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Phytochemical and Physicochemical Analysis of the Invitro Antimicrobial and Antifungal Activities of *Juglans Regia* L. Bark (Walnut Bark)

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Abstract: Preliminary phytochemical and physicochemical screenings of *Juglans regia* are conducted to assure its purity, safety, and efficacy, as well as to determine the antibacterial and fungal activities and inhibitory zones of extracts against certain bacterial and fungal strains, with the aim of overcoming resistance. *J. regia* Linn, also known as *akhrot* in Pakistan, is a member of the Juglandaceae family, with a dense, long-lasting bark that is fragrant and resinous. The tree has a history of medical usage in the treatment of multiple ailments. The present research focuses on using preliminary phytochemical and physicochemical analyses to standardize *Juglans regia* Linn bark and to explore its antibacterial and antifungal activities. These analyses were conducted through microscopic analysis of the bark and determination of its quality parameters. Four different extracts of *J. regia* bark were tested for antimicrobial activity against a variety of harmful fungi and bacteria. Phytochemical analysis indicated the existence of reducing sugars, alkaloids, tannins, phenols, steroids, and saponins. Stone cells with sclereids, phloem fibers, oil cells, and lignified fibers are some of the observed microscopic characteristics of the powder. Antimicrobial analysis of *J. regia* bark extracts at concentrations of 300 and 400 μg/ml revealed that the ethanol and chloroform extracts had a considerable inhibitory effect on the development of pathogenic bacteria and fungi. This research will serve as a reference point for correctly identifying crude medications. It will also support the use of natural products as medications and validate the antibacterial and antifungal properties of *J. regia* (walnut bark) through clinical trials.

Keywords: antimicrobial activity, antifungal activity, *Juglans regia*.

胡桃树皮（核桃皮）体外抗菌和抗真菌活性的植物化学和物理化学分析

摘要：对核桃进行初步植物化学和物理化学筛选，以确保其纯度、安全性和有效性，并确定提取物对某些细菌和真菌菌株的抗菌和真菌活性和抑制区，以克服耐药性。J.帝王，在巴基斯坦也被称为阿赫罗特，是胡桃科的一员，树皮致密，树皮持久，芳香而树脂质。这棵树在治疗多种疾病方面有医学用途的历史。本研究的重点是使用初步的植物化学和物理化学分析来标准化胡桃树皮并探索其抗菌和抗真菌活性。这些分析是通过对树皮进行显微镜分析并确定其质量参数来进行的。测试了四种不同的王树皮提取物对多种有害真菌和细菌的抗菌活性。植物化学分析表明存

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在还原糖、生物碱、单宁、酚、类固醇和皂苷。具有硬化的石细胞、韧皮部纤维、油细胞和木质纤维是观察到的粉末的一些微观特征。对浓度为300和400微克/毫升的王树皮提取物进行抗菌分析表明，乙醇和氯仿提取物对病原菌和真菌的生长具有显着的抑制作用。这项研究将作为正确识别生药的参考点。它还将支持使用天然产品作为药物，并通过临床试验验证J.帝王（核桃皮）的抗菌和抗真菌特性。

关键词：抗微生物活性，抗真菌活性，核桃。

1. Introduction

Juglans regia Linn, also known as *akhrot* in Pakistan, is a part of the Juglandaceae family and is native to Eastern Europe and Northern Asia [1]. It is a woody, deciduous frost-tender tree that can grow as high as 20 meters [2]. Its dense, long-lasting bark is fragrant and resinous. This tree has a long history of use in the treatment of several diseases [3], with almost every part of the plant having a traditional medicinal use. Anthelmintic, astringent and detergent properties are found in the root and stem bark [4]; the dried stem bark can be used for tooth cleaning [5–6].

Herbal medicine is famous for its therapeutic variety among the general public. Plants have been utilized for medicine since the dawn of history because they meet urgent personal needs and are readily available and affordable. Plants have been used to heal numerous ailments in humans and animals for thousands of years in places all over the world [7]. Herbal medications have the potential to be a superior alternative to current synthetic treatments, as they have few negative outcomes and are thought to be comparatively safe [8].

Herbal formulations typically include the use of either dried or fresh plant materials. Proper information about these crude drugs is a critical part of the manufacture, safety, and efficacy of herbal products. Phytochemistry is a straightforward and reliable approach for obtaining detailed information about crude medicine [9].

