groups. PKEO has moderate antioxidant activity, with IC50 values of 98.19 µg/mL in DPPH scavenging and 42.72% inhibition in the ABTS assay. It also exhibits dose-dependent inhibition of protein denaturation and HRBC stabilizing activity. Antimicrobial tests show PKEO inhibits E. coli growth, indicating potential antibacterial properties. **Conclusion:** These findings highlight PKEO's promising bioactive profile, suggesting potential therapeutic and cosmetic formulation applications. The antifungal activity also shows the potential antifungal effects of the PKEO.

## *INTRODUCTION*

*Picrorhiza kurroa* (Kutki) is a perennial herbaceous plant native to the Himalayas and known for its numerous therapeutic virtues. It is a perennial herb that belongs to the Plantaginaceae family. The Kutki plant is a popular subject in herbal medicinal research due to its numerous therapeutic characteristics and traditional usage. This perennial herb has been used in many traditional medical systems, particularly Ayurveda, for its hepatoprotective, immunomodulatory, and anti-inflammatory properties [1 – 3]. The plant is high in iridoid glycosides, such as picrosides I and II, which are principally responsible for its therapeutic properties, including treating liver and respiratory

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problems [2]. Research has shown that Kutki has a variety of pharmacological properties. It has been demonstrated to have antioxidant capabilities, which are important in treating oxidative stress-related disorders [4]. Kutki can be prepared in various forms, including phytosome complexes, which improve its solubility and bioavailability, making it more useful as a medicinal agent. Furthermore, the plant has been recognized for its involvement in traditional practices, where it is used as a tonic for anemia and constipation and in folk medicine to treat coughs and fevers [3, 4]. The conservation of Kutki is critical, as it has become endangered due to overharvesting and habitat destruction [2, 3]. Sustainable practices and conservation efforts are necessary to ensure this valuable medicinal resource remains available for future generations. Integrating ethnobotanical knowledge with modern pharmacological research can facilitate the development of new herbal formulations that leverage the therapeutic potential of Kutki while promoting biodiversity conservation [5]. Kutik's multifaceted medicinal properties, combined with its traditional significance and the urgent need for conservation, underscore its importance in herbal medicinal studies. The ongoing research into its phytochemical composition and therapeutic applications continues to reveal its potential as a valuable resource in modern medicine. Kutki (*Picrorhiza kurroa*) is traditionally known for its hepatoprotective, anti-inflammatory, and antioxidant activities, attributed to its bioactive components picrosides I and II and Kutki [6]. Root oil, derived from the plant *Picrorhiza kurroa*, is recognized for its significant antioxidant properties, which are critical in combating oxidative stress and related diseases. *Picrorhiza kurroa*, also known as Kutki or katuka, is a perennial herb used in traditional medicine for centuries. Its pharmaceutical applications are diverse and well-documented. Key bioactive compounds are Picroside I and II, which are Iridoid glycosides with anti-inflammatory, antioxidant, and hepatoprotective properties. Kutkoside: A phenolic glycoside with anti-inflammatory and antioxidant activities. Apolignans: Lignan glycosides have anti-inflammatory, antioxidant, and immunomodulatory effects. The antioxidant activity of kutki root oil can be attributed to its rich phytochemical composition, which includes various bioactive compounds known for their radical scavenging abilities. Research indicates that *Picrorhiza kurroa* exhibits a range of biological activities, including potent antioxidant effects. For instance, a study highlighted that extracts from kutki roots demonstrated substantial antioxidant activity through various assays, including DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assays, which are commonly employed to evaluate the free radical scavenging capacity of plant extracts [7,8,9]. The presence of phenolic compounds and flavonoids in kutki is particularly noteworthy, as these substances are well-documented for their ability to neutralize free radicals, thereby mitigating oxidative damage [7, 8]. The antioxidant potential of *Picrorhiza kurroa* root oil has been compared favorably with other essential oils. For example, essential oils from various plant roots have shown varying antioxidant activity, with some studies indicating that root oils often possess higher antioxidant capabilities than those derived from leaves or stems [10]. This is attributed to the higher concentrations of specific antioxidant compounds, including monoterpenes and sesquiterpenes, which are prevalent in root oils [10]. In the case of *Picrorhiza kurroa*, the oil's composition likely includes these beneficial compounds, enhancing its efficacy as an antioxidant. The anti-inflammatory properties of *Picrorhiza kurroa* are significantly linked to the presence of apocynin. This methoxy-substituted catechol has been shown to inhibit NADPH oxidase activity, thereby reducing the production of reactive oxygen species (ROS) [11,12]. This inhibition is crucial as excessive ROS production contributes to inflammation. Studies have demonstrated that apocynin can effectively mitigate inflammatory responses in various experimental models, including colitis, by modulating the immune response and reducing the levels of pro-inflammatory cytokines [12]. Additionally, the ethanolic and aqueous extracts of kutki have demonstrated the ability to stimulate humoral immune responses, further supporting its role in managing inflammatory conditions [13]. The antibacterial activity of *Picrorhiza kurroa* root oil has been documented against a range of pathogenic bacteria. Research indicates that the extracts significantly inhibit bacteria such as Propionibacterium acnes, which are associated with acne and inflammatory skin conditions [7, 14]. The mechanism of action is believed to involve the disruption of bacterial cell membranes and inhibition of lipase activity, which is essential for bacterial proliferation and inflammation [14]. Furthermore, the broad-spectrum antibacterial properties of kutki may be attributed to its diverse phytochemical constituents that exhibit synergistic effects against bacterial pathogens [7]. In addition to its antibacterial properties, *Picrorhiza kurroa* also exhibits antifungal activity. The root oil has been shown to inhibit the growth of various fungal strains, including those responsible for skin infections

