

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

Zehra Ashraf<sup>1</sup>, Anwar Ejaz Beg<sup>2</sup>, Mirza Tasawer Baig<sup>3</sup>, Nudrat Fatima<sup>4</sup>, Tehmina Sohail<sup>5</sup>, Samina Iqbal<sup>5</sup>

<sup>1</sup>Dr., Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan; Ph.D. Scholar, Department of Pharmacy, Ziauddin University, Karachi, Pakistan

<sup>2</sup>Dr., Professor, Department of Pharmaceutics, Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan

<sup>3</sup>Dr., Associate Professor, Department of Pharmacy Practice, Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan

<sup>4</sup>Dr., Professor, Department of Pharmacognosy, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan

<sup>5</sup>Dr., Senior Scientific Officer, Pharmaceutical Research Centre, PCSIR Labs Complex, Karachi, Pakistan

**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

Received: February 11, 2022 / Revised: March 25, 2022 / Accepted: April 14, 2022 / Published: May 30, 2022

About the authors: Zehra Ashraf, Dr., Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan; Ph.D. Scholar, Department of Pharmacy, Ziauddin University, Karachi, Pakistan; Anwar Ejaz Beg, Dr., Professor, Department of Pharmaceutics, Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan; Mirza Tasawer Baig, Dr., Associate Professor, Department of Pharmacy Practice, Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan; Nudrat Fatima, Dr., Professor, Department of Pharmacognosy, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan; Tehmina Sohail, Samina Iqbal, Dr., Senior Scientific Officer, Pharmaceutical Research Centre, PCSIR Labs Complex Karachi, Pakistan

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著的抑制作用。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 physicochemical 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, dichloromethane, 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].

$Moisture\ content\ (\%) = \frac{w1-w2}{weight\ of\ sample} * 100$   
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:

$ash\ (\%) = \frac{weight\ of\ ash}{weight\ of\ sample} * 100$

### 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.

$$(w3-w1) \times 100 / (w2-w1)$$

#### 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:

$$\text{Bulk and Tapped Densities} = \text{Mass/Volume}$$

## 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. In vitro 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<sub>2</sub>.2H<sub>2</sub>O 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. fumigatus* 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-inoculated 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°C for 24 hours, and for fungus, plates were incubated at 25°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.

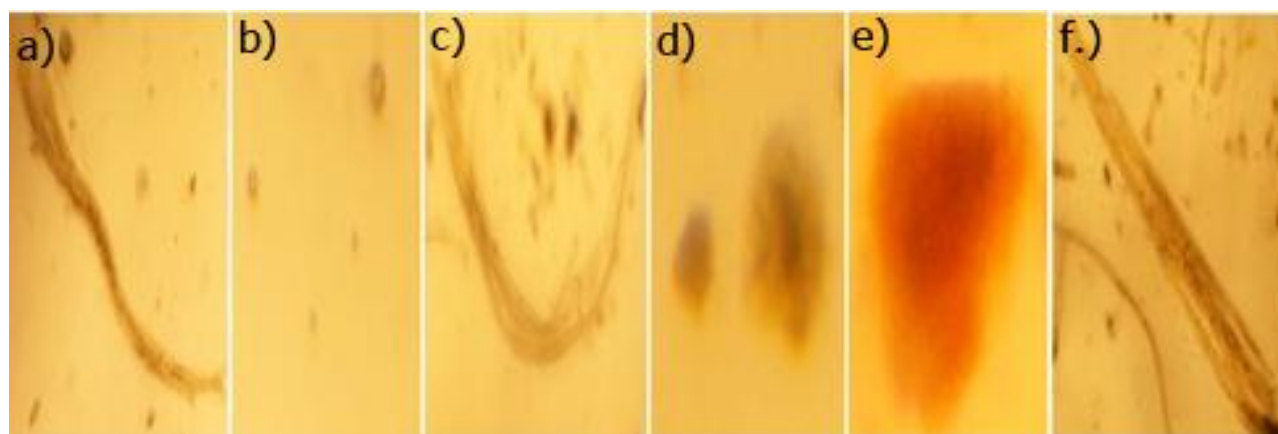


Fig. 1 a). Phloem fiber; b). Oil cells; c). Lignified fibers; d). Stone cells with sclereid; e). Resin cell; f). Septate fiber