Proper management of starting material is essential for ensuring the repeatable quality of herbal medications. Authentication is the initial step towards ensuring this quality for the beginning material, and it is followed by the creation of numerical values for standard comparison [10]. Pharmacognostical characteristics for ease of identification, such as leaf constant, microscopy, and physicochemical studies, are just a few of the essential protocols for herbal standardization [11].

Over the past few years, there has been an upsurge in the number of reports of individuals suffering unfavorable health effects as a result of using herbal medication. A range of reasons for this difficulty have been discovered through research and analysis [12]. Poor-grade herbal drugs and raw medicinal plant materials are closely linked to these most common causes of reported side effects [13]. Only by properly documenting plants' botanical and physiochemical features and medical uses in the form of monographs can traditional knowledge about them be passed down to future generations [14]. Such plant monographs are written in accordance with WHO principles and offered as herbal pharmacopoeia. The plant material can be identified, authenticated, examined for adulterants and standardized using these principles [15].

The focus of this research is on the use of preliminary phytochemical and physicochemical analyses to standardize Juglans regia Linn bark and determine its antifungal and antibacterial activities.

1.1. Antibacterial and Antifungal Activities

The antibacterial and antifungal activities of extracts taken from different parts of the walnut plant been researched.

The antibacterial activities of walnut phenolic compounds have long been investigated. The aqueous extract of a walnut husk was evaluated against *S. aureus* and *E. coli*, and the end outcomes were very promising. Several studies have also shown that various components of the walnut have antibacterial capabilities [16].

Traditional medicines have employed *J. regia* seeds as bactericidal, anti-parasitic, and anti-diarrheal agents. The antibacterial activities of ethyl-acetate, di-chloromethane, methanol, and the aqueous extracts of the *J. regia* epicarp and seed coat were cross-examined against gm-positive and gm-negative bacteria [19]. A walnut husk was tested for antibacterial movements against gm-positive and gm-negative bacteria, as well as fungus. Gm-positive bacteria, such as *Staphylococcus aureus*, could be found anywhere in the extract. This finding suggests that walnut green husks may become a viable source of antibacterial agents [20].

On diverse *Candida* species, researchers have studied the powerful anti-fungal effects of *Salvador apersica* and

J. regia bark. *The in vitro* activity against *Candida* species was tested via different solutions of fresh and dried *Salvador apersica* and *J. regia* bark [21]. These plants were chosen because they have a long history of being used to treat mouth and fungal diseases. The findings of this study reveal that the use of *J. regia* bark can aid developing countries in limiting oral infections, particularly oral *candidiasis* [22].

These findings indicate that plant material may be exploited as a low-cost, easily available source of natural antifungal and antioxidant agents [23].

Previous research examined the antioxidant and antifungal properties of natural and treated *J. regia* L. bark on a variety of vaginal *Candida* and *Candida* type strains [24]. In-vitro activity against vaginal *Candida* strains were tested using methanol, water, ethyl acetate and dil. solvent acetone extracts of natural and dyed *J. regia* barks [25].

2. Methodology

2.1. Plant Material Collection

This study employed fresh and dried plant samples. Fresh walnut was obtained from a botanical shop in Karachi, Pakistan, and confirmed by the Department of Botany, University of Karachi, Pakistan. After washing and drying the bark, it was milled into a fine powder.

2.2. Extract Preparation

Powdered Juglans bark was extracted in methanol, chloroform and water (100 g test material in 500 ml of each solvent). The extract was then filtered and evaporated using a rotary vacuum evaporator under vacuum at 40°C. The remnants were refrigerated until further testing [26].

2.3. Collection of Microorganisms

Different walnut extracts were evaluated for antibacterial activity against gm (–ve) bacteria *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and gm (+ ve) bacteria *S. aureus* (ATCC 23235).The bacteria were reactivated from stock cultures and kept at 37°C for 18 hours in nutrient broth.

The fungi employed in this investigation were *Aspergillus niger* (*ATCC 16404), Candida albicans* (ATCC 10231), and *Aspergillus fumigates* (ATCC 9197). Dermatophytes are usually caused by this fungus, causing human diseases [27].