and other mycotic diseases [3, 15]. The antifungal effects are likely due to iridoid glycosides and other phenolic compounds that disrupt fungal cell wall integrity and metabolic processes [3]. The efficacy of kutki in combating fungal infections highlights its potential as a natural therapeutic agent in treating conditions caused by fungal pathogens.

## *MATERIAL AND METHODOLOGY*

### Materials

The root of the *Picrorhiza kurroa* plant was obtained from the local market in the Nashik district of Maharashtra. All the reagents, solvents, and chemicals are analytical grade and obtained from HI media PVT. Ltd and Loba chemicals, which are used without further purification.

### Authentication of raw material

Dr. N.J. Wadkar at Infinite Biotech Sangli identified and authenticated *Picrorhiza kurroa* roots, and the voucher number is 996502.

### Extraction of PKEO

Hydrodistillation is a combination of steam distillation and solvent extraction. The process involves collecting and washing 100gm of freshly collected and thoroughly washed roots in a 500ml round bottom flask, adding water and steam, and boiling for 8 hours at 55°C. The essential oil is extracted from the plant tissue, and the vapor mixture is condensed. The distillate is then separated from the water, and the oil is collected and stored in an amber-colored glass vial/15ml centrifuge tube covered with aluminum foil for use at 2-8ºC.

### Compositional characterization of PKEO

### Phytochemical investigation

The isolated PKEO was confirmed through qualitative testing, identifying secondary metabolites such as flavonoids, glycosides, carbohydrates, saponins, alkaloids, steroids, phenols, terpenoids, starch, proteins, and more. Tests were conducted using various methods, including Keller Killani, Raymond, and Legal tests for glycosides, Wagner's test, Mayer's test, and Hager's reagent for alkaloids, and multiple tests for flavonoids, steroids, phenols, terpenoids, saponins, carbohydrates, proteins, tannins, anthocyanins, starch, and flavanol. The presence of steroids was confirmed using chloroform and Salkowski tests, phenols and terpenoids using the Salkowski and Liebermann-Burchard tests, saponins using foam, hemolysis, and Raymond's assays, carbohydrates using Molisch's, Barfoed's, and Benedict's tests, proteins using Biuret, xanthoproteic, and Millon's assays, tannins using NaOH and Gelatin tests, and anthocyanins using the HCl test.

### Gas chromatography-tandem spectrophotometry-MS (GC–MS/MS) analysis

The analysis used a Shimadzu 17A GC and a QP5050A (quadruple) Mass Spectrometer (Shimadzu, Japan). It was equipped with EI and a fused silica column DB-5 (30 m × 0.25 mm) with a film thickness of 0.25 µm. The oven temperature was set to 50 °C for 5 minutes before gradually increasing to 280 °C for 40 minutes. A 2 ml/min helium flow rate was maintained, with a split ratio 1:30 for a 1 µl sample injection. MS analysis was performed using the EI technique with a 70-eV ionization voltage. The constituents of PKEO were identified by comparing their MS and retention index data to those of typical ethnic spectra and their fragmentation pattern in mass spectra to those of WILEY 139.LIB and NIST 12.LIB. The retention indices were calculated using Kovats' method.

### Total-content analysis

*Phenols.*

The Folin-Ciocalteu method was used to estimate the total phenolic content of PKEO. In brief, 2.5 mL of 10% (w/v) Folin-Ciocalteu reagent was mixed with 1 mL of PKEO (300-900 µg/mL). After five minutes, Na2CO3 (2.0 mL; 75% concentration) was added to the blend, followed by a 10-minute incubation at 50 °C with periodic stirring. After cooling, the absorbance at 765 nm was measured using a UV Spectrophotometer (Shimadzu, UV-1800, Tokyo, Japan). The results were expressed in milligrams per gram (µg GAE/g) of dry material using a gallic acid standard curve (200-1000 µg/mL).

*Flavonoids.*

The study used a colorimetric method to estimate the total flavonoid content in PKEO. The mixture was mixed with $NaNO_2$, distilled water, NaOH, and $AlCl_3.H_2O$ solution. The absorbance was measured at 510 nm using a spectrophotometer. Standard curves of quercetin were prepared and plotted against their respective concentrations. The total flavonoid concentration in PKEO was determined by comparing its absorbance value with the standard curves expressed in quercetin equivalents per gram of extract. This method

effectively quantifies the flavonoid content in PKEO, facilitating its characterization and potential applications in various fields.

**Analytical characterization of PKEO:**

*UV visible spectroscopy*

The UV-visible spectrometer (V-570, Jasco, Japan) with the 200-800 nm scanning range was used to record the UV-visible absorption spectra of PKEO. The extracted PKEO was scanned for a spectral run using the UV 1800 Model of Shimadzu from 800 to 200 nm scanning range for 120 s run time. For this purpose, 2 mL of PKEO in liquid form was kept in the UV-visible cuvette.

