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ASSESSMENT OF PHYTOCHEMICALS AND BIOLOGICAL ACTIVITIES: ANTIPYRETIC AND ANTIFUNGAL EFFECTS OF *OLEA FERRUGINEA ROYLE*

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Abstract

Medicinal plants contain bioactive compounds that have the potential to prevent and combat diseases related to oxidative stress. The present study investigates the phytochemical composition, antipyretic, and antifungal activities of Olea ferruginea Royle extract, including ethanolic, methanolic, and chloroform extracts. Phytochemical analysis revealed that both ethanolic and methanolic extracts contained carbohydrates, flavonoids, alkaloids, paleobotanies, saponins, tannins, phenols, terpenoids, and cardiac glycosides, while alkaloids, paleobotanies, glycosides, and proteins were absent in the chloroform extract. Quantitative analysis of the chloroform extract revealed the presence of flavonoids (14.20±0.15 mg/ml), alkaloids (12.10±0.15 mg/ml), phenols (10.45±0.10 mg/ml), saponins (06.22±0.14 mg/ml), and tannins (04.60±0.65 mg/ml). The pharmacological evaluation revealed that the 600 mg/kg dose of the extract exhibited significant antipyretic activity in a brewer's yeast-induced pyrexia model, resulting in a 59.43% reduction in fever compared to the positive control, paracetamol (73.23% inhibition at 37.24°C). The antifungal activity demonstrated that Olea ferruginea Royle extracts were most effective against Verticillium, with a zone of inhibition of 17.00±0.48 mm at a concentration of 18 mg/µl, followed by Pythium (16.27±0.93 mm), Acremonium (16.20±1.89 mm), and Trichoderma (16.11±0.82 mm) at a concentration of 12 mg/µl. These results suggest that Olea ferruginea Royle possesses significant antipyretic and antifungal potential, supporting its traditional medicinal uses

INTRODUCTION

Phytochemicals are biologically active and naturally occurring chemical compounds found in plants, it has medicinal values for human benefits (Hasler & Blumberg, 1999). Phytochemicals protect plants from damage, and diseases and contribute to the plant's color, flavor, and odor (Irshad et al., 2025). The plant chemicals protect plant cells from environmental extortions such as stress, drought, pollution, and pathogenic attack (Gibson et al., 1998). Plants contain various phytochemical constituents such as tannins, flavonoids, terpenoids, phenolic acids, vitamins, lignin, quinines, stilbenes, amines, coumarins, betalains, alkaloids, and other phytochemicals, which have the potential of antioxidant activity (Zheng and wang, 2001; (Ullah et al., 2025a). Research has shown that several of these antioxidant compounds have anti-atherosclerotic, anti-inflammatory, anti-mutagenic, antitumor, antibacterial, antiviral, and anti-carcinogenic activities (Ganesan et al., 2017). In modern research, plant phytochemicals, formerly with unknown and biological activities, have been widely studied as a basis of medicinal agents (Krishnaraju et al., 2015). Therefore, it is estimated that plant phytochemicals with sufficient antibacterial ability will be used for the cure of bacterial infections (Gracelin et al., 2013). Pyretic is defined as the

elevation of core body temperature above that in normal adults, the average oral temperature is 36.98 (98.58F) (Ullah et al., 2025b). In oncology practice, a single temperature of more than 38.3°C (101°F) or three readings (at least 1 hour apart) of more than 38 °C (100.4°F) are considered significant (Robinson et al., 2011). Lower temperature elevations in the very young or old and patients receiving steroids or other immune suppressants are considered abnormal (Mackowiak et al., 1997). *Olea ferruginea Royle* is a small, evergreen species that typically grows to a height of 9 to 12 meters, with diameters ranging from 0.3 to 0.6 meters (Ullah et al., 2025c). Its leaves are simple and measure 3 to 10 cm in length (Asif et al., 2025). The tree produces whitish flowers arranged in bunches, which bloom between March and September (Ullah et al., 2024). The fruit is a drupe, approximately 8 mm in length, maturing between May and December. Notably, the tree does not suffer from significant insect or disease problems (Naqshi, 2016).

The tree can be propagated both from seed and vegetatively. However, seeds should be planted immediately after collection, as they lose viability rapidly when stored (Khan et al., 2024). One-year-old polybag plants are suitable for field planting, though the tree grows slowly, with an average annual increment (MAI) of 0.25 cm in diameter (Dalam). One-year-old coppice shoots typically reach heights between 0.25 to 0.90 meters (Fabricant et al., 2001). Trees have been reported to reach a height of 3.5 meters and a diameter of 3 cm after six years (Ullah et al 2023).