2.4. Nutritional Analysis

2.4.1. Moisture Content Determination

The AACC approach was used to determine the moisture content. The 2 gm sample was placed in a pre-heated and weighted glass Petri plate, dried for 2 hours at 130°C in a hot air oven, then placed in desiccators until it became cool. The Petri plate was reweighted. The moisture content percentage was calculated using the formula below [27].

*Moisture content (%) = w1-w2/weight of sample*100*

where w1 - sample weight (g) before drying, w2 - sample weight (g) after drying.

2.4.2. Ash Content Determination

Ash content is determined by using the AACC technique. An amount of 2 g of the sample was placed in a pre-weighed crucible, and it was allowed to incinerate in a muffle furnace uncovered for 4 h at 820°C. The crucible was then cooled in a desiccator and weighed again. The ash content is determined by using the following formula:

*ash (%) = weight of ash/weight of sample*100*

2.5. Physicochemical Testing of Active Powdered Juglans Regia Bark

2.5.1. Physical Appearance Analysis

Material used: Powder, black paper sheet.

Equipment used: Magnifying glass.

Method: Weigh a small amount of powder in a black paper sheet and observe keenly the appearance of *J. regia* powder with a magnifying glass.

2.5.2. Water Content Determination

Material used: Methanol (Merck Cat No 106012), Karl Fischer reagent-5 (Merck Cat No 109248).

Equipment used: Karl Fischer, weighing balance.

Method: Transfer about 40 ml of dried methanol (Merck Cat No 106012) into a titration vessel. Press the knob to start the neutralization with standard Karl Fischer reagent-5 (Merck Cat No 109248). Observe the drift value; it should always be less than 50. If the drift value is more than 50, restart the instrument to a value of less than 50. After neutralizing, weigh 0.1-0.2 g of the sample to be tested and carefully transfer it into a titration vessel. Run the instrument as per standard operating procedure (QC/PR/OP/036) and note the water content displayed in percentage [27].

2.5.3. Residue on Ignition

Material used: Porcelain crucible, desiccator.

Equipment used: Furnace.

Method: Use 1 g of substance. Ignite a porcelain crucible for 10 min, allowing cooling in a desiccator and weighing (w1). Weigh accurately 1 g of the substance to be tested in a porcelain crucible, again weighing the sample with a crucible (w2). Heat gently until the substance is thoroughly charred, and then cool and

moisten the residue with 1 ml of nitric acid (conc.) and 5 drops of sulphuric acid (conc.). Subsequently, ignite gently until the white fumes no longer appear and then ignite at 750 °C until the black particles disappear. Cool the crucible and weigh (w3). Again ignite for 15 minutes and repeat this procedure until two successive weighings do not differ by more than 0.5 mg.

(w3-w1) X100/ (w2-w1)

2.5.4. Bulk and Tapped Densities

The material used: Powder, measuring cylinder.

Equipment used: Weighing balance.

Procedure: Weigh 5.0 gms of sample and transfer it into a measuring cylinder of 50 ml through a funnel. Note the bulk volume of powder and calculate the bulk density using the formula given below. Then tap the cylinder 20-25 times to remove the trapped air until further changes in the volume are not observed. Record this volume and calculate the tapped density. Bulk and tapped densities are calculated using the formula given below:

Bulk and Tapped Densities = Mass/Volume

2.6. Phytochemical Analysis

2.6.1. Total Phenolic Content Determination

It is determined using the Folin-Ciocalteu assay. The absorbance is estimated at 765 nm, and the results are demonstrated in gallic acid equivalents.

2.6.2. Total Flavonoid Content Determination

The number of flavonoids is assessed by using the aluminum chloride colorimetric method. The absorbance is estimated at 510 nm, and the results are expressed in catechin equivalents.

2.6.3. Determination of Tannins

Tannins are determined by a method described by Robinson and Van-Buren. The absorbance is estimated at 605 nm, and the results are expressed in tannic acid equivalents [28].

