



Research Article

Antimicrobial Activity of *Olea ferruginea* Leaf Galls Extractsa

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Abstract | The aim of this study was to determine and compare the extraction potential and *in vitro* antimicrobial activity of methanol, ethanol, chloroform, aqueous and acetone extracts of *Olea ferruginea* leaf nut galls against bacteria and fungi of gastrointestinal tracts. Cold maceration procedure was used to check the extraction potential of the active biomolecules. Qualitative Phytochemical screening for primary and secondary metabolites were carried out for all of the extracts and were analyzed for the presence of active constituents such as flavonoids, terpenoids, tannins, carbohydrates, proteins, steroids and saponins. Various extracts of *Olea ferruginea* leaf nut galls were screened for antimicrobial activity at 100mg/ml concentration level against four bacterial species including two gram positive bacteria (*Staphylococcus aureus* and *Streptococcus mutans*), two gram negative bacteria (*Escherichia coli* and *Salmonella typhi*) and one yeast species (*Candida albicans*) using agar well diffusion method. Among the extracts, methanol was found best solvent for extraction followed by acetone. Qualitative Phytochemical screening of leaf nut galls extracts confirmed the presence of primary and secondary metabolites i.e., tannins, steroids, terpenoids, proteins, carbohydrates, saponins and flavonoids. Results of antimicrobial activity demonstrated that various extracts were active against the selected test microorganisms. The acetone and ethanol extracts were most active against all the tested microorganisms. *Candida albicans* was most susceptible (36.00 mm) in acetone extract among the handpicked microorganisms to different crude extracts of nut galls, followed by *Staphylococcus aureus* (34.00mm) in acetone extract, *Streptococcus mutans* (33.00mm) in acetone extract, *Salmonella typhi* (32.67mm) and *Escherichia coli* was found least susceptible to nut galls extract (27.00 mm) in ethanol. However Chloroform extract was found comparatively least effective against microorganisms. In conclusion, *Olea ferruginea* leaf nut galls showed promising phytochemicals and antimicrobial properties, making them a potential source for further research in developing antibacterial and antifungal agents.

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Introduction

Throughout history, humans have delved into the natural world, specifically medicinal plants, in their quest for novel pharmaceuticals. Approximately 80% of the global population has relied on medicinal plants for their fundamental health needs. Phytochemicals, which naturally occur in plants, leaves, vegetables, and roots, operate as a defence mechanism and safeguard against various diseases. They also impart distinct sensory attributes and colour to the plants (Wadood *et al.*, 2013). In contemporary times, pathogens have developed resistance to antibiotics, prompting the exploration of herbal remedies. To address this demand, a recent study was conducted to investigate the phytochemical composition and antimicrobial properties of extracts from *Olea ferruginea* leaf nut galls.

Olea ferruginea (Oleaceae) is commonly known as wild Olive. Nut galls arise on the lower surfaces of the leaves caused by gall midges or gall flies (Naveed *et al.*, 2012). Various plants and galls have been screened and reported to possess antimicrobial and antioxidant activity e.g., first ever report on olive leaf nut gall caused by gall midges or gall flies (Naveed *et al.*, 2012), Phytochemical content and antioxidant activity of *Q. infectoria* galls (Kamarudin *et al.*, 2021), Gall of *Pistacia integerrima* for antimicrobial activity (Ramachandra *et al.*, 2010) Phytochemistry and pharmaceutical properties of *Olea europaea* (Hashmi *et al.*, 2015) and leaves and bark of *Olea ferruginea* Royle (Mehmood and Murtaza, 2018). As no such study is done on the *Olea ferruginea*, so the aim of this project work was to investigate phytochemical profile and antimicrobial efficacy of extracts derived from *Olea ferruginea* leaf nut galls.

Materials and Methods

Chemicals

Benedict solution, Molisch reagent, Iodine solution, Distilled water, Bromine water, Sodium carbonate (Na_2CO_3), Acetic Acid, Sulphuric acid, NaOH, HCl, HNO_3 , Lead acetate, Chloroform, Ferric chloride (FeCl_3), Methanol, Acetone, Ethanol.

Test micro-organisms

Fungal strain: *Candida albicans*

Bacterial strains

Gram (-ve) bacterial strains

- *Escherichia coli*
- *Salmonella typha*

Gram (+ve) bacterial strains

- *Streptococcus mutans*
- *Staphylococcus aureus*

Plant collection

The leaf nut galls of *Olea ferruginea* were collected from Palosasar Shamshaki District Karak KP-Pakistan. The test microorganisms and antimicrobial activity was performed by PCSIR.

Preservation and grinding

Leafnut galls were dried in the lab at room temperature. Dried mass was ground by electric grinder and stored in air tight bottle.

