# PHYTOCHEMICAL ANALYSIS AND FT-IR OF LEAF AND STEM EXTRACTS OF OLEA FERRUGINEA ROYLE

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**ABSTRACT:** The latest study was performed to investigate the ethno pharmacological potential of ethnobotanical significant plant, *Olea ferruginea* Royle that is a member of the Oleaceae family. The stem of this plant and leaf powders were macerated in different (polar and non-polar) solvents. The phytochemical study showed the occurrence of saponins, alkaloids, anthraquinonines, flavonoids, minimizing sugars, cardiac glycosides, tannins and terpenoids in reasonable quantities in *Olea ferruginea*, as verified by Fourier Transform Infrared (FT-IR) analysis. Based on the findings of the current research, the traditional use of this targeted plant of the *Oleaceae* family as food, fodder, feed, and medicinal seems acceptable and therefore justifiable.

Key words: O. ferruginea Royle, FT-IR, Oleuropin, Spectroscopy, Antiviral potential, Cytotoxic activity, Cell culture technique.

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# **INTRODUCTION**

Ethnopharmacology is branch of а pharmacology concerned with the identification of medications from plants. Plants are used as renewable resources in pharmacology for a number of reasons, such as, bioactivities isolation, synthesis as well as pharmaceutical development. In the ethnobotanical field, the Indian Subcontinent is regarded as a crucial region. Pakistan is especially rich in medicinal plants and their development is aided by the country's diverse climate and biological zones. Medicinal plants have been identified in approximately 6000 species of plants (Mahmood et al., 2003).

Herbal medications have become extremely popular in both developing and underdeveloped nations as a result of their low negative effects and natural origins. (Verma and Singh, 2008). Plants are utilized in the medicine and the community health in a variety of different ways such as active antimicrobial compounds like Rauwolfia serpent is a resource of reserpine, a tranquilizer used to treat hypertension and anxiety, as a supplemental agents such as stabilizers and binders such as align by sea weeds used in tablets and ointments as binding agents. Plants offer building resources for pharmaceutical manufacturing, like steroidal sapogenins, which are taken from the Mexican Yam and used to make progestin, androgens, cortison and hydrocortisone (Basit *et al.*, 2018). Contagious infections are quickly treated using soil medicines and antimicrobial medicine. A variety of phytoconstituents is applied in combination with organic herbal medications throughout the prevention of multiple contagious diseases (Khan *et al.*, 2021).

Spectroscopy is used to check the existence of different plants secondary metabolites, providing useful information about the quantitative, subjective and composition characteristic of such biomolecules (Hussain *et al.*, 2009). FT-IR is high-precision scientific approach for distinguishing compound components and clarifying structural compounds.

Olive tree and its descendants belong to the Oleaceae dicotyledonous class, which contains 29 genera including roughly 600 known species of the shrubs and deciduous trees. *Forsythieae, Fontanesieae, Jasmineae, Oleeae* and *Myxopyreae* are the tribes that make up the family. The Oleaceae family thrives throughout Malaysia and Asia particularly in temperate and tropical regions (Hashmi *et al.*, 2015). It has eight genera and roughly 30

species in Pakistan, with around 22 of them being cultivated (Grohmann, 1974).

Olea ferruginea Royle, also known as an Indian Olive and Kao that grows upto 10 meters tall and is greyish green in color. Fresh leaf decoction strengthens gums and reduces hoarseness and toothache and pain in throat (Shabir *et al.*, 2015). A large number of bioactive chemicals in olive plants may have good potential like antihypertensive and antioxidant agents (Hansen *et al.*, 1996), anti-bacterial, anti-inflammatory (Nora *et al.*, 2012).

# MATERIALS AND METHODS

#### Physio-chemical Tests.

AlCl <sub>3</sub>	Folin-Ciocalteu	Olive oil
NH4OH	reagent	KCl
$C_{40}H_{56}$	$(HO)_3C_6H_2COOH$	КОН
Bi <sub>5</sub> O(OH) <sub>9</sub> (NO <sub>3</sub> ) <sub>4</sub>	CH <sub>3</sub> COOH	KI
CHCl <sub>3</sub>	HCl	$KNaC_4H_4O_6.4H_2O$
CuSO <sub>4</sub> .7H <sub>2</sub> O	Iodine: I	Rutin: $C_{27}H_{30}O_{16}$
$H_2O$	$Pb(C_2H_3O_2)_2$	CH <sub>3</sub> CO <sub>2</sub> Na.3H <sub>2</sub> O
C <sub>2</sub> H <sub>5</sub> OH	Mg	$Na_2CO_3$
FeCl <sub>3</sub>	$HgCl_2$	NaOH
H <sub>2</sub> SO <sub>4</sub>	C <sub>6</sub> H <sub>14</sub>	NaNO <sub>2</sub>