### 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	0.224 gm/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  $\mu\text{g/ml}$ . 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  $\mu\text{g/ml}$  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  $\mu\text{g/ml}$ , 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  $\mu\text{g/ml}$ , 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	CONCENTRATIONS ( $\mu\text{g/ml}$ )			
		100 $\mu\text{g}$	200 $\mu\text{g}$	300 $\mu\text{g}$	400 $\mu\text{g}$
1.	Water	0	0	0	0
	Zone of inhibitions in mm				
2.	Methanol	15	15	16	16
	Zone of inhibitions in mm	14.5	15	15.8	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
	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
	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	CONCENTRATIONS ( <i>S.aureus</i> )			
		100 µg	200 µg	300 µg	400 µg
1	Water	12	15	15	16
	Zone of inhibitions in mm	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	CONCENTRATIONS ( <i>Ps.auro</i> )			
		100 µg	200 µg	300 µg	400 µg
1.	Water	10.5	13	13	14
	Zone of inhibitions in mm	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*

Test	Fungus	ANTIFUNGAL ACTIVITY				
		EXTRACTS (SOLVENTS)				
		Water Extract	Chloroform Extract	Ethanol Extract	Methanol Extract	
Compound 1	<i>C. albicans</i> ATCC10231	Results (Zone of Inhibition in mm)				
Ketoconazole (10 mg/ml)		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	<i>A.fumigatus</i> ATCC#9197	Ketoconazole (10mg/ml)	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	<i>A.niger</i> ATCC#16404	Ketoconazole (20mg/ml)	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.

## References

- [1] ARADHYA M., VELASCO D., IBRAHIMOV Z., TOKTORALIEV B., MAGHRADZE D., MUSAYEV M., BOBOKASHVILI Z., and PREECE J. E. Genetic and ecological insights into glacial refugia of walnut (*Juglans regia* L.). *PLoS ONE*, 2017, 12(10): e0185974. <https://doi.org/10.1371/journal.pone.0185974>
- [2] BERNARD A., LHEUREUX F., and DIRLEWANGER E. Walnut: past and future of genetic improvement. *Tree Genetics and Genomes*, 2018, 14(1): 1-28. <https://doi.org/10.1007/s11295-017-1214-0>
- [3] SAVILL P. S. *The silviculture of trees used in British forestry*. CABI, 2019.
- [4] EBRAHIMI S., JAMEI R., NOJOOMI F., and ZAMANIAN Z. Persian walnut composition and its importance in human health. *International Journal of Enteric Pathogens*, 2018, 6: 3-9. <https://doi.org/10.15171/ijep.2018.02>
- [5] JAHAN N., & MAKBUL S. A. Phytochemical and pharmacological appraisal of barks from Unani medicinal plants. In: SWAMY M. (ed.) *Plant-Derived Bioactives*. Springer, Singapore, 2020: 523–548.

[https://doi.org/10.1007/978-981-15-1761-7\\_22](https://doi.org/10.1007/978-981-15-1761-7_22)