Extraction

The extraction process of crude plant drugs involves isolating bioactive compounds from plant material using appropriate solvents and extraction techniques (Evens, 2009). The extraction process followed the cold maceration technique with a selection of five dissimilar solvents: Water, ethanol, methanol, acetone, and chloroform. 10g of leaf gull powder were soaked in 200ml for 10 days. They were regularly shaken after 12 hours and filtered with filter paper. The filtrate was evaporated at room temperature and in water bath to remove extra solvent and dried to semi-dried crude extracts (Wado *et al.*, 2022).

Estimation of the extraction yield

The extraction yield (%) was calculated using the formula:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the extract after evaporating solvent and freeze drying}}{\text{dry weight of the sample powder}} \times 100$$

Phytochemical analysis

Phytochemical analysis of aqueous, methanol, ethanol, acetone and chloroform extract of the screened olive leaf galls were done for the presence or absence of active bioactive secondary metabolites or different components such as Carbohydrates, Proteins, Tannins, Saponins, Steroids, Flavonoids, and Terpenoids following the procedures of Evans (2009), Khalid *et al.* (2018), Yadave and Agarwala (2011) and Banu and Cathrine (2015).

Table 1: *Phytochemical screening of various crude extracts obtained from the nut galls of Olea ferruginea.*

S. No	Phytochemicals	Tests	Extracts results				
			Aqueous extract	Methanol extract	Ethanol extract	Acetone extract	Chloroform extract
1	Tannins	Ferric chloride test	+++	+++	+++	+++	+
		Bromine water test	+	+++	+++	+++	-
		Lead acetate test	+++	+++	+++	+++	-
2	Carbohydrates	Mulish test	+	+++	+	+	+++
		Iodine test	+	++	-	+++	++
		Benedicts test	+++	+++	++	+	-
3	Protein	Xanthoprotic test	+++	++	+++	+++	-
4	Saponins	Test for Saponins	-	+++	+++	-	-
5	Terpenoids	Test for terpenoids	+++	+	+++	+	+
6	Steroids	Test for steroid	+	-	+++	+++	+
7	Flavonoids	Test for flavonoids	+++	+++	+++	+++	+++

Note: “+”least intense, “++” moderate amount.

Antimicrobial activity

The antimicrobial effectiveness of different organic and inorganic solvent extracts was evaluated using the agar well diffusion method. The test microorganisms were initially cultured in nutrient broth for 24 hours before the experiments. To create the agar plates, nutrient agar (Mueller-Hinton Agar) was melted, cooled to 40°C, and poured into sterilized petridishes wells were formed in the agar using a sterile metal cork borer. The microorganisms were streaked onto the agar plates using a non-pathogenic cotton swab. This process was repeated three times, with the plate rotated by 60° between each streaking. Following this, approximately 1ml of the respective extracts (at a concentration of 100mg/ml), dissolved in DMSO, was added to the wells and allowed to diffuse at room temperature. The plates were then incubated at 37°C for 24 hours. As a standard for gall extracts of different solvents, ciprofloxacin at 25µg/ml was used as control. The diameter of the zone of inhibitions was measured in millimeters.

Statistical analysis

The experimental outcomes were expressed as mean ± Standard deviation (STD) from three repetitions in each trial. Statistically ANOVA one way and Dunnett’s tests were applied to data for analysis in MS Excel 2007.

Results and Discussion

Estimation of the extractive yield

Extractive values are crucial in medicinal plants because they determine the concentration of bioactive

compounds, such as alkaloids, flavonoids and phenolic compounds, which possess therapeutic properties. Extractive values of olive leaf nut galls can vary depending on the solvent used for extraction and the specific components being targeted. Here extractive values are expressed in %. The most extractable solvent was methanol followed by acetone while the rest of the solvents showed moderate extraction i.e., 7.7% for ethanol, 6% for aqueous and 4.7% for chloroform.

Phytochemical analysis

Phytochemical analysis plays a significant role in drug development by identifying bioactive compounds in medicinal plants that can lead molecules for pharmaceutical research. Here’s a brief overview of the phytochemical analysis of olive leaf nut galls.

Olive leaf nut galls are rich in tannins, flavonoids, terpenoids, proteins, etc. tannins are polyphenolic compounds known for their antioxidant and antimicrobial properties (Omar, 2010). Flavonoids are another group of phytochemicals found in olive leaf nut galls. These compounds have various biological activities, including antioxidant, anti-inflammatory and antimicrobial effects (Jemai *et al.*, 2009). These studies provide insights into the phytochemical composition and potential health benefits of olive leaf nut galls.