2.7. Invitro Antibacterial and Antifungal Testing

2.7.1. Preparation of Inoculum

Anti-microbial activities were performed using fresh bacterial/fungal cultures. The strains' ATCC colonies were inoculated on nutrient broth and Sabouraud dextrose agar and incubated at 37°C for 22 to 24 hours. The turbidity suspension of the actively growing broth culture was adjusted with sterile broth to achieve optical turbidity comparable to the 0.5 McFarland standards. The turbidity was adjusted to match 0.5 McFarland standard barium sulfate, which was made by mixing 0.5 mL, i.e., 1.175 percent w/v (0.048 mol/L) hydrated $BaCl_2.2H_2O$ with sulfuric acid [29].

2.7.2. Antibacterial and Antifungal Susceptibility Test

The anti-microbial screening properties of the herbal extract were examined in-vitro using the well diffusion method on nutrient agar against *S. aureus, E. coli, and P. aeruginosa.* The antifungal activity of the test compounds against *A. niger, Candida albicans*, and *A. fumigates* was also assessed using the agar well diffusion method. Sabouraud dextrose agar was used to cultivate all fungal organisms. The stock solution (500 µg/ml) was made by dissolving the compounds in DMSO and diluting them in various concentrations (100, 200, 300, and 400 µg/ml). A well was bored into the pre- inoculate agar medium using an 8 mm sterile borer in a standard process. Each well 6mm diameter was filled with the test solution and a standard antibiotic and antifungal drug, which was then left at room temperature for 30 minutes to allow the chemicals to disperse.

For bacteria, plates were incubated at 37^{o}C for 24 hours, and for fungus, plates were incubated at 25^{o}C for 72 hours. During this period, the test solution diffused, impacting the development of the injected bacteria. The inhibitory zone was tested three times, and the concentration was noted. The extract inhibition zone was compared with the standard antibiotic ciprofloxacin and ketoconazole for *A. fumigatus, Candida albicans, A. niger*, and *A. fumigatus*.

2.8. Ethical Approval

The study was approved by the Ethical Review Committee of Ziauddin University (Reference Code: 4451021ZAPHA, December 27, 2021).

3. Results

3.1. Microscopy of Powders

Stone cell with sclereid, Phloem fiber, oil cell, and lignified fiber were found in *J. regia* powder, which was brown in color.

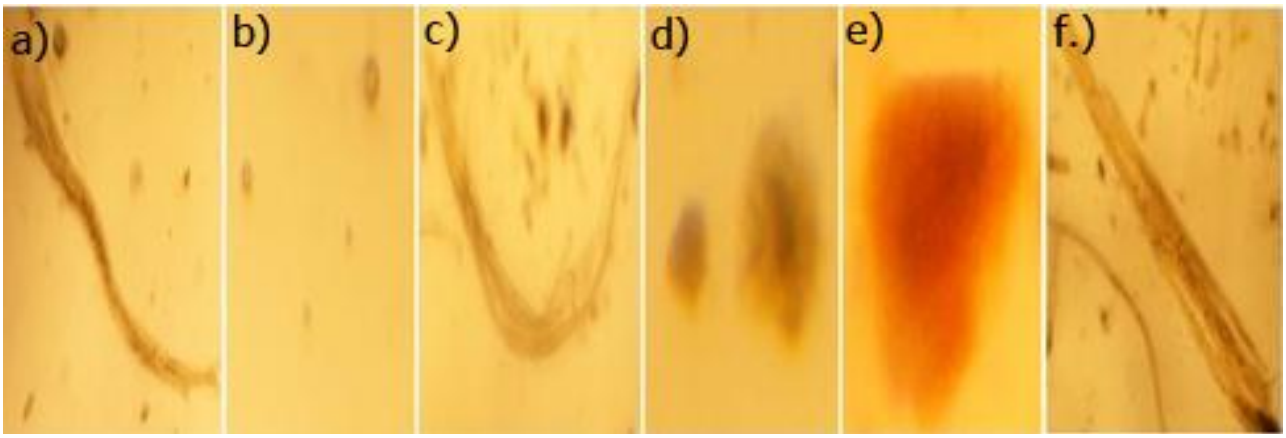

Fig. 1 a). Phloem fiber; b). Oil cells; c). Lignified fibers; d). Stone cells with sclereid; e). Resin cell; f). Septate fiber

3.2. Phytochemical Screening (Preliminary)

A preliminary phytochemical test on the drug's ethanol extract was performed. Reducing sugar, alkaloids, tannins, phenols, steroids, and saponins were all tested on the extract and the results are displayed in Table 1.