*FT-IR spectroscopy*

The FTIR spectrum of PKEO was measured using a Bruker ALPHA II spectrophotometer, which can scan wavelengths from 4000 to 400 cm−1 with a resolution of 2 cm−1. KBr pellets were used as sample holders, and a drop of PKEO was carefully placed on the surface of a KBr pellet to avoid increasing sample thickness. Excess PKEO was removed using a capillary tube to achieve a uniform and thin layer of the sample. The pellets were dried to remove any residual moisture or solvent, ensuring optimal conditions for FTIR analysis. FTIR spectra were recorded for the dried PKEO-loaded KBr pellets, allowing for the identification of characteristic absorption bands corresponding to functional groups present in the PKEO sample. This process ensures optimal conditions for FTIR analysis.

*Zeta potential*

The zeta potential of PKEO was measured using a Horiba Scientific SZ-100 instrument. After extraction, the sample was diluted with DMSO, and electrophoretic mobility measurements were performed at 25°C and 78.5°D. The zeta potential was calculated using Horiba Version 2.40 software.

**Antioxidant activity**

*Antioxidant activity by DPPH radical scavenging assay*

The antioxidant activity of PKEO was assessed using a DPPH free radical scavenging assay. Various concentrations of PKEO were prepared, along with standard ascorbic acid solutions and positive controls of Chlorhexidine gluconate. A 0.1% methanolic DPPH solution was added to each test tube, which was incubated in darkness for 30 minutes. The level of discoloration in each tube was visually evaluated, indicating antioxidant activity. The level of discoloration correlates with PKEO's antioxidant activity, with more discoloration indicating stronger DPPH radical scavenging activity. The antioxidant potential of PKEO was assessed by comparing absorbance values to those of standard solutions and positive controls. Radical scavenging activity was determined using Equation 1

$$Scavenging\ activity\ (\%) = \frac{Absorbance_{control} - Absorbance_{test}}{Absorbance_{control}} \times 100 \quad (1)$$

*ABTS radical scavenging assay*

The antioxidant activity of PKEO was evaluated using a modified ABTS+ radical cation scavenging test. The ABTS radical cation was created by mixing 7 mM 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) with 2.45 mM potassium persulfate (K2S2O8). The ABTS+ solution was mixed with PKEO, ascorbic acid, and a positive control of CHX 0.2%. The reaction mixture was rested for 6 minutes to neutralize the ABTS+ radicals. The absorbance of the reaction mixture was measured spectrophotometrically, indicating PKEO's scavenging efficiency against ABTS radicals. The antioxidant potential was assessed by comparing PKEO's scavenging activity to standard solutions and positive control. The % inhibition was calculated using equation (2).

$$\%\ inhibition = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control} \times 100 \quad (2)$$

In Free Radical Scavenging, the ABTS assay measures a substance's ability to scavenge free radicals, specifically the ABTS radical cation (ABTS+). ABTS is oxidized to form a stable radical cation, which is chromogenic, meaning it absorbs light at a specific wavelength (734 nm). When an antioxidant is present, it donates electrons to neutralize the ABTS radical cation, reducing its absorption at 734 nm. The decrease in absorption at 734 nm is directly proportional to the antioxidant activity of the tested substance.

**Anti-inflammatory activity**

*Protein denaturation activity*

A solution of PKEO (100 µL), phosphate-buffered saline (PBS) adjusted to pH 6.4 (5.6 mL), and fresh hen's egg albumin (0.4 mL) was produced, providing a final volume of 10 mL. After thorough mixing, the solution was incubated for 15 minutes at 37°C to promote interaction between components. The mixture

was then heated for 5 minutes at 70 °C to denaturation the protein. After cooling to ambient temperature, the solution's turbidity was measured at 660 nm with an Optima SP-3000 UV/VIS spectrometer (Tokyo, Japan). Diclofenac sodium was used as the standard reference, with PBS as a control to establish baseline turbidity levels. The capacity of PKEO to suppress protein denaturation was assessed by comparing the turbidity of the PKEO-treated samples to that of the control and standard. The proportion of protein denaturation inhibition was calculated using Equation 3.

$$\%\ inhibition\ of\ denaturation = 100 \times \left(1 - \frac{T}{c}\right) \quad (3)$$

**Human red blood cell (HRBC) stabilizing assay**

The study involved a blood sample from a healthy donor who had abstained from NSAIDs for at least 15 days. The blood was mixed with an Alsever solution containing dextrose, citric acid, sodium citrate, and sodium chloride dissolved in distilled water. The mixture was centrifuged at 3000×g for 15 minutes to separate the blood cells. The resulting cell pellet was washed with an isosaline solution to remove residual components. An assay mixture was prepared, including 0.15 M PBS, HRBC suspension, 0.36% hyposaline solution, and PKEO. The assay mixture was incubated in a BOD incubator at 37°C for 30 minutes to allow for stabilization.