Antimicrobial assay

The antimicrobial efficacy of diverse extracts of *Olea ferruginea* leaf nut galls was carried out at a 100mg/mL concentration level against different bacterial and fungal strains, i.e. *Escherichiacoli*, *Salmonella*

typha, *Staphylococcus aureus*, *Streptococcus mutans*, and *Candida albicans* using the well diffusion method. The bacterial strains were gram positive and gram-negative species that often cause infectious diseases. The result from the different solvents extracts of *Olea ferruginea* leaf nut galls against various bacteria and fungi are presented in the above Table 2.

The efficiency of different extracts was expressed by measuring the zone of inhibition in millimeters. Acetonic crude extract was less active against *Escherichia coli* i.e., 15.67 ± 1.53 mm however it showed appreciable antimicrobial activity against all other strains. Ethanolic extract induced higher bactericidal activity against *Escherichia coli*. It also showed a considerable amount of activity against rest of the strains. Methanolic and acetonic extracts also inhibited the growth of the *Streptococcus mutans* i.e. (32.00 ± 2.00 , 33.00 ± 1.00 mm), respectively. Acetone and ethanol crude extracts also showed greater efficiency against *Staphylococcus aureus* bacteria (34.00 ± 1.00 , 25.67 ± 0.58 mm). Aqueous extract induced higher antimicrobial activity against *Escherichia coli*, *Candida albicans* and *Salmonella typha* (21.67 ± 1.53 , 24.00 ± 1.00 mm), respectively. It also showed a greater amount of activity against *Candida albicans* (24.00 ± 1.00 mm). Chloroform extract inhibited the growth of any of all the tested organisms less efficiently. The highest activity (36.33 ± 1.53 mm) was found for acetone crude extract against *Candida albican*.

The treatment of diseases through plant derived medicines is immemorial. According to (WHO) the qualities of safety, efficacy, low-cost rate and easily availability have made natural products as an important source in killing primary health problems on earth (Hussain *et al.*, 2023; Atta *et al.*, 2023). The research employed two categories of test microorganisms:

Bacterial and fungal strains. Specifically, the study focused on four bacterial variations (two positive and two negative) and one type of fungus. To assess phytochemicals screening and antimicrobial activity, five solvents of variable polarity were utilized.

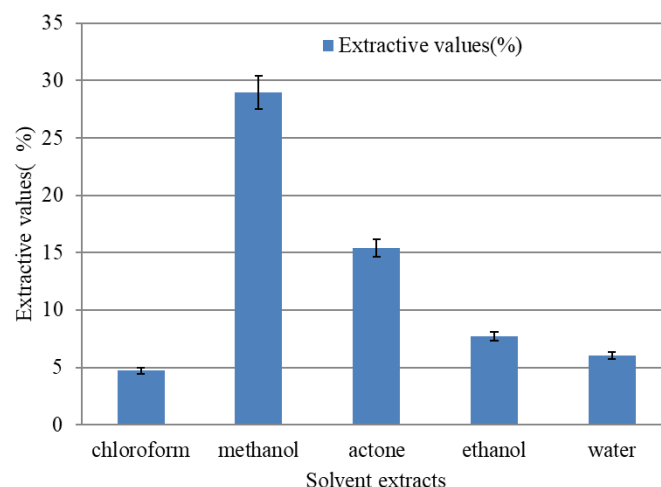


Figure 1: Graphical representation of % yield of various crude extracts of *Olea ferruginea* leaf nut galls .

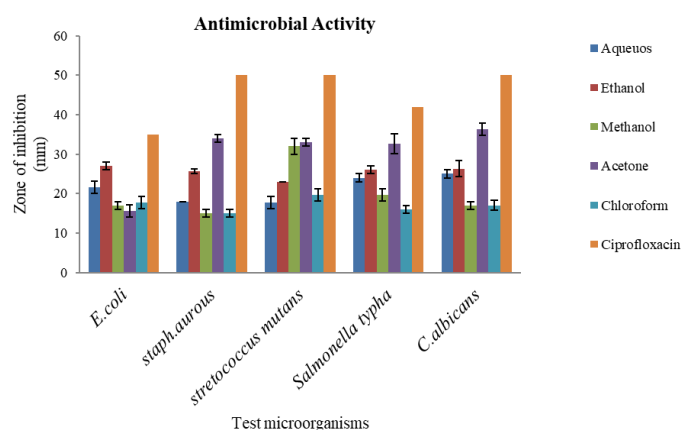


Figure 2: Microbial inhibition exhibited by various crude extracts against the specified microorganisms.

Atta *et al.* (2023) explored the extraction yield using distilled water, methanol, and ethyl acetate extracts of different nine plants by sonication assisted maceration

Table 2: Zone of inhibition(mm) of different crude extracts of *Olea ferruginea* leaf nut galls against selected microorganisms.