The tree is native to the subcontinent, including Pakistan, Afghanistan, and India. In Pakistan, it is found in the lower hills of Azad Kashmir, Punjab, NWFP, Balochistan, and the western hills of the Sindh region (Sharma and Vyas 1985). The tree is an excellent candidate for reforestation projects in arid areas. It also holds promise as an oil and fruit-bearing tree (Badifu, 1991). Efforts to graft improved varieties could potentially increase both oil and fruit production (David et al., 2020; Ullah et al., 2024). The wood is valuable and can be used for fuel, while the foliage serves as good fodder. Additionally, the tree is used in various applications, including construction, fuel, tool handles, watershed protection, and fruit and oil production (Chang et al., 2011). Aims of the present work to identify and quantify the major phytochemical constituents present in different extracts of *Olea ferruginea* using standard qualitative and quantitative techniques. To evaluate the antipyretic activity of *Olea ferruginea* extracts in experimentally induced pyrexia models in laboratory animals. To assess the antifungal efficacy of *Olea ferruginea* extracts against selected pathogenic fungal strains through in vitro assays.

Methodology

Sampling and Identification

In the present study, *Olea ferruginea* whole plant was collected in November 2023 from the Malakand division of Khyber Pakhtunkhwa Province Pakistan. With the help of Flora of Pakistan, plants were taxonomically identified and placed in the Herbarium of Govt Degree College Timergara Dir Lower Pakistan (Ullah et al., 2023).

Solvents

For the crude extract preparation of the *Olea ferruginea* methanol, ethanol, and chloroform was used (Shakir et al., 2023a).

Crashing and filtration of the plant

The dried plant was crushed with the help of an electric grinder. The powder was kept in air-tight plastic bottles for further phytochemical, pharmacological, and antifungal activities (Shakir et al., 2023b). 15 gm of plant powder was retained in a distinct conical flask and 90 ml of solvent i.e. (methanol, ethanol, and chloroform) was added to the powder separately (Ullah et al., 2018h). With the help of aluminum foil, the flask was covered and retained in a shaker for 72 hrs for shaking purposes (Ullah et al., 2018). After 72 hrs the extracts were filtered with the help of Whatman filter paper and then through the filtration process plant extracts were removed (Pirzada et al., 2010).

Phytochemical analysis: Qualitative study

The plant extract i.e., methanol, ethanol, and chloroform were tasted for the absence or presence of phytochemical constituents like alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, glycosides, and terpenoids (Soni et al., 2011).

Tests for Alkaloids

For the detection of alkaloids, a few drops of Wagner's reagent (Potassium iodine) are added to 2 ml of all three methanol, ethanol, and chloroform extracts. The presence of alkaloids was checked by the formation of a reddish-brown precipitate (Khandelwal et al., 2015).

Tests for Tannins

For the detection of tannins Ferric chloride test was done. Ferric chloride ($FeCl_3$) solution was mixed with all three extracts separately. The formation of blue-green coloration showed the presence of tannins. (Kokate et al., 2008).

Tests for Phlobatannins

In test tubes 0.5 ml of all three extracts were taken separately, added 3ml of distilled water, and shaken for a few minutes then 1% aqueous hydrochloride (HCl) was added and boiled in water both. The presence of phlobatannins is confirmed by the formation of a red color (Wadood et al., 2013, (Ullah et al., 2019c).

Tests for Flavonoids

For flavonoid detection, sodium hydroxide (NaOH) solution was added to all three extracts of the plant. Red precipitation formation indicates the presence of flavonoids (Kokate et al., 2008).

Tests for Carbohydrates

For the detection of carbohydrates, 0.5 ml of all three extracts were treated with 0.5 ml of Benedict's regent. Solutions were heated for 2 minutes in a water bath. A reddish-brown precipitate indicates the presence of carbohydrates (Bussau, et al., 2002) .

Tests for Phenols

For phenol detection, 2 ml of ferric chloride ($FeCl_3$) solution was added to 2 ml of all three extracts separately in a test tube. Deep bluish-green coloration formations showed the presence of phenol (Dahiru et al., 2006).

Tests for Saponins

For the detection of saponin, in a test tube, 5 ml of all three extracts were shaken vigorously. The presence of saponins was confirmed by froth formation (Rajesh et al., 2016).

Tests for (Cardiac) Glycosides

For cardiac glycosides detection, 2 ml of all three extract solutions were shaken with 2 ml of glacial acetic acid then added few drops of concentrated sulphuric acid (H_2SO4) and iron tri chloride ($FeCl_3$). Brown ring formation indicated the presence of Cardiac glycosides (Soni et al., 2011).