The suspension then underwent centrifugation at 3000×g for 20 minutes. Aspirin and deionized water were included as positive and negative controls, respectively. The amount of hemoglobin released into the supernatant was measured using a UV–visible spectrophotometer. The % HRBC membrane stabilization was calculated using Equation (4). The study aimed to determine the effect of NSAIDs on the blood-brain barrier (BBB) membrane.

$$Inhibition\ (\%) = 100 - ({}^{ODs}/_{ODc}) \times 100 \quad (4)$$

**Antimicrobial Activity**

*Antibacterial activity by well diffusion method*

The microorganism's inoculum was produced using bacterial cultures. 15ml of nutritional agar (Hi media) medium was put into clean, sterilized Petri plates and allowed to cool and harden. Pipette out 100 µg of bacterial strain broth and uniformly spread it over the medium with a spreading rod until dry. Wells 6mm in diameter were bored with a sterile cork borer. Compound solutions (100µl/ml) were produced in DMSO, followed by 100µl of test solution and standard added to the wells. The petri plates were incubated at 37°C for 24 hours. Streptomycin (1mg/ml) was made as a positive control, whereas DMSO was used as a negative control. Antibacterial activity was assessed by measuring the diameters of the zone of inhibitions (ZI), with all measurements conducted in triplicate. Antibacterial activity was assessed by measuring the diameters of the zone of inhibitions (ZI), with all measurements conducted in triplicate.

**Antifungal activity by well diffusion method**

The microorganism's inoculum was produced using bacterial cultures. 15ml of Saubroad agar (Hi media) medium was put into clean, sterilized Petri plates and allowed to cool and harden. To prepare the medium, 100 µl of fungal strain broth was pipetted and evenly spread with a spreading rod until fully dried. Wells 6mm in diameter were bored with a sterile cork borer. Compound solutions (100µl/ml) were produced in DMSO, followed by 100µl of test solution and standard added to the wells. The petri plates were incubated at 37°C for 24 hours. Miconazole (1mg/ml) was created as a positive control, whereas DMSO was used as a negative control. Antibacterial activity was tested by measuring the diameters of the zone of inhibitions (ZI); all determinations were conducted in triplicate.

***RESULT***

**Extraction**

Pale yellow-colored PKEO was isolated using hydro-distillation (Figure. 1 A, B). 1 mL of oil was obtained per 100 gm of roots. The resulting oil’s color was observed to be light yellowish.

**Phytochemical investigation**

Steroids, triterpenoids, alkaloids, phenols, proteins, flavonoids, and tannins were obtainable.

**Composition characterization**

*Gas chromatography-mass spectrophotometry-MS (GC–MS MS)*

The GC-MS study of PKEO revealed two phenolic compounds, namely 2-Methoxyphenol and 2, 3, 5, 6-tetramethylphenol, as well as 3, 7, 11, 15-Tetramethylhexadec-2-en-1-yl acetate, a sesquiterpene ester. These molecules work together to enhance the essential oil's bioactivity, aroma, and stability. Phenols boost the oil's antioxidant capacity, while the sesquiterpene ester promotes anti-inflammatory properties and offers a characteristic fragrant profile.

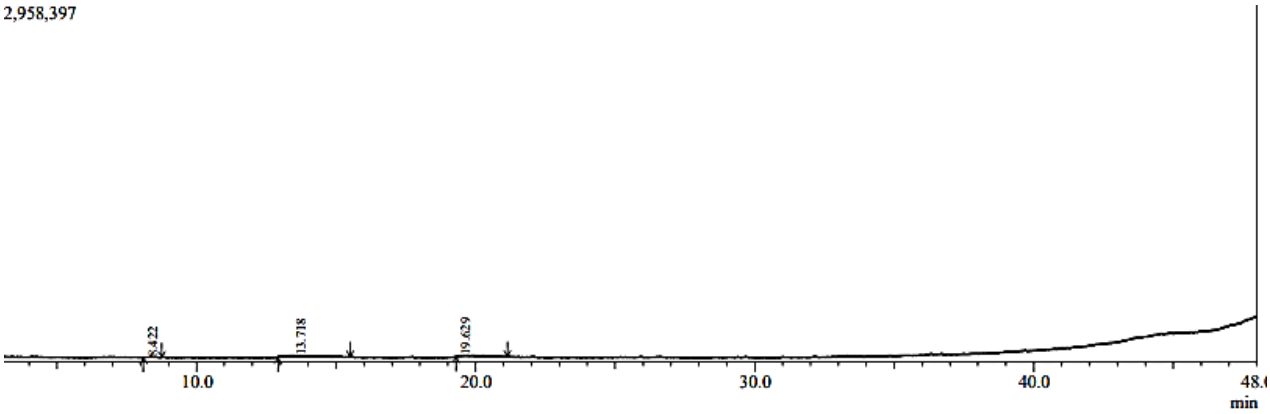



**Figure 1: GCMS chromatogram of PKEO**

*Total-content analysis*

*Phenol:* TPC was found to be 250.47µg GAE/gm. PKEO's TPC was calculated using the Folin-Ciocalteu reagent. Results were obtained using Gallic acid's (200-1000 µg/mL) calibration curve (y=0.0005x+0.0184 R2=0.992), and are presented as GAE per gm dry extract weight (Table 1). TPC was discovered to be 250.47 µg GAE/gm.