Strains	Aqueous extract	Ethanol extract	Methanol extract	Acetone extract	Chloroform extract	Ciprofloxacin (25µg)
<i>Escherichia coli</i>	21.67 ± 1.53	$27.00 \pm 1.00^{***}$	17.00 ± 1.00	15.67 ± 1.53	17.67 ± 1.53	35
<i>Staphylococcus aureus</i>	18.00 ± 0.00	25.67 ± 0.58	15.00 ± 1.00	$34.00 \pm 1.00^{***}$	15.00 ± 1.00	50
<i>Streptococcus mutans</i>	17.67 ± 1.53	23.00 ± 0.00	32.00 ± 2.00	$33.00 \pm 1.00^{***}$	19.67 ± 1.53	50
<i>Salmonella typha</i>	24.00 ± 1.00	26.00 ± 1.00	19.67 ± 1.53	$32.67 \pm 2.52^{***}$	16.00 ± 1.00	42
<i>Candida albicans</i>	24.00 ± 1.00	26.33 ± 2.08	17.00 ± 1.00	$36.33 \pm 1.53^{***}$	17.00 ± 1.29	50

Note: Values are expressed as mean $\pm$ STD.DE. ANOVA followed by Dunnett's post hoc test at alpha level 0.01, 0.05 and 0.10 compared to control group (ciprofloxacin). *** indicated the highest inhibition zone in all of the extracts against selected test micro-organisms.

method. Out of this methanol extract showed higher extraction efficiency. And greater extractive yield may not always refer to definite biological inhibition and more pronounced activity may be observed in lower yield of extracts. This depends upon the nature of the extracted phytochemicals.

In the current study using distilled water, methanol, acetone, ethanol, and chloroform were used as a solvent for cold maceration process. The extraction efficiency of methanol was higher in all the extracts followed by acetone. Chloroform has least extractive value and acetone show more pronounced antimicrobial activity followed by ethanol. Tannins and terpenoids are being assessed for their potential as analgesic and anti-inflammatory agents. Tannins also heal the wounds and inflamed mucous membrane (Thomas and Krishnakumari, 2015; Hussein *et al.*, 2023).

Saponins are cardiogenic in nature and reported to have antifungal and antidiabetic activities. Plant steroids are effective for insecticidal and antimicrobial properties. It also used as nutrition, herbal medicine and in cosmetics (Hussein *et al.*, 2018).

Flavonoids dissolve poorly in water but readily dissolve in organic solvents (Kamarudin *et al.*, 2021). Flavonoids also used as antiviral, antibacterial spasmolytic activities (Hussein *et al.*, 2018). Mehmood and Murtaza (2018) worked on *Olea ferruginea* Royle leaves and bark extracts. The results showed that both leaf and bark chloroform extracts did not stop the growth of any test species, while in present study, leaf nutgall of chloroform extract exhibited considerable inhibition values for microbial growth but least among all of the other extracts.

Ramachandra *et al.* (2010) reported from the investigation of *Pistacia integeryima* leaf gall using ethanol and aqueous extract. The ethanol extract appears to more potent in antibacterial activity than the aqueous extract. Ethanol extract showed highest suppressive impact on the growth of *Escherichia coli* in the current investigative work. This is because of two reasons: First one is the nature of biologically active chemicals and secondly the stronger capacity of extraction of corresponding active constituents of antibacterial activity. Studies on antibacterial properties of medicinal plants by Adil *et al.* (2020), Hashim *et al.* (2022) and Noori *et al.* (2023) are in support of our current studies. For anti-fungal activity,

Hussein *al.* (2018) reported the antifungal activity of *Q. infectoria* galls against *Candida albicans* at different concentrations. The highest antifungal activity was noted as 28mm at concentration of 100mg/ml and 20mm at 6.25mg/ml. The present study illustrating that inhibition zone value of *Candida albicans* was high in all of the extracts than the others test microorganisms (36.33mm in acetone extract specifically). This high antifungal activity of all the experienced extracts is associated with the flavonoids and other phenolic content that are confirmed by phytochemical screening (Atta *et al.*, 2023).

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Novelty Statement

This study pioneers the investigation of *Olea ferruginea* leaf gall extracts, revealing their potent antimicrobial properties and underscoring their potential as a natural remedy against microbial infections.

Author's Contribution

All authors contributed to the conception and design of the study. Madeeha collected the plant material. Madeeha, Jalwa and Ayosha processed the plant material, while Dr. Gul Rose and Dr. Abdul Haq conducted the antimicrobial activity tests. Dr. Shahida Naveed designed and supervised the whole research activity. Madeeha, Jalwa and Ayosha analyzed the data. Zunaira helped in outline of the manuscript. InayatUllah helped financially in this research activity. All authors reviewed and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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