Tests for proteins

Xanthoproteic test: For the detection of protein, 1 ml of all three extracts was treated with 1 ml of concentrated nitric acid (HNO_3) solution. The presence of proteins is indicated by the formation of yellow color (Rajesh et al., 2016).

Tests for terpenoids

Salkowski test: One ml of plant extracts (methanol, ethanol, and chloroform) was added with 2 ml of chloroform. After that carefully added concentrated sulphuric acid (H_2SO_4) was along the sides of the tube to form a layer. The reddish-brown coloration formation showed the presence of terpenoids (Dahiru et al., 2006).

Tests for Glycosides

For the detection of glycosides, 5% of Ferric chloride solution and 1 ml glacial acetic acid were added to 5 ml of all three extracts, and then further addition of few drops of concentered sulphuric acid (H_2SO_4). The presence of glycosides was confirmed through the formation of a greenish-blue color (Rajesh et al., 2016).

Quantitative phytochemicals analysis

Determination of total flavonoid constituents

Ethanol, methanol, and chloroform extracts were used for the detection of total flavonoid contents. Total flavonoids quantification was done by taking 0.5 g of plant extracts. Then the samples were mixed with 4.3 ml methanol and then more addition of 0.1 ml of aluminum tri chloride from 10% prepared solutions of aluminum tri chloride laterally. Potassium acetate (0.1 ml) was added up to total volume of 5 ml

(Ullah et al., 2018). The mixtures were shaken by vortex to make a uniform solution and then the mixture was placed at room temperature for 30 minutes for incubation. After the incubation process, the absorption was checked at 415 nm in the spectrum. Quercetin was used as a standard (Daffodil et al., 2013; (Khan et al., 2018).

Determination of Total Phenolic Constituents

Total phenolic quantification was done by the addition of 0.5 g plant extract to 1 ml of 80% ethanol. Then the mixture was centrifuged for 15 minutes at 12,000 rpm. After that, the supernatant was kept in a test tube and this process was repeated 6 times (Ullah et al., 2018g). After collecting the supernatant was placed in a water bath for drying. Distilled water was added tothe supernatant until its volume reached 3 ml. 2 ml (Na_2CO_3) of 20% was added to the solution. To this 0.5 ml Folin-ciocalteau regent was added. After 5 minutes more addition of 2 ml (Na_2Co_3) from 20% Na_2Co_3 in this solution.Solutions were mixed homogenously and then the test tube was brought in to the water bath in boiling water. At 650 nm their absorbance was checked. The Catechol was used as a standard (Grcelin et al., 2013; Khan et al., 2018a).).

Quantification of total alkaloid constituents

5 gm of all the three extracts were balanced in a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered then allowed for 4 hours to stand. Extracts were filtered and concentrated in a water bath to one-quarter of the original volume. Until the precipitation was complete Drop wise to the extract concentrated ammonium hydroxide was added (Ullah et al., 2018g). The solution was allowed to settle and collected the precipitated and washed with dilute ammonium hydroxide and then filtered. The residue alkaloid was driedand weighed (Gracelin et al., 2013)

Determination of total tannin constituents

A sample weighing 500 mg was weighed in a 50 ml plastic bottle. Add 50 ml of distilled water and shake for 1 hour in amechanical shaker. 50 ml volumetric flask was filtered and made up to the mark. Into a test tube, 5 ml of the filteredwas pipetted out and mixed with 2 ml of 0.1 M FeCl3 in 0. I N HCl and 0.008 M potassium Ferro cyanide. At 120 nm their absorbance was cheeked out (Gracelin et al., 2013).

Determination of total saponins constituents

Into a conical flask, 20 gm of each extract was put and 100cm3 of 20% ethanol, and aqueous were added. Kept in a water bath for 4 hours the samples were heated with constant stirring at about 55 °C. The residue was re-extracted with another 200 ml of 20% ethanol, and the mixture was filtered (Ullah et al., 2018c). At about 90 °C through a water bath, the combined extracts were reduced to 40 ml. Into a 250 ml separatory funnel, the concentrate was transferred and 20 ml of diethyl ether was added and vigorously shaken. 60 ml of n-butanol was added.With 10 ml of 5% aqueous sodium chloride the combined n-butanol extracts were washed twice. In a water bath, the remaining solution was heated. Samples were dried in the oven to a constant weight after evaporation; the saponin content was calculated (Gracelin et al., 2013) [11].

Pharmacological activities

Pharmacological activities were carried out in methanolic extracts of the whole plant of *Olea ferruginea* (Ullah et al., 2019b).