Table 1 Results of phytochemical screenings of ethanol-ic extract of J. regia bark

Test	Observations
Reducing sugars	+
Carbohydrates	+
Amino acids	-
Alkaloid	+
Tannins	+
Phenols/ Flavonoids	+
Steroids	-
Saponins	+
Anthraquinones	-

3.3. Physicochemical Analysis

The results of the physicochemical analysis are presented in Table 2. In this test, we analyze the interaction of physical arrangement and composition. It comprises of physical appearance analysis, water content, residue on ignition, total ash percentage, and bulk and tapped density. Ash value percentage essentially shows how pure a drug is, while extractive value indicates the polarity of the compound.

Table 2 Results of physicochemical analysis of dried bark powder of *Juglans regia*

Physicochemical properties	Results
Physical appearance analysis	A light brown to dark brown fibrous powder.
Water content	8.4315%.
Residue on ignition	91.48%
Total ash	9.51%
Alcohol soluble Ash (Extractive Value)	6.03%
Water soluble Ash (Extractive Value)	4.02%
Bulk density	0.224gm/ml.
Tapped density	0.348 gm/ml.

3.4. Antimicrobial Activity

Tables 3-5 present the antimicrobial assay findings of several extracts of *J. regia* bark at different concentrations ranging from 100 to 400 µg/ml. When compared to the chloroform and aqueous extracts, the efficacy of ethanol and methanol extracts has a significant inhibitory effect on the growth of *E. coli* at concentrations of 200, 300, and 400 µg/ml with the average zone of inhibition 16.3-17.9 mm. The sequence of inhibitory effects of various extracts is ethanol extract > methanol extract > chloroform extract > aqueous extract, as shown in Table 3.

When compared to methanol and aqueous extracts, the efficacy of chloroform and ethanol extracts have a substantial inhibitory impact on the development of *S. aureus* at concentrations of 200, 300, and 400 µg/ml, with the average zone of inhibition 20-21.7 mm. Table 4 shows the zone of inhibition of different extracts with the sequence as chloroform extract > ethanol extract > methanol extract > aqueous extract.

Compared with chloroform and aqueous extracts, ethanol and methanol extracts exhibited a substantial inhibitory effect on the development of *Ps. auro* at concentrations of 200, 300, and 400 µg/ml, with the average zone of inhibition 23-25 mm. The order of inhibitory effect is ethanol extract > methanol extract > chloroform extract > aqueous extract) (Table 5).

Table 3 Effect of walnut bark powder extracts of various concentrations on *E. coli*

S.No	SOLVENT USED	CONCERNTRATIONS /E.coli			
1.	Water	100 µg	200 µg	300 µg	400 µg
	Zone of inhibitions in mm	0	0	0	0
2.	Methanol	15	15	16	16
	Zone of inhibitions in mm	14.5	15	15.8	16
		14.8	15.5	16	16
	Average	14.76667	15.16667	15.93333	16
3.	Ethanol	16	16.5	17	17.8
	Zone of inhibitions in mm	16.3	16.4	17	18
		16	16	17.3	18
	Average	16.1	16.3	17.1	17.93333
4.	Chloroform	10	11	13	13
	Zone of inhibitions in mm	10.3	11.5	13	13
		10	11	12.8	13
	Average	10.1	11.16667	12.93333	13

Table 4 Effect of walnut bark powder extracts of various concentrations on *S. aureus*

S.No.	SOLVENT USED	CONCERNTRATIONS *(S.aureus)*			
1	**Water**	**100 µg**	**200 µg**	**300 µg**	**400 µg**
	Zone of inhibitions in mm	12	15	15	16
		12.5	14.8	15	17
		13	14.8	15	16.8
	Average	12.5	14.86667	15	16.6
2	**Methanol**	11	19	20	20
	Zone of inhibitions in mm	10	18.8	20	20.3
		10	19	20	20
	Average	10.33333	18.93333	20	20.1
3	**Ethanol**	19.5	20	20	20
	Zone of inhibitions in mm	19	20	20.3	20.5
		19	20.2	20.4	20.4
	Average	19.16667	20.06667	20.23333	20.3
4	Chloroform	18	18	20	21.8
	Zone of inhibitions in mm	17.5	18	20	22
		17.8	18	20	21.5
	Average	17.76667	18	20	21.76667