*Flavonoid:* Flavonoid concentrations in PKEO were analyzed quantitatively using a colorimetric technique with aluminum chloride. Results were derived from the calibration curve (y=0.000x+0.288; R2=0.973) of quercetin (0-100 µg/mL) and reported as QE per gram EO weight (Table 1). The total favonoid content of PKEO at 900 µg/mL was 245.26 (µg QE/g).

**Table 1: Total phenolic and flavonoid content**

| Conc. of PKEO | TPC µg GAE/gm. | TFC (µg QE/g) |
|---|---|---|
| 300 | 89.12 | 73.89 |
| 600 | 93.25 | 115.36 |
| 900 | 250.47 | 245.26 |

**Analytical characterization of PKEO**

*UV-visible analysis*

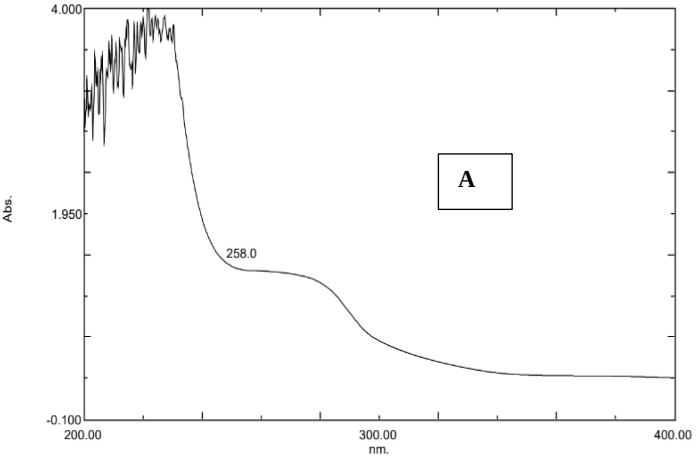
The UV-Vis spectrum of *Picrorhiza kurroa* essential oil (PKEO) reveals absorbance peaks in the ultraviolet region, indicating the presence of conjugated systems or aromatic groups. Key peaks include 1.383 at 258.0 nm, indicating phenolic compounds, 227.4 nm, and 220.0 nm, indicating conjugated unsaturated compounds. The UV spectrum confirms the presence of phenolic compounds and terpenoid esters, highlighting PKEO's complex composition and potential therapeutic properties.

*IR spectroscopy*

The IR analysis of PKEO reveals a complex profile of functional groups, indicating the presence of various bioactive compounds. Broad peaks in the 3360.85 to 3779.50 $cm^{-1}$ suggest hydroxyl (O-H) groups, commonly associated with alcohols or phenols, indicating hydrogen bonding. Peaks at 2921.51 and 2853.75 $cm^{-1}$ correspond to C-H stretching vibrations, suggesting aliphatic (alkyl) groups typical of terpenoids in essential oils. A strong peak at 1743.92 $cm^{-1}$ indicates carbonyl (C=O) groups, likely from ester or ketone compounds, aligning with ester components such as 3, 7, 11, 15-Tetramethylhexadec-2-en-1-yl acetate. The fingerprint region (1015.84 - 678.31 $cm^{-1}$) contains a series of complex peaks, providing unique structural details specific to this essential oil. Overall, the IR spectrum confirms the presence of hydroxyl, aliphatic, carbonyl, unsaturated, and ester functional groups, aligning with phenolic compounds and terpenoid esters identified in GC-MS analysis.

*Zeta potential*

The zeta potential of PKEO was around 0 mV, indicating a low surface charge. This low number indicates probable instability and limited effectiveness in applications needing a surface charge.