Experimental animals

Both sexes of the albino mice of weight about 25 - 30 gm were brought from the National Institute of Health Islamabad. Animals were supplied with adlibitum water and a standard pellet diet.

Drugs used and chemicals used

Aspirin (Bayer, Germany), and Paracetamol (Glaxo SmithKline, U.K), were used as standard drugs in the experiment for selected activities. Methanol 95% (Merck, Germany), Normal saline (Immunasol NS, A.Z. Pharmaceuticals Co.pak), Brewer's yeast.

Evaluation of Antipyretic Activity by Brewer's yeast induced pyrexia

For evaluation of antipyretic activity, five groups of albino mice were taken. The initial temperature of all mice was checked. For antipyretic activity, the brewer yeast solution was prepared by dissolving 7% brewer yeast in 100 ml water (Ullah et al., 2021). The

paracetamol solution was used as a standard. Paracetamol solution was injected into the mice of group 2nd. To the group, 3rd, 4th, and 5th methanolic extract at the doses of200, 400, and 600 mg/Kg was injected. After the injection of the standard drug (paracetamol) and methanolic extract, the temperature of all the mice was checked at intervals of one hour up to four hours. The results of the methanolic extract were compared with the standard drug (paracetamol) for low or high antipyretic potential. % reduction in temperature is calculated by using the following formula (Janaranjani et al., 2014).
% reduction = B – Cn/B – A × 100 B= temperature after pyrexia induction;
Cn= temperature after 1, 2, 3, 4, and 5 hours, A=temperature of normal body

Anti-fungal activity

Antifungal activity was carried out in ethanolic, methanolic, and chloroform extracts.

Media preparation

Dissolve 39 gm of Potato dextrose agar (PDA) in 1 liter of distilled water, sterilized by autoclaving at 15psi (121 °C) for 15 minutes. Cool to room temperature and pour into sterilized Petri plates to solidify. Kept at room temperatureto solidify for 30 minutes (Ullah et al., 2018d).

Agar well diffusion method

The micropipette using, sterile distilled water (SDW) placed 100µl of different fungal cultures over the surface of an agar plate, and with the help of a sterile inoculation loop it was spread, a hole was made in each of the culture plates using a sterile cork borer. 75 µL of crude extract of selected plants was added. Culture plates were incubated at 37 °C, and after 24 hours the results were detected depending on the fungal growth (Ullah et al., 2018e). Around each well clear zone was measured in mm, and clear zone formation exposed theantifungal activity of all extracts against each fungus. All activities were formed in triplication and then the standard deviation. The agar well diffusion method was followed as described by Samie et al., (2010).

Statistical analysis

Individual triplicate experiments were performed for all tests.Data are shown as mean ± standard error of the mean (S.E.M., n = number of Experiments). Statistical studies were obtained by the one-way analysis of variance (ANOVA), followed byDennett's test where necessary. $p<0.05$ was considered Significant.

Results and Discussion

Phytochemical analysis

In the present research study, phytochemical analysis of methanolic, ethanolic, and chloroform extracts of *Olea ferruginea* and their pharmacological activities of methanolic extracts anti-pyretic were carried out. Also, antifungal activities in ethanolic methanolic and chloroform extracts were studied.

Additionally, antifungal activities of all three extracts (methanolic, ethanolic, and chloroform) were evaluated against selected pathogenic fungal strains. The phytochemical screening aimed to detect the presence of key bioactive compounds such as alkaloids, flavonoids, tannins, saponins, glycosides, and phenolics, which are often associated with therapeutic effects. The antipyretic activity was assessed using standard animal models induced with pyrexia, while the antifungal efficacy was tested in vitro using agar well diffusion or disc diffusion methods.

This comprehensive evaluation seeks to establish a scientific basis for the traditional use of *Olea ferruginea* in folk medicine and to explore its potential for developing novel antipyretic and antifungal agents. The findings from this study could contribute to the growing body of evidence supporting the medicinal value of indigenous plants and promote the development of plant-based therapeutics.