Plant specimens from the Oleaceae family, *Olea ferruginea* Royle, were taken from the Botanic Garden, Government College University, Lahore. Stem and leaves were used for analysis. Then plant sample was grounded to convert this into the powder form that was dipped into the solvents.

#### **Maceration of Plant's Material**

The estimated amount of finely ground organic material being homogeneously packed in impermeable glass vials and soaked into the corresponding solvent (non-polar and polar) termed menstruum in the maceration technique (Seidel, 2006 and Al-Dahmash, 2021). The plant portion chosen was crucial since it verifies the bioactive phytochemical components extracted.

The followings are the characteristics of a fantastic solvent for plant distillation:

- Negligible combustibility
- Minute toxic effects
- Little problem of the detonation
- Effortlessly reprocessed from evaporation
- Economical

Solvents utilized included the n-hexane, chloroform, ethanol with filtered water, which were ordered by divergence grade by the non-polar to polar. At room temperature the glass tank was kept for nearly 7 - 15 days. The constant shaking helps the solvents to release the relevant chemical ingredients. The substance was purified utilizing filter paper no. 4. Finally, the extracted materials were dried and concentrated using a magnetic stirrer for chloroform and n-hexane ethanol extracts and a lyophilized for the water distillates. After that, the extraction was kept at 20°C. The accompanying

formula was used to calculate the percent extraction yield:

Wt. of dried plant extract

% Extraction yield = ------ ×100 Wt. of powdered plant sample

## **Chemical Tests**

The results of a thorough chemical investigation of the *Olea ferruginea* Royle liquid fuels macerated in various solvents based on their polarity grade were presented.

# **Qualitative Estimations of the Chemical Constituents:**

#### (a) Test for the Alkaloids

### **Dragendorff's Experiment**

Waldi's (1965) design was used to do with the essential premise that an atom of a heavy metal contained inside each individual reagent is coupled to nitrogen, forming a particle matching that is unneglectable. 2mL of the 2mg/mL of plant concentrated being taken to the reaction tube, then took into it from adding 2M HCl 0.2mL and the Dragendorff's 1mL. Presence of the alkaloids confirmed by the production of the orange-brownish precipitations.

## Mayer's Testing

For the specific process, Sethi's (2003) technique was used. In a reaction tube, 1mL of the 2mg/mL plants concentrate being mixed with the 0.2 mL of the 2M hydrochloric acid (HCl) plus 1mL of Mayer's reagent. Occurrence of the alkaloids was confirmed by the production of the cream from the yellow color precipitate.

#### Wagner's Test

For alkaloid analysis, Wagner's technique (1993) was used. 1 mL of plant macerate being added to the test tube, followed by the 0.2 mL 2M HCl with 1 mL of Wagner's solution. The existence of alkaloids was confirmed by the formation of the rosy-cocoa precipitate.

### (b) Test for the Anthraquinones

### Born Trager's Testing.

After Evans, Born Trager's approach was utilized to detect anthraquinonoid (2009). 0.25g of plant extract dissolved in the 5mL diluted sulphuric acid (H<sub>2</sub>SO<sub>4</sub>), boiled for the 2 minutes until boiling point and sieved. Than 2.5mL of chloroform was added to the reaction tube, followed by agitation, resulting in the isolation of a specific natural layer. Finally, the stratification was removed to a new tube containing the 0.5mL of a 10 percent ammonium hydroxide (NH<sub>4</sub>OH) concentration. Regular appearance of red, pink and violet demonstrates that plant macerates tints have anthraquinones.