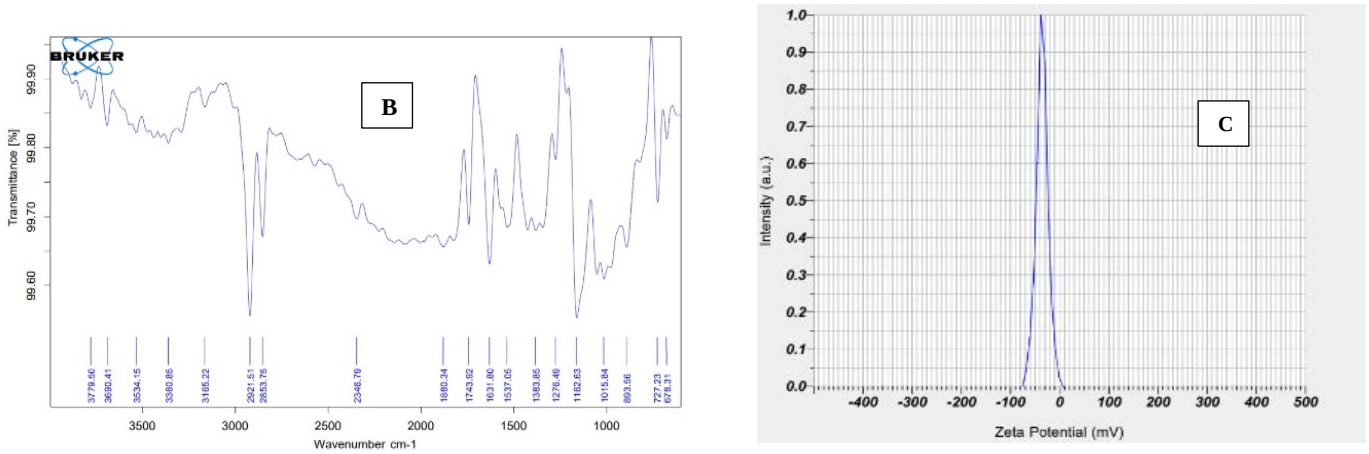



**Figure 2: (A) UV Vis Spectrum of PKEO. (B) FT-IR Spectrum of PKEO. (C) Zeta potential of PKEO**

**Antioxidant activity**

**DPPH scavenging assay**

Five concentrations of PKEO (20-100 µg/mL) were tested to determine their inhibitory effects on the target. Figure (3 A) shows a clear dose-dependent response, with higher concentrations of PKEO resulting in higher inhibition percentages. At the highest measured concentration of 100 µg/mL, PKEO achieved a peak inhibition of 52.84%. This finding is significant compared to the inhibition level attained by the standard ascorbic acid, which is 66.83%. The IC50 values highlight the relative potency of PKEO. Ascorbic acid has an IC50 of 58.16 µg/mL, and PKEO has an IC50 of 98.19 µg/mL, showing the efficacy of PKEO as an antioxidant.

**ABTS Assay:**

The percentage of inhibition for PKEO was 42.72% at 100 µg/mL as compared to the standard (62.72%) at 100 µg/mL (Fig. 3B). However, as concentrations of PKEO increased from 20 to 100 µg/mL, the percentage of inhibition increased, respectively. The highest radical scavenging activity for PKEO was observed at 100 µg/mL.

**Anti-inflammatory activities**

*Protein denaturation activity*

The anti-inflammatory assay results demonstrate PKEO's medical potential in lowering inflammation, as indicated by concentration-dependent inhibition across five concentrations (200, 400, 600, 800, and 1000 µg/mL). At 200 µg/mL, PKEO showed 7.59% inhibition, which rose to 48.73% at 1000 µg/mL. At the same time, PKEO's inhibition percentage was compared with the inhibition percentage of normal diclofenac, which was 72.78%. The steady increase in PKEO activity with concentration suggests it can control inflammatory responses. These data imply that PKEO has considerable anti-inflammatory characteristics that may contribute to its usage in therapeutic applications targeted at regulating inflammation.

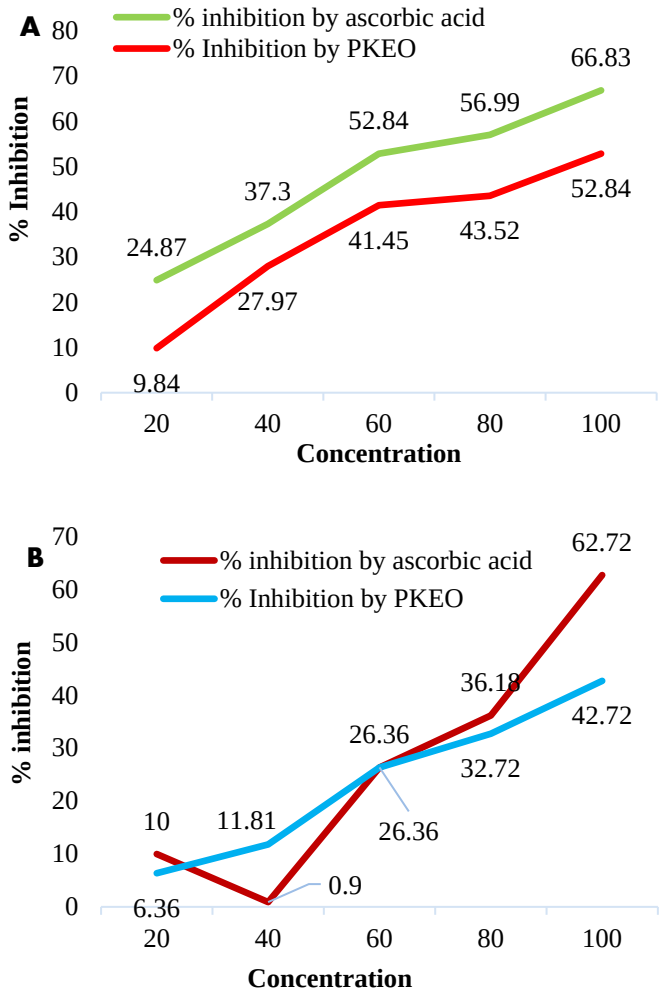



**Figure 3: Graphical representation of % of inhibition against oxidants by (A) DPPH method and (B) ABTS method**

*HRBC stabilizing assay:*

Its percentage of stabilization assessed the anti-inflammatory activities of PKEO at increasing concentrations (200, 400, 600, 800, and 1000 µg/mL). At the lowest concentration (200 µg/mL), PKEO had an initial stabilization effect of 45.09%, gradually improving as concentrations increased. At 400 µg/mL, stabilization was 50%, followed by 53.34% at 600 µg/mL and 60.66% at 800 µg/mL. The highest concentration examined, 1000 µg/mL, demonstrated a strong stabilization effect of 82.21%. This concentration-dependent increase in stabilization percentage highlights PKEO's potential anti-inflammatory activity, implying that it could be useful in applications to reduce inflammation. However, more research is needed to compare its efficacy with standard anti-inflammatory agents directly.