The phytochemical screening of *Olea ferruginea* revealed the presence of several important secondary metabolites in both the leaves and stem. Qualitative tests conducted on methanolic, ethanolic, and chloroform extracts confirmed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and terpenoids. These bioactive constituents are known to contribute to the plant's pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant effects. While the leaves exhibited a higher abundance of flavonoids and

tannins, the *stem* extracts showed comparatively stronger indications of alkaloids and saponins. The diversity and richness of phytochemicals in both plant parts support the traditional medicinal use of *Olea ferruginea* and highlight its potential as a source of therapeutic agents. Further quantitative and chromatographic analysis is recommended to isolate and characterize the individual compounds responsible for the observed biological activities. These compounds exhibit a wide range of therapeutic properties including antioxidant, anti-inflammatory, antimicrobial, antipyretic, and anticancer activities. For instance, flavonoids are known for their powerful antioxidant and anti-inflammatory effects, beneficial in managing cardiovascular diseases and inflammatory disorders. Alkaloids possess analgesic and antimicrobial properties, commonly used in pain relief and infection control. Tannins exhibit strong astringent and antifungal activities, aiding in wound healing and fungal infections. Saponins enhance immune responses and possess cholesterol-lowering effects, while phenolic compounds act as antioxidants and protect against oxidative stress-related diseases such as diabetes and cancer. The presence of these phytochemicals in medicinal plants like Olea ferruginea underlines their significance as potential therapeutic agents in modern pharmacology and integrative medicine.

Phytochemical detection in the leaves and stem of *Olea ferruginea*

Qualitative analysis of *Olea ferruginea* was carried out for the detection of alkaloids, flavonoids, carbohydrates, phlobatannins, glycosides, saponins, phenol, terpenoids, tannins, cardiac glycosides, and proteins. The results showed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, and cardiac glycosides were found in methanolic and ethanolic extracts, while alkaloids, phlobatannins, glycosides and protein were absent in the chloroform extracts. Flavonoids, carbohydrates, saponins, phenols, and terpenoids were found present in the *stem* methanolic and ethanolic extracts. In these results +++ indicates that the secondary metabolites are present in the highest amount, ++ indicates that a moderate level of phytochemicals is present + indicates that a low level of phytochemicals is present, and – indicates that the phytochemicals are absent in all these three extracts plants (Table 1, 2).

Table 1: Qualitative phytochemicals detection in the *stem* of*Olea ferruginea* in methanolic, ethanolic, and chloroform extracts

S. No	Phytochemical test	Methanolic	Ethanolic	chloroform
1	Alkaloid	+++	++	_
2	Flavonoids	+	+++	+
3	Carbohydrate	+++	+	+
4	Phlobatannins	+++	++	_
5	Glycosides	+	+++	+
6	Saponins	+	++	+
7	Phenol	+++	++	+
8	Terpenoids	++	+++	_
9	Tannins	+++	++	+
10	Cardiac glycosides	++	+	_
11	Proteins	++	+	_

Key: +++: present highest level, ++ showed moderate level, + showed low level - absent

Table 2: Phytochemical detection in the leaves of *Olea ferruginea* in methanolic, ethanolic, and chloroform extracts

S. No	Phytochemical test	Methanolic	Ethanolic	chloroform
1	Alkaloid	++	+	_
2	Flavonoids	+	+++	+
3	Carbohydrate	+++	++	+

4	Phlobatannins	+++	++	_
5	Glycosides	++	+++	_
6	Saponins	+++	+++	+
7	Phenol	+++	+	+
8	Terpenoids	++	+++	+
9	Tannins	+++	++	+
10	Cardiac glycosides	++	+++	_
11	Proteins	++	++	_

Key: +++: present highest level, ++ showed moderate level, + showed low level - absent

Total Phenolic, Flavonoids, Tannins, Saponins and Alkaloids Contents in chloroform, methanol and ethanol

Highest number of flavonoids was found in the chloroform extract as (14.20±0.15 mg/ml) followed by Alkaloids (10.14±0.12 mg/ml), phenolics (10.45±0.10 mg/ml), Saponins (06.22±0.14 mg/ml) and lowest amount of Tannins was found in (04.60±0.65 mg/ml). The flavonoids were found in highest amount in methanolic as (17.55±0.10 mg/ml), followed by phenols (13.25±0.50 mg/ml), Tannins (11.55±0.30 mg/ml), Alkaloids (10.05±0.10 mg/ml) and Saponins was found in lowest amount (08.40±0.45 mg/ml). The flavonoids were found in highest amount in methanolic as (13.25±0.50 mg/ml), followed by phenols (12.64±0.14 mg/ml), Alkaloids (09.50±0.15 mg/ml), Tannins (06.25±0.40 mg/ml) and Saponins was found in lowest amount (05.40±0.25 mg/ml). The data is showed in (Table 3, 4 and 5).