Table 5 Effect of walnut bark powder extracts of various concentrations on *Ps. auro*

S.No.	SOLVENT USED	CONCERNTRATIONS *(Ps.auro)*			
1.	**Water**	**100 µg**	**200 µg**	**300 µg**	**400 µg**
	Zone of inhibitions in mm	10.5	13	13	14
		11.5	13	13	14.5
		10.8	13	13	14
	Average	10.93333	13	13	14.16667
2.	**Methanol**	18	19	20	22
	Zone of inhibitions in mm	18	18.8	20	22
		18	19	20	22.3
	Average	18	18.93333	20	22.1
3.	**Ethanol**	20	23	23	25
	Zone of inhibitions in mm	20	23	23.5	25
		20	23.2	23	25
	Average	20	23.06667	23.16667	25
4.	**Chloroform**	15	20	20	21
	Zone of inhibitions in mm	14.8	20.5	21	21
		15	20	21	21
	Average	14.93333	20.16667	20.66667	21

3.5. Antifungal Activities

The greater growth inhibition zone widths for ethanol and chloroform extracts in *C. albicans* (ATCC10231) were 14 and 16 mm, respectively, at 300 and 400 µg/ml concentrations. Compared to ethanol and chloroform extracts, methanol extract showed a lower zone of inhibition, while water had no impact at any concentration (Table 6).

At concentrations of 300 and 400 µg/ml, the highest growth inhibition zone diameters for ethanol and chloroform extracts in *A. fumigatus* (ATCC#9197) were 15 and 18.2 mm and 11-14 mm, respectively. Methanol extract had a lower zone of inhibition than ethanol and chloroform extracts, and water had a very minimal influence at just 400 mg/ml concentration.

In *A. niger* (ATCC#16404), at concentrations of 300 and 400 µg/ml, the highest growth inhibition zone diameters for ethanol and chloroform extracts were 16 and 18.0 mm and 10-12 mm, respectively. In any concentration, methanol and water extracts had no inhibitory effect.

Table 6 Effect of walnut bark powder extracts of various concentrations on *fungus*

Antifungal Activity					
Test	**Fungus**	**EXTRACTS(SOLVENTS)**			
		Water Extract	**Chloroform Extract**	**Ethanol Extract**	**Methanol Extract**
Compound 1	***C. albicans***	**Results (Zone of Inhibition in mm)**			
Clotrimazole (10 mg/ml)	**ATCC10231**	20mm	20mm	20mm	20mm
100 mg/mL		–	10.0 mm	–	–
200 mg/mL		–	11.2 mm	–	–
300 mg/mL		–	12.0 mm	14mm	10.0 mm
400 mg/mL		5mm	14.0 mm	16mm	11.0 mm
Compound 2	***A.fumigatus***				
Ketoconazole (10mg/ml)	**ATCC#9197**	22mm	22mm	22mm	22mm
100 mg/mL		–	–	–	–
200 mg/mL		–	–	–	–
300 mg/mL		–	11.0 mm	15.0mm	12.0 mm
400 mg/mL		8mm	14.0 mm	18.2mm	14.0 mm
Compound 3	***A.niger***				
Ketoconazole (20mg/ml)	**ATCC#16404**	30mm	30mm	30mm	30mm
100 mg/mL		–	–	–	–
200 mg/mL		–	–	–	–
300 mg/mL		–	10.0 mm	16.0mm	–
400 mg/mL		–	12.0 mm	18.0mm	–

4. Discussion

The data gathered from the preliminary phytochemical screening will be important in determining the drug's genuineness. Initial phytochemical screening of *J. regia* bark extract revealed the presence of alkaloids, carbohydrates, flavonoids, tannins, tri-terpenes/sterols, and saponins, while amino acids, steroids, and anthraquinones were absent (Table 1). Alkaloids, carbohydrates, and tannins have a therapeutic effect against various maladies, including pathogenic organisms, justifying their usage as traditional medicines [28]. Tannins are polyphenols with anti-carcinogenic and antibacterial properties, while alkaloids have a therapeutic effect in humans and are used as muscle relaxants, pain relievers, and anti-microbials [27].