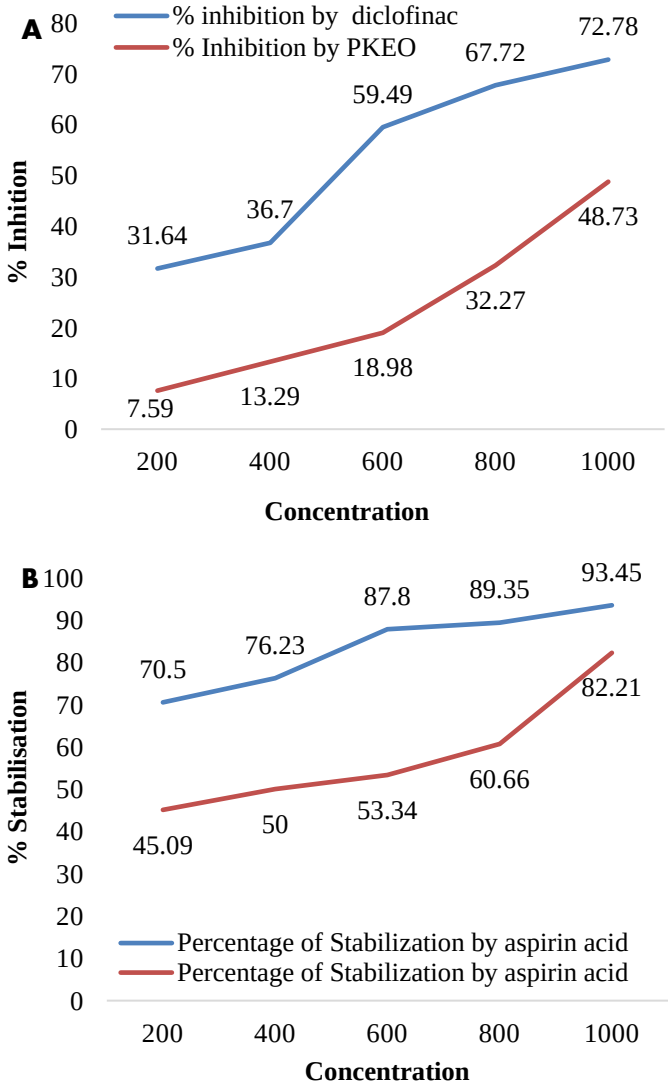



**Figure 4: Graphical representation of the % of inhibition against oxidants by (A) PDA method and (B) HRBC method**

**Antimicrobial activity**

*Antibacterial activity of PKEO against E. coli*

The antibacterial efficacy of PKEO against E. coli was evaluated by measuring the inhibition zone in millimeters. PKEO exhibited an inhibition zone of 8 mm, demonstrating its capacity to inhibit the growth of *E. coli* partially. While this effect was less significant than that of the standard antibiotic streptomycin, which presented a 26 mm inhibition zone, PKEO's activity indicates that it possesses some antibacterial characteristics. The control sample, which showed no inhibition zone (0 mm), further highlights the specific action of PKEO against E. coli. These results underscore the potential of PKEO as a natural antibacterial agent.


**Figure 5: Images depicting zones of inhibition of streptomycin, CHX 0.2%, & PKEO against bacterial strains**

*Antibacterial activity of PKEO against Staphylococcus aureus*

The antibacterial properties of PKEO against Staphylococcus aureus were assessed by measuring the diameter of the inhibition zone in millimeters. In this experiment, PKEO revealed a minimum inhibition zone of 2 mm, which indicates a limited antibacterial effect on Staphylococcus aureus. Conversely, the standard antibiotic streptomycin displayed a significant inhibition zone of 24 mm, demonstrating its potent antibacterial action. The control sample recorded no inhibition (0 mm), emphasizing the specific antibacterial effects of both PKEO and streptomycin. Although PKEO's inhibition zone is considerably smaller than that of streptomycin, these findings imply that PKEO possesses a mild antibacterial effect against Staphylococcus aureus.

*The antifungal activity of PKEO against Candida albicans*

The antifungal activity of PKEO against Candida albicans was evaluated by measuring the inhibition zone in millimeters. PKEO had a modest antifungal activity against Candida albicans, with an inhibition zone of 10 mm. In contrast,

Miconazole, the common antifungal medication, showed a significantly greater inhibitory zone of 29 mm, indicating its potent antifungal properties. The control sample confirmed the specific antifungal effect of miconazole and PKEO, which showed no inhibition (0 mm). The 10 mm zone indicates that PKEO has significant antifungal capabilities and promise as a natural antifungal agent but with less efficacy than traditional antifungal therapies, even if its inhibition zone is smaller than miconazole.

*The antifungal activity of PKEO against Candida albicans*

The zone of inhibition was measured at a dose of 1 mg/ml to evaluate the antifungal activity of PKEO (Plant Extract Oil) against Aspergillus niger. With a zone of 0 mm, the control, which lacked an active component, displayed no inhibition. Miconazole, a common antifungal medication, showed good action with a 29 mm zone of inhibition at the same dose. By contrast, PKEO showed a 9 mm zone of inhibition and modest antifungal efficacy. These findings imply that although PKEO has some antifungal qualities against Aspergillus niger, Miconazole is more effective.