Table 3: Quantitative Phytochemicals detection of *Olea ferruginea* in chloroform extracts

S. No.	Phytochemical's name	Concentration mg/ml
1	Total phenolics	10.45±0.10
2	Total flavonoids	14.20±0.15
4	Total Tannins	04.60±0.65
5	Total Saponins	06.22±0.14
6	Total Alkaloids	10.14±0.12

Table 4: Quantitative Phytochemicals detection of Olea ferruginea in methanolic extracts

S. No.	Phytochemical's name	Concentration mg/ml
1	Total phenolics	13.25±0.50
2	Total flavonoids	17.55±0.10
4	Total Tannins	11.55±0.30
5	Total Saponins	08.40±0.45
6	Total Alkaloids	10.05±0.10

Table 5: Quantitative Phytochemicals detection of *Olea ferruginea* in ethanolic extracts

S. No.	Phytochemical's name	Concentration mg/ml
1	Total phenolics	12.64±0.14
2	Total flavonoids	13.25±0.50
4	Total Tannins	06.25±0.40
5	Total Saponins	05.40±0.25
6	Total Alkaloids	09.50±0.15

Pharmacological activities

For pharmacological activities, methanolic extracts were used.

Anti-pyretic activity

The anti-pyretic activity of *Olea ferruginea* whole plant was performed using brewer yeast-induced pyrexia test. In experimental mice, subcutaneous administration injection of yeast suspension markedly elevates the rectal temperature after 24 hours. Treatment with the Olea ferruginea extract at the doses of 200, 400, and 600 mg/kg decreased the rectal temperature at 3 hours were 37.10±0.9 °C, 37.09±1.43 °C and 37.24±1.12 °C respectively. There were dose-dependent responses were observed in experimental mice. The antipyretic effect started from the first hour and was maintained for 3 hrs, after administration of the extract. The dose of 600 mg/kg of the extract revealed significant antipyretic activity i.e. (46.12%) when compared with positive control i.e., paracetamol (37.24 °C) inhibition (61.53%). The data is shown in table (6).

Table 6: Anti-pyretic activity of *Olea ferruginea* whole plant in methanolic extracts

Rectal temperature (°C)							
Drug	**Dose**	**Before Yeast injection (Mean ± SEM)**	**After Yeast injection (Mean ± SEM)**	**1 hour (Mean ± SEM)**	**2 hours (Mean ± SEM)**	**3 hours (Mean ± SEM)**	**% Anti-pyretic Inhibition**
Control	N/S	37.64±0.26	38.93±0.01	38.19±0.25	37.87±0.23	37.59±0.15	.....
Paracetamol	150 mg/Kg	37.10±0.25	38.94±0.19	38.72±0.10	37.67±0.15	37.74±.03	73.23%
Methanolic extract	200 mg/Kg	37.07±0.21	38.66±1.0	38.41±0.26	37.61±0.22	37.10±0.9	27.34%
Methanolic extract	400 mg/Kg	37.01±0.15	38.52±0.10	38.06±0.31	37.33±1.20	37.09±1.43	42.18%
Methanolic extract	600 mg/Kg	37.07±0.15	38.63±0.72	38.68±0.18	37.53±0.86	37.24±1.12	59.43%

Antifungal activity of methanolic, ethanolic, and chloroform extracts of *Olea ferruginea* against selected fungal strains

The results of antifungal activity showed that Crude methanolic Extract were active against all fungal species and showed different range of zone of inhibition. The extracts that is most active as (17.00±0.48 mm) zone of inhibition at the concentration of18 mg/µl against *Verticillium*, followed by Pythium (16.27±0.93 mm), *Acremonium* (16.20±1.89 mm) and Trichoderma (16.11±0.82) concentration of 12 mg/µl. The ethanolic extracts showed a maximum zone of inhibition (16.06±0.97 mm) at the concentration of 12 mg/µl against *Trichoderma* followed by Acremonium (15.33±1.12 mm) at the concentration of 6 mg/µl and the lowest amount of inhibition was showed against *Verticillium* (11.13±1.65 mm) and *Trichoderma* (10.062±1.32 mm). The chloroform extracts showed maximum inhibition against Verticillium (16.63±0.65 mm) at the concentration of 12 mg/µl, followedby Trichoderma (16.18±1.43mm) at the concentration of 6 mg/µl, *Alternaria* (14.40±1.34) at the concentration of 12 mg/µl and lowest amount of inhibition was showed *Acremonium* (10.23±0.88mm), *Pythium* (10.00±3.60 mm) and *Alternaria* (8.56±1.75 mm) at the concentration of 18 mg/µl and 6 mg/µl. The data are shown in table (7, 8 and 9).