### (c) Cardiac Glycosides Test:

### Keller-Killiani Test

Method employed from Onwukaeme *et al.* (2007) originally inspired by the Keller-Killiani testing, which was created in the nineteenth century by H. Kiliani and C.C. Keller. In a test tube consist of 0.5mL of 10mg/mL of Plant macerate, 2mL of glacial acetic acid through one strand of the ferric chloride (FeCl<sub>3</sub>) was emptied. Finally, 1mL of the concentrated sulphuric acid was added to the test tube's edge. The existence of the deoxy-sugars was detected by a greenish-blue coloration in acidic corrosive phase, a cocoa brown band just at interface or a violet band beneath to brown ring.

### (d) Test for Flavonoids

## Shinoda Testing

Shinoda test was considered getting Jonathan (2009) plot also for impression of the flavonoids. If the presence of flavonoids was detected, the yellow tinting was gradually converted into the red. In test tube, the 1mL the10mg/mL of plant macerates poured, HCl, 5mL the 95 percent of ethanol and the 0.5 grams magnesium were taken. Existence of the flavonoid in the plant macerate being evaluated by the orangish tint towards the red and reddish to the blood red, then the red towards fuchsia coloration

# Sodium Hydroxide Test

NaOH test was carried out after Bello *et al.* (2011) approach. 5 milligram of plant macerates in the 2mL of the refined water was taken. After a while, the 2mL of the 10% sodium was included in reaction tube. Yellowish shading shows the nearness towards flavonoids so it was next checked by adding diluted acid.

Color was changed from yellowish to the vapid (colorless). 1mL of the 10mg/mL plant's macerate, 5mL of 95 percent ethanol, and 0.5g magnesium were placed into a test tube to protect against the enlargement of a few drops of concentrated HCl.

#### **Ferric Chloride Test**

Mace's approach was used to conduct the FeCl<sub>3</sub> test (1963). In the 2mL of ethanol, 5mg of plant material was got broken up. Few droplets of 10% of the ferric chloride sample were added in just this way. Greenish blue coloring shows presence of the flavonoid.

#### Lead Acetate Test

Ngameni *et al.* (2013) methodology was used. 5mg of plant's macerate was taken in the reactions tube that took from expansion by 10 percent lead acid liquid solutions. The growth of the yellow precipitates confirms nearness of the flavonoids

# **Test for Reducing Sugars**

## (e) Fehling's Test

The trial was presided over by German scientist Fehling (1849). In basic requirements, due to the presence of aldehyde, royal blue copper sulphate liquid was tinted to rosy red accumulates of intractable copper oxide during the assessment. 2mL Fehling's solution was poured into reaction tube along with three plant macerate pellets and test tubes were placed in a water shower at 60°C for a few minutes. Yellow to the brown-red precipitations confirms the presence of the reducing sugar.

## (f) Test for the Saponins

### Frothing Testing

The Akinjogunla *et al.* (2010) approach was used to identify saponins in the plant specimens during investigation. 5ml distilled water plus 0.5g of plant macerate were placed in a test tube, warmed in the water shower and vigorously shaken. Saponins that are identified by bubbles are produced. The bubbles were mixed with three olive oil drops, which resulted in descriptions of the emulsion.

### (g) Testing for Tannins

### **Ferric Chloride Testing**

Evans (2009) has used to indicate the existence of the tannins in the plant material, with the hypothesis that Gallic and elegiac acid were supplied as a result of tannin hydrolysis, which had been transformed to the pyrogalol after sifting and then reacted with the FeCl<sub>3</sub> to produce a greenish to blue shade. 0.25g of plant extract was bubbled with the 5mL of the disinfected water in a test tube and three drops of the 0.1 percent ferric chloride were added to the filtrates. Growth of the tannish caramel is the greenish or the blue-dark tinting to confirms the nearness to the tannins.

#### Matchstick Test

Evans (2009) methodology was used for the individual trial when it was discovered that when matchstick wood is exposed to HCl, the tannins are hydrolyzed, resulting in pink coloration as a result of the phloroglucinol detailing. The matchstick's backside was soaked in the 10mg/mL of plant extract, blown dry and then soaked in concentrated HCl. Finally, the test piece was delivered close to soul's light. Productions of the pink to the hinder edge of the matchstick test is occurrence of tannins.

#### (h) Test for the Terpenoids

#### Salkowski Testing

Following Harborne, the Salkowski method, named for German scientist was considered (1973). 0.5g

of plant concentration and 2mL of chloroform were added to the each reaction tube, followed by expansions of the 3mL concentration.  $H_2SO_4$  form a layer. Yellow to the cocoa pigmentations showed the proximity of the torpenoid.