Physical appearance, water content, ash content percentage, residue on ignition, bulk density, and tapped density are some of the parameters commonly used to determine the purity and standard of a crude medication. In this study, we looked at the physical arrangement and composition of walnut bark extract in Table 2. Walnut bark is light to dark brown fibrous powder. The existence of polar components is suggested by alcohol soluble extractive values, which were found to be 6.03%. The residue on ignition is usually used to determine the content of inorganic impurities in an organic substance. The residue on ignition value was 91.48%. Inter-particulate interaction that influences a powder's bulking qualities also obstructs powder flow; comparing the bulk and tapped densities can measure the relative relevance of these interactions in a given powder. The compressibility index and the Hausner ratio are two examples of such a comparison used as an index of the powder's ability to flow. The values for bulk and tapped densities were 0.224 gm/ml and 0.348 gm/ml,

respectively.

According to a previous study, the morphology and anatomical investigations that have been presented can be used as a distinguishing factor in determining the genuineness of this medicine. A layperson can use these simple but dependable standards as medication for home treatment [28].

This in-vitro study aimed to investigate how effective the bark of *J. regia* extract was against three different concentrations of bacteria and fungus. The current in vitro investigation found that all the extracts of walnut bark had a stronger inhibitory impact on microorganisms (bacteria and fungus) (Tables 3-6). However, the findings in the current study were almost identical to those of a previous study, which found that the acetone and aqueous extract of this plant reduced bacterial growth in vitro [28].

Compared to the current study, in vitro tests were carried out previously on the green hull of *J. regia* to investigate its anti-microbial and antioxidant characteristics. As a result, it was shown that the ethanol extract of this plant had the highest antibacterial activity compared to other extracts [29].

In addition, the ethanol extract of *J. regia* bark had high antifungal activity against *A. niger, C. albicans* and *A. fumigatus*, but the chloroform, methanol and water extracts had minimal antimycotic activity as compared to the ethanol extract (Table 6). The obtained results serve as a foundation for further research into walnut bark preparation for antifungal properties.

In contrast to the current study, researchers previously worked on the antifungal potential of four extracts obtained from *J. regia* leaves against pathogenic *C. albicans* strains. The antifungal activity of ethanol extract from walnut leaves was the highest, whereas ethyl acetate and hydrolyzed methanol suppressed the development rate of the fungal pathogens to the lowest degree [30, 31].

In comparison to the existing study, researchers examined the antifungal and antioxidant activities of *J. regia* natural bark on a variety of vaginal *Candida* isolates and *Candida* type strains [18]. The anti-candidal activity of various dilution extracts of natural *J. regia* barks was investigated *in vitro*, and the results revealed that the ethanol extract of walnut bark had the highest anti-candidal activity [17].

In contrast to the present study, the ethanol extract of natural walnut exhibited the best antifungal action against vaginal *Candida* strains, according to one of the researchers. The findings suggested that *J. regia* could be employed as a low-cost, easily accessible natural antifungal and antioxidant agent [17].

5. Conclusion

The information gathered in this study will aid in the production of a monograph on the crude drug and its inclusion in various pharmacopoeias. It could also be used by manufacturers to identify and choose raw materials for drug synthesis. Walnut bark extracts—based on the findings of this study—could be a viable therapy for the treatment of bacterial and fungal infections in the future.

The early findings acquired provided information that could support the article's scientific novelty, which is based on the conceptual study of phytochemical and microscopic characteristics of *J. regia* bark extract. The study's physicochemical parameters were used to evaluate the efficacy and quality of *J. regia*. Therefore, the antibacterial and antifungal activity of extracts of *J. regia* bark has been established against pathogenic bacteria and fungal activity of extracts. This study has validated and justified the usage of *J. regia* bark among the general public, particularly in rural areas.

The novel findings show that *J. regia* bark extracts have a considerable effect on the cell membrane of gram +ve and –ve bacteria, as well as fungal species. As a result, *J. regia* bark has a lot of potential as a natural antimicrobial.

The limitation of this study is that it was conducted under *in vitro* screening. The proper approval of this antifungal herbal drug would require the study to be extended to the necessary clinical trials, so new antifungal medications that would increase efficacy or minimize the toxicity of existing antifungal treatments could then be developed. Despite their infancy, all these strategies will improve the clinician's ability to care for patients with fungal infections.

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