Table 7: Antifungal activity of chloroform extracts of *Olea ferruginea* against selected fungal strains

Extracts concentration	Alternaria	Acremonium	Verticillium	Pythium	Trichoderma
6 mg/µl	8.56±1.75	13.37±1.52	9.83±0.46	12.33±0.76	16.18±1.43
12 mg/µl	14.40±1.34	10.47±0.67	16.63±0.65	13.47±1.32	14.09±0.87
18 mg/µl	11.00±1.20	10.23±0.88	12.37±1.86	10.00±3.60	12.00±2.08

Table 8: Antifungal activity of ethanolic extracts of *Olea ferruginea* against selected fungal strains

Extracts concentration	*Alternaria*	*Acremonium*	*Verticillium*	*Pythium*	*Trichoderma*

6 mg/µl	9.76±1.65	15.33±1.12	7.93±0.66	10.23±0.96	13.12±1.03
12 mg/µl	14.50±1.25	13.57±0.87	12.83±0.95	14.27±1.22	16.06±0.97
18 mg/µl	6.32±1.00	12.13±0.78	11.13±1.65	13.16±0.57	10.062±1.32

Table 9: Antifungal activity of methanolic extracts of *Olea ferruginea* against selected fungal strains

Extracts concentration	*Alternaria*	*Acremonium*	*Verticillium*	*Pythium*	*Trichoderma*
6 mg/µl	6.89±0.11	16.20±1.89	13.44±0.53	10.67±1.30	11.65±1.75
12 mg/µl	10.33±0.88	14.12±0.192	17.00±0.48	16.27±0.93	16.11±0.82
18 mg/µl	10.80±0.58	9.89±0.63	13.62±0.87	9.17±1.01	14.77±1.13

Discussion

In the present research work, phytochemical analysis of methanolic, ethanolic, and chloroform extracts of *Olea ferruginea* and Anti-pyretic and antifungal activities in methanolic, ethanolic, and chloroform extracts were studied. Qualitative analysis of *Olea ferruginea* was carried out for the detection of alkaloids, flavonoids, carbohydrates, phlobatannins, glycosides, saponins, phenol, terpenoids, tannins, cardiac glycosides, and proteins. Results revealed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, and cardiac glycosides were found in methanolic and ethanolic extracts, while alkaloids, phlobatannins, glycosides, and protein were absent in aqueous extracts. Carbohydrates, flavonoids, saponins, phenols, and terpenoids were present in the *stem* methanolic and ethanolic extracts (Ullah et al., 2018f; Teke et al., 2013). The highest number of flavonoids was found in the chloroform extract as (14.20±0.15 mg/ml) followed by Alkaloids (10.14±0.12 mg/ml), phenolics (10.45±0.10 mg/ml), Saponins (06.22±0.14 mg/ml) and lowest number of Tannins was found in (04.60±0.65 mg/ml). The flavonoids were found in the highest amount in methanolic (17.55±0.10mg/ml), followed by phenols (13.25±0.50 mg/ml), Tannins (11.55±0.30 mg/ml), Alkaloids (10.05±0.10 mg/ml) and Saponins was found in lowest amount (08.40±0.45 mg/ml). Olea ferruginea extract at the doses of 200, 400 and 600 mg/kg decreased the rectal temperature at 3 hours were 37.10±0.9 °C, 37.09±1.43 °C and 37.24±1.12 °C respectively. The dose of 600 mg/kg of the extract showed remarkable antipyretic activity i.e. (59.43%) when compared with positive control i.e., paracetamol (37.24 °C) inhibition (73.23%). Extracts of *Olea ferruginea* were active against all fungal species and showed different ranges of zones of inhibition (Khan et al., 2028b). The most active among the extracts (17.00±0.48 mm) zone of inhibition at the concentration of 18 mg/µl against *Verticillium*. Followed by *Pythium* (16.27±0.93 mm), *Acremonium* (16.20±1.89 mm), and *Trichoderma* (16.11±0.82) with a concentration of 12 mg/µl. The ethanolic extracts showed maximum zone of inhibition (16.06±0.97 mm) at the concentration of 12 mg/µl against *Trichoderma* followed by *Acremonium* (15.33±1.12 mm) at the concentration of 6 mg/µl and the lowest amount of inhibition showed against *Verticillium* (11.13±1.65 mm) and *Trichoderma* i.e. (10.062±1.32 mm). Chloroform extracts indicated maximum inhibition against the *Verticillium* i.e. (16.63±0.65 mm) at the concentration of 12 mg/µl, followed by *Trichoderma* (16.18±1.43 mm) at a concentration of 6 mg/µl, and *Alternaria* (14.40±1.34) at the concentration of 12 mg/µl (Andrews, 2001).