- [6] BAKHT J., KHAN S., SHAFI M., and IQBAL A. Fractionation of crude extracts from controlled dried and commercially available stem bark of *Juglans regia* and their antimicrobial effects. *Pakistan Journal of Pharmaceutical Sciences*, 2017, 30(5): 1581-1588. <http://www.pjps.pk/wp-content/uploads/pdfs/30/5/Paper-9.pdf>
- [7] MENSAH M. L., KOMLAGA G., FORKUO A. D., FIREMPONG C., ANNING A. K., and DICKSON R. A. Toxicity and safety implications of herbal medicines used in Africa. In: BUILDERS P. F. (ed.) *Herbal Medicine*. IntechOpen, London, 2019. <https://dx.doi.org/10.5772/intechopen.72437>
- [8] DAR R. A., SHAHNAWAZ M., and QAZI P. H. General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, 2017, 6(6): 349-351. <https://doi.org/10.31254/phyto.2017.6608>
- [9] JAMSHIDI-KIA F., LORIGOOINI Z., and AMINI-KHOEI H. Medicinal plants: Past history and future perspective. *Journal of Herbmmed Pharmacology*, 2018, 7(1): 1-7. <https://doi.org/10.15171/jhp.2018.01>
- [10] SANTINI A., TENORE G. C., and NOVELLINO E. Nutraceuticals: A paradigm of proactive medicine. *European Journal of Pharmaceutical Sciences*, 2017, 96: 53-61. <https://doi.org/10.1016/j.ejps.2016.09.003>
- [11] LIU C., GUO D.-A., and LIU L. Quality transitivity and traceability system of herbal medicine products based on quality markers. *Phytomedicine*, 2018, 44: 247-257. <https://doi.org/10.3389/fphar.2021.705498>
- [12] HUANG L.-Q., ZHAO Y., and YUAN Y. Emerging Molecular Pharmacognosy. In: HUANG L. (ed.) *Molecular Pharmacognosy*. Springer, Singapore, 2019: 1-12. <https://doi.org/10.1007/978-981-32-9034-1>
- [13] VILLA F., QUARTO R., and TASSO R. Extracellular vesicles as natural, safe and efficient drug delivery systems. *Pharmaceutics*, 2019, 11(11): 557. <https://doi.org/10.3390/pharmaceutics11110557>
- [14] KAMRAN R. M., KHALIQ H. A., and UZAIR M. Pharmacognostic and phytochemical studies on *Plumeria obtusa* L. *The Journal of Phytopharmacology*, 2020, 9(2): 120-124. <https://doi.org/10.31254/phyto.2020.9208>
- [15] ZHOU X., LI C.-G., CHANG D., and BENSOUSSAN A. Current status and major challenges to the safety and efficacy presented by Chinese herbal medicine. *Medicines*, 2019, 6(1): 14. <https://doi.org/10.3390/medicines6010014>
- [16] KESKIN D., CEYHAN N., and UGUR A. Chemical Composition and in vitro Antimicrobial Activity of Walnut (*Juglans regia*) Green Husks and Leaves from West Anatolia. *Journal of Pure and Applied Microbiology*, 2012, 6(2): 583-588. [https://microbiologyjournal.org/archive\\_mg/jmabsread.php?snoid=830&month=&year=](https://microbiologyjournal.org/archive_mg/jmabsread.php?snoid=830&month=&year=)
- [17] KHADHRI A., AOUADHI C., HUSEIN N., MASSON E., and PIZZI A. Characterization of essential oil composition of *Juglans regia* bark, comparison of secondary metabolites and biological activities of its extracts. *Open Access Research Journal of Biology and Pharmacy*, 2022, 4(1): 60-68. <https://doi.org/10.53022/oarjbp.2022.4.1.0025>
- [18] AYADI HASSAN S., GHADAM P., and ABDI ALI A. One step green synthesis of Cu nanoparticles by the aqueous extract of *Juglans regia* green husk: assessing its physicochemical, environmental and biological activities. *Bioprocess and Biosystems Engineering*, 2022, 45(3): 605-618. <https://doi.org/10.1007/s00449-022-02691-2>