## Attenuated Total Reflectance (ATR) Fourier Transform Infrared (FT-IR) Spectroscopy:

#### Procedure

Tiny quantity of the powder sample material deposited directly by germanium crystal like an infrared spectrometer with continuous pressure exerted, then infrared absorber data was acquired over a wavelength range of the 4000 cm<sup>-1</sup> to the 550 cm<sup>-1</sup>. Ultrasonic waves travel via an attenuated crystal then travel as traveling waves to that same sample

# RESULTS

Table 1: Chemical	analysis of the stem extra	ct of Olea ferruginea Royle.

Constituent	Phytochemical Test	Stem extracts			
		<i>n</i> -hexane	Chloroform	Ethanol	Aqueous
Alkaloids	Dragendorff's Test	+	+	+	+
	Mayer's Test	+	+	+	+
	Wagner's Test	+	+	+	+
Anthraquinones	Born Trager's Test	-	+	+	-
Cardiac glycosides	Keller-Killiani Test	-	+	-	+
Flavonoids	FeCl <sub>3</sub> Test	+	+	+	+
	Lead acetate Test	+	+	+	+
	NaOH Test	+	+	+	+
	Shinoda Test	Flavonoids	Flavonoids	Flavonoids	Flavonoids
		+	+	+	+
Reducing sugars	Fehling's Test	-	-	-	+
Saponins	Frothing Test	+	+	+	+
Tannins	FeCl <sub>3</sub> Test	Gallic	Gallic	Gallic	Gallic
		+	+	+	+
	Matchstick Test	+	+	+	+
Terpenoids	Salkowski Test	+	+	+	+

Anthraquinones, alkaloids, tannins, saponins, reducing sugars, flavonoids along with terpenoids were checked in all preparations of the *Olea ferruginea* Royle stem macerates. The phytochemical processing is carried out on all the fractions including its *Olea ferruginea* Royle leaf. The results showed that alkaloids, phenolic and flavonoids were present in chloroform, water soluble and ethanol (Mehmood, 2018). Cardiac glycosides are most abundant in chloroform, aqueous soluble and ethanol and least abundant in the n-hexane extracts. Saponins and sugars were found absent in the chloroform extract, although sugars were present in the aqueous macerate. The highest concentrations of terpenoids were

found in the n-hexane solubility macerates, with lower levels in other extracts. In water extract terpenoids were observed absent.

FT-IR examination of the *Olea ferruginea* stem revealed numerous structural features at different peaks, including O-H (polyphenols) at the 3284 cm-1, at 2916 cm-1 C-H (phenols), C-O at 2848 cm-1, at 1730 cm-1 (saponins) and at 1631 cm-1 (flavonoids). Peak 1454 cm-1 showed presence of C-H (terpenes), nitro compounds were observed at 1360 cm-1. Glycosides and esters were confirmed at 2349 and at 1157 cm-1 to 1022 cm-1 esters were examined. Pakistan Journal of Science (Vol. 73 No. 4 December, 2021)

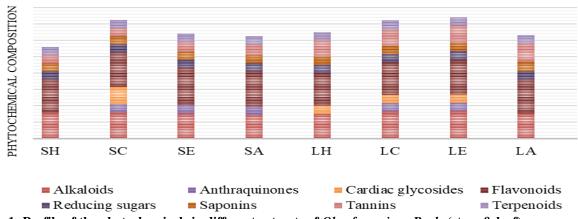


Figure 1: Profile of the phytochemicals in different extracts of *Olea ferruginea Royle* (stem & leaf)

 Table 2: Analysis of chemicals in the leaf macerates of Olea ferruginea Royle

<b>O A</b> <sup>1</sup> <b>A A</b>	Phytochemical Test -	Stem extracts			
Constituent		<i>n</i> -hexane	Chloroform	Ethanol	Aqueous
	Dragendorff's Test	+	++	+	++
Alkaloids	Mayer's Test	+	++	++	+
	Wagner's Test	++	++	+	+
Anthraquinones	Born Trager's Test	-	++	+	-
Cardiac glycosides	Keller-Killiani Test	+	+++	++	++
Flavonoids	FeCl <sub>3</sub> Test	++	+	++	+
	Lead acetate Test	++	++	++	++
	NaOH Test	+	+	+	+
Shinoda Test	Shinoda Test	Flavonoids	Flavonoids	Flavonoids	Flavonoids
	Shinoda Test	+	+	+	+
Reducing sugars	Fehling's Test	+	-	++	++
Saponins	Frothing Test	+	+	+	+++
Tannins	FeCl <sub>3</sub> Test	Gallic	Gallic	Gallic	Gallic
		+	+++	+++	+
	Matchstick Test	-	-	+	+
Terpenoids	Salkowski Test	+++	+	+	-