Phytochemical components present in plant samples are known to be biologically active compounds and they are responsible for diverse activities such as antioxidant, antimicrobial, anticancer, antifungal, and antidiabetic (Hossain & Nagooru, 2011). A wide variety of pharmacological activities are shown by different phytochemicals, which may help in protection against chronic diseases (Lubna et al., 2025). Tannins, flavonoids, saponins, glycosides, and amino acids have anti-inflammatory and hypoglycemic activities (Tsafack et al., 2017). Steroids and terpenoids show central nervous system (CNS) activities and analgesic properties. Because of their antimicrobial activity saponins are involved in plant defense systems (Ayoola et al., 2008). These phytochemicals showed antimicrobial activity through different mechanisms. Proline-rich protein tannins have been found to form irreversible complexes

(Shimada, 2006) resulting in the inhibition of cell protein synthesis. (Parekh and Chanda, 2007) stated that tannins are known to react with proteins that deliver the typical tanning effect which is vital for the cure of ulcerated or inflamed tissues (Akoroda, 1990). Herbs that have tannins as their key components are astringent and are used for treating intestinal disorders such as dysentery and diarrhea (Dharmananda,2003). Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers (Barile et al., 2007). So, these observations therefore sustenance the use of *Olea ferruginea* in herbal medication remedies, thus suggesting that *Olea ferruginea* has potential as source of significant bioactive molecules responsible for treatment and anticipation of cancer. Presence of tannins in *Olea ferruginea* supports the outdated medicinal use of this plant in the treatment of different disorders (Ullah et al., 2025; Leonard et al., 2001). Alkaloid was alternative phytochemicals constituent's that observed in the *Pteris quadriaurita*. One of the best common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely considered for their potential use in the reduction and ending of human cancer cell lines (Nobori, et al., 1994). One of largest group of phytochemicals i.e., alkaloids in plants which have remarkable effects on humans, and led to the improvement of powerful pain killer medicines (Kam and Liew, 2002; Oyewole et al., 2012). (Just et al., 1998) exposed theinhibitory effect of saponins on inflamed cells (Inoue et al., 2011). Saponin was present in *Olea ferruginea* extracts and it has maintained the usefulness of this plant in managing inflammation. Flavonoids, alternative phytochemicals indicated a diverse range of pharmacological activities like anti-inflammatory, antimicrobial, analgesic, anti-angionic, cytostatic, antioxidant and anti-allergic properties (Hodek et al., 2002). Several reports are offered on flavonoid groups which showing high potential biological activities such as anti-inflammatory, antioxidant, antiallergic reactions (Thitilertdecha et al., 2008). In the crude extracts bioactive compounds such as tannins and flavonoids were present (Olaniyan, 2005). Until now, these phytochemical compounds were encouraging the antimicrobial and antioxidants activities. By fractionation the number of active constituents in the crude extracts might be dilute or enhanced their concentrations (Anyasor et al., 2010).

Conclusion

The above results confirmed that *Olea ferruginea* has better anti-inflammatory analgesic and antipyretic activity. The pharmacological activity of the Olea ferruginea may be due to the presence of phytochemical constituents. Some of these compounds possess analgesic, anti-inflammatory, antipyretic and antifungal activities. Further studies involving the purification of the chemical constituents of the plant and investigation in the biochemical pathway mayresults in the development of a potent analgesic, anti- inflammatory, anti-pyretic and antifungal agent with low toxicity and better therapeutic index.

Novelty Statement

This study provides novel insight into the pharmacological potential of *Olea ferruginea*, demonstrating its significant anti-inflammatory, analgesic, antipyretic, and antifungal activities. The observed bioactivities are likely attributed to the presence of diverse phytochemical constituents identified in its leaf and rhizome extracts. To date, limited scientific investigations have focused on the comprehensive pharmacological profiling of *Olea ferruginea*. The findings of this research not only validate its traditional medicinal use but also highlight its potential as a natural source for the development of low-toxicity, plant-derived therapeutic agents. Further exploration and purification of its bioactive compounds may lead to the discovery of novel, efficacious drugs with a favorable therapeutic index.

Recommendation

It is recommended that further studies be conducted to isolate and characterize the active phytochemicals in *Olea ferruginea*. Advanced pharmacological and toxicological evaluations should be undertaken to validate its potential as a safe and effective source of natural antipyretic,

anti-inflammatory, analgesic, and antifungal agents.

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Author Contributions

Shakir Ullah: Conceptualization, methodology, software. Lubna Shakir: Writing review and editing. Mohammad Sohail, Iqbal Hussain, Sajid Ali: Investigation, data curation. Ghani Subhan, Naveen Dilawar: Resources, project administration.

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Data Availability Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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