**Figure 6: Images depicting zones of inhibition of miconazole, CHX 0.2%, and NSEO against *Candida albicans* and *Aspergillus niger***

***DISCUSSION***

*Picrorhiza kurroa* is a highly valued medicinal plant in ancient Ayurvedic medicine, known for its numerous therapeutic effects. The main focus of the current study is on root oil as this plant root oil is particularly well-known for its hepatoprotective, immunomodulatory, antioxidant, anti-inflammatory, and antidiabetic properties, making it an important ingredient in various herbal compositions. Root oil has not been studied very extensively. The antioxidant activity of the root extract of *Picrorhiza kurroa* has been the subject of multiple studies, highlighting its potential as a natural antioxidant source. One significant study by Nisar et al. evaluated the antioxidant properties of different extracts of *Picrorhiza kurroa* roots, including ethanolic, methanolic, and aqueous extracts. The study utilized the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay to measure the radical scavenging activity, revealing that the extracts exhibited substantial antioxidant activity, which was attributed to their high phenolic and flavonoid content, which is in agreement with the current study. However, the significant difference between the two studies is that the current study is entirely focused on the essential oil extracted from the roots, and the extraction method employed was Clevenger's oil extraction method. The previous study concentrated solely on the various solvent extracts obtained through the maceration process [7]. *P. kurroa*'s antioxidant benefits have been related to its high concentration of bioactive chemicals, including iridoid glycosides, particularly picrosides and kutkin, which have been shown to reduce oxidative damage [2, 16]. The current study results are consistent with previous studies, demonstrating the presence of phenols and terpenes in the PKEO. *Picrorhiza kurroa* is well renowned for its anti-inflammatory properties.

The in vitro protein denaturation assay is a standard approach for determining the anti-inflammatory properties of different substances. This assay evaluates a substance's capacity to suppress protein denaturation, commonly caused by heat or other stresses. *P. kurroa* has shown considerable protective effects in this situation. *P. kurroa* extracts have been demonstrated to reduce protein denaturation, indicating a potential for anti-inflammatory effects [7, 17]. The mechanism behind this action is likely related to the antioxidant properties of the bioactive compounds present in *P. kurroa*, such as picrosides, which can scavenge free radicals and reduce oxidative stress, thereby preventing protein denaturation [18]. Various assays, including the DPPH (2, 2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) assays, which assess the antioxidant capacity of the extracts, have supported the effectiveness of *P. kurroa* in reducing inflammation. These assays have shown that *P. kurroa* possesses significant antioxidant potential, further contributing to its anti-inflammatory effects by scavenging free radicals and reducing oxidative stress [7].

The antibacterial activity of *P. kurroa* has been evaluated using various methods, including the agar well diffusion method and

broth microdilution assays. Studies have shown that extracts of *P. kurroa* exhibit inhibitory effects against both Gram-positive and Gram-negative bacteria. For instance, Sinha et al. reported that the methanolic extract of *P. kurroa* demonstrated significant antibacterial activity against Staphylococcus aureus and Escherichia coli, with zones of inhibition comparable to standard antibiotics [17].

These bacteria' minimum inhibitory concentration (MIC) values indicate potent antibacterial properties [7]. *P. kurroa* also exhibits notable antifungal activity, particularly against pathogenic fungi such as Candida albicans. The antifungal properties have been assessed using similar methodologies, including the broth microdilution method, which measures the effectiveness of the extracts against fungal growth. Research indicates that *P. kurroa* extracts can inhibit the growth of C. albicans, with MIC values suggesting that the extracts are effective at relatively low concentrations [7, 17]. However, all these past studies were conducted on the P. kurroa extracts, not on oil. The present study is undertaken purely on the PKEO.

## *CONCLUSION*

The study focuses on the bioactive properties of Plant Extract Oil (PKEO) obtained from *Picrorhiza kurroa* roots. PKEO was successfully extracted and characterized via hydrodistillation, exhibiting a diverse phytochemical profile that included phenolic chemicals, flavonoids, steroids, and terpenoids. The GC-MS analysis found significant bioactive compounds, such as 2-Methoxyphenol and 3, 7, 11, and 15-Tetramethylhexadec-2-en-1-yl acetate, contributing to the therapeutic characteristics. PKEO showed modest antioxidant activity, substantially suppressing the DPPH and ABTS assays. It had dose-dependent anti-inflammatory effects, as protein denaturation and HRBC stabilization tests demonstrated.

Furthermore, PKEO shows antibacterial activity against *E. coli* and antifungal activity against *Aspergillus niger*, but with lesser potency than conventional medicines. These findings highlight the potential of PKEO as a natural bioactive ingredient for use in pharmaceutical and cosmetic formulations. We can develop injectable formulations containing *Picrorhiza kurroa* essential oil for severe inflammatory conditions and autoimmune diseases. However, more research is required to fully realize its medicinal potential, particularly in vivo testing and formulation optimization.

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NIL

## *CONFLICT OF INTEREST*

The authors declare no conflict of interest.

## *AUTHOR CONTRIBUTION*

Avish D. Maru designed the research study and interpreted the associated experimental data, while Amol R. Patil conducted laboratory work, analyzed the data, interpreted the results, and wrote the manuscript.

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