 Table 3: FT-IR Peak Values and Functional groups

Wave number	Bonds & compounds	
3290	O-H Polyphenols	
3280	-	
3275	-	
3273	-	
2919	C-H Methylene alkanes	
2918	Phenols	
2916	Phenols	
2850	C-H Terpenes	
2848	C-O Carboxylic acid	
2357	C=O Glycosides	
2355	C=O Glycosides	
2353	C=N Nitriles	
2351	C=N Nitriles	
1730	C=O Saponins	
1728	Quinones	
1633	Primary amines	

1631	C=O Flavonoids
1612	Unknown
1602	Alkenes
1600	Primary amines
1514	N-H Alkaloids
1454	C-H Terpenes
1315	Nitro compounds
1305	S=O Sulphate esters
1234	C-N Amines
1232	C-N Amines
1157	C-O Esters
1155	C-O Esters
1012	C-O Esters

In leaf extracts *Olea ferruginea* FT-IR research revealed that the different functional sets such as the O-H at the peak 3273 cm<sup>-1</sup>, C-H at 2918 cm<sup>-1</sup>, at 2848 cm<sup>-1</sup> carboxylic acid, C=N at 2351 cm<sup>-1</sup>, C=O at 1728 cm<sup>-1</sup>, C-N at 1633 cm<sup>-1</sup> and N-H at 1514 cm<sup>-1</sup> were stretched. Peaks at 1155 cm<sup>-1</sup> to 1016 cm<sup>-1</sup> indicated the presence of polyphenols and esters (Fig 4).

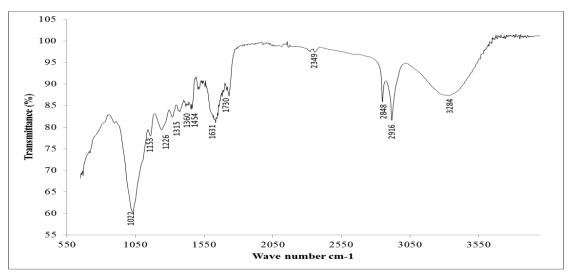


Fig 2: FT-IR spectra of Olea ferruginea stem

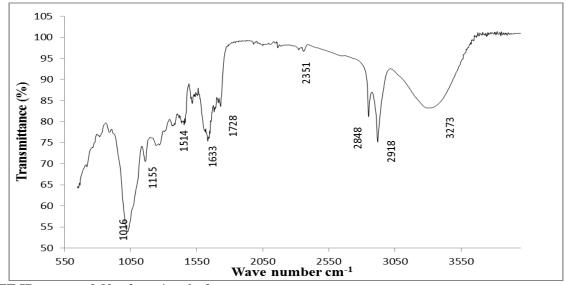


Fig 3: FT-IR spectra of Olea ferruginea leaf

*Olea ferruginea* Royle extract contained alkaloids, flavonoids, glucose and fructose, tannins and saponins but the degrees of the existence differed by one extract to the next (Malik, 2015). Alkaloids have been linked to pharmacological and biological activities like antibacterial, anticancer, spasmolytic, insecticidal, herbicidal, antiviral, anti-oxidant and anti-inflammatory properties, in addition to their cytotoxic effect (Kokoska *et al.*, 2002). Because of their capacity to operate as free radical scavengers, metal chelating activities or the electron and the hydrogen donation capacity, alkaloids were known to have antioxidant properties (Singh *et al.*, 2012).

Flavonoids with a yellow tint can be applied to screen atherosclerosis and hypertension (Nailiet et al., 2010). Because of the placement of functional groups around the nuclear structure, flavonoids may be considered potent antioxidants (Montoro et al., 2005). Tannins can be extracted from a variety of plant components and while a few can be found throughout the aqueous solution of certain plants, they are most commonly produced by treating them with less solvent. Tannins are employed in the leather industry and contain antiviral properties. (Lin et al., 2004), antibacterial (Funatogawa et al., 2004). The interloping of tannin compounds with bacterial cell walls inhibited bacterial growth, causing bacterial colonies to disintegrate. (Erasto et al., 2004). Tannins are anti-inflammatory that work as an antidote in the treatment of alkaloid poisoning, gonorrhea, leucorrhoea, piles, diarrhea and burns (Akinmoladun et al., 2007). Saponins are present in the hemolytic and the antibacterial activates (Sparg et al., 2004). Saponins protect the membrane bilayer against free radicals that promote membrane integrity by inhibiting the generation of oxygen free radicals, preventing cellular malfunction (Akinpelu et al., 2014). Cardiac glycosides have been shown to apoptotic and anti-proliferative effects in cancer cell lines such as leukemia, melanoma and neuroblastoma, melanoma, renal adenocarcinoma, but with much less adverse aftershocks than typical cytotoxic medicines. (Lopez-Lazaro, 2005). Anthraquinons also reported as the antibacterial many times (Dastgir et al., 2012). Terpenoids have been shown to own the capacity to repair wounds and irritated mucus membranes as well as protect blood fluids from oxidative stress. (Okwu and Josiah. 2006).

The FT-IR analysis of the *Olea ferruginea* powders and the leaf were utilized to document the presence of the functional groups within those plant components. Similarity and variation seen between various portions of *O. ferruginea* are recognized based on functional categories and fingerprint traits of peak positions. In present research different function sets identified like as C=O, C-H, C-O, C-N, C=N, N=O and C-O at the different absorption peaks such as the 3290

cm<sup>-1</sup>-3273 cm<sup>-1</sup>, 2919 cm<sup>-1</sup>- 2916 cm<sup>-1</sup>, 2850 cm<sup>-1</sup>-1305cm<sup>-1</sup>, 1234cm<sup>-1</sup>- 1232cm<sup>-1</sup>, 1157cm<sup>-1</sup> and 1012 cm<sup>-1</sup> are responsible for the formation of the deoxyribose. alcohols, anhydrides and alkyl groups (Sohrabi et al., 2005). Present investigation shows an agreement to Mariswamy et al., 2012 who studied the FT IR research on Aervalanata (L.) Juss.Ex Schulte. The current results are in line to the Maobe and Nyarango, 2013 and Bobby et al., 2012 who presented these functional sets in the Utricadioica and leave Albizialebbeck Bent had various relevant absorption edges respectively. *Oleuropein* is chemical in the Olea plant and related along with the antibacterial activities so it may be because of its surfaceactivity qualities which lead cell membrane to bacteria. Tannins may involve to inactivation of the microbial enzyme and proteins into the cell envelope with formation of the complex with the polysaccharides (Devprakash et al, 2011). Current studies of tannins present in the plant extracts can be considered to antibacterial action.

# CONCLUSION AND RECOMMENDATIONS

The plant from the family Oleaceae, namely Olea ferruginea Royle was used in the current research. Maceration of the examined plants' stem and leaf powder was carried out using n-hexane, chloroform, alcohol, and distilled water as solvents. O. ferruginea stem aqueous extracts had the highest percent extraction yield of any plant. Different phytochemical analysis showed the presence of alkaloids, anthraquinonines, tannins, *flavonoids*, reducing sugars, cardiac glycosides, saponins, and torpedoed at higher concentrations in alcohol and aqueous extracts of *O. ferruginea*. FT-IR analysis revealed the functional groups of various compounds and verified the presence of all of these chemical components in the tested plant. In comparison to O. ferruginea, the stem ethanol and n-hexane extracts had the highest antibacterial activity. Different extracts of examined plants showed a negative association among inhibition zones and Minimum Inhibitory Level. At 1.25mg/mL, stem n-hexane extracts of O. ferruginea inhibited growth. The O. ferruginea that was shown to have higher antioxidant potential. The highest phenolic content was stable in the leaf n-hexane extract. Olea ferruginea leaf ethanol extract showed high total antioxidant activity. The current research has shown the significance of the herbal route for the efficient diagnosis of specific illnesses caused by bacteria and viruses due to their enormous potential pharmacological actions. The phytochemicals in the examined plant, O. ferruginea, need to be popularized for widespread use and adoption so that their maximum capabilities for livestock health and productivity may be achieved.

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