- [19] RAJA V., AHMAD S. I., IRSHAD M., WANI W. A., SIDDIQI W. A., and SHREAZ S. Anticandidal activity of ethanolic root extract of *Juglans regia* (L.): Effect on growth, cell morphology, and key virulence factors. *Journal de Mycologie Médicale*, 2017, 27(4): 476-486. <https://doi.org/10.1016/j.mycmed.2017.07.002>
- [20] OLIVEIRA I., SOUSA A., FERREIRA I. C., BENTO A., ESTEVINHO L., and PEREIRA J. A. Total phenols, antioxidant potential and anti-microbial activity of walnut (*Juglans regia* L.) green husks. *Food and Chemical Toxicology*, 2008, 46(7): 2326-2331. <https://doi.org/10.1016/j.fct.2008.03.017>
- [21] ROHILLA S., BHATT D. C., and GUPTA A. Therapeutic potential of phytomedicines and novel polymeric strategies for significant management of candidiasis. *Current Pharmaceutical Design*, 2018, 24(16): 1748-1765. <https://doi.org/10.2174/1381612824666180524102933>
- [22] IKE C. O., ORJIOKE N. M., OKORO U. C., OGBUANU C. C., CHUKWUEMEKA-OKORIE H. O., AGUOMA C. C., NWELOKE C. R., NSUDE O. P., UGWU M. C., and BENJAMIN P. A. Comparative Studies on the Antimicrobial Properties of Seed and Bark of Walnut. *Pharmacologyonline*, 2021, 1: 262-269. [https://pharmacologyonline.silae.it/files/archives/2021/vol1/PhOL\\_2021\\_1\\_A033\\_Ike.pdf](https://pharmacologyonline.silae.it/files/archives/2021/vol1/PhOL_2021_1_A033_Ike.pdf)
- [23] DEVI T. N., APRAJ V., BHAGWAT A., MALLYA R., SAWANT L., and PANDITA N. Pharmacognostic and Phytochemical Investigation of *Juglans regia* Linn. Bark. *Pharmacognosy Journal*, 2011, 3(25): 39-43. <https://doi.org/10.5530/pj.2011.25.7>
- [24] BENNACER A., SAHIR-HALOUANE F., AITSLIMANE-AITKAKI S., OUKALI Z., OLIVEIRA I. V., RAHMOUNI N., and AISSAOUI M. Structural characterization of phytochemical content, antibacterial, and antifungal activities of *Juglans regia* L. leaves cultivated in Algeria. *Biocatalysis and Agricultural Biotechnology*, 2022, 40: 102304. <https://doi.org/10.1016/j.bcab.2022.102304>
- [25] NISTANE N. T., CHAURIYA C. B., and GAJBHIY V. R. A comparative pharmacognostic and anti-microbial evaluation of different parts of *Mimusops elengi* for dental associated problems. *Journal of Pharmacognosy and Phytochemistry*, 2019, 8(4): 772-779. <https://www.phytojournal.com/archives/2019/vol8issue4/PartN/8-3-651-110.pdf>
- [26] PURI A. V. Microscopical Evaluation, Phytochemical Screening, and High-Performance Thin-Layer Chromatography Fingerprinting of Leaves of *Diospyros montana* (Roxb.). *International Journal of Green Pharmacy*, 2018, 12(3): 205-211. <https://doi.org/10.22377/IJGP.V12I03.1953>
- [27] AYURVEDIC PHARMACOPOEIA COMMITTEE. *The ayurvedic pharmacopoeia of India*. Department of AYUSH, Government of India, Ministry of Health and Family



Welfare, New Delhi, 2001.

[28] SINGH T. G. Phytochemical Activities and Pharmacology of Herbal Drug: Nardostachys Jatamansi. *Plant Archives*, 2020, 20(1): 3842-3848. [http://www.plantarchives.org/SPECIAL%20ISSUE%2020-1/241\\_3842-3848.pdf](http://www.plantarchives.org/SPECIAL%20ISSUE%2020-1/241_3842-3848.pdf)

[29] JAFER F. N., and NASER L. A. The Biological Activity of Aqueous and Methanolic Extracts of Juglans Regia on Yeasts and Pathologic Bacteria. *Archives of Clinical Microbiology*, 2020, 11(3): 2405-2410. <https://doi.org/10.36648/1989-8436.11.3.113>

[30] HEIM L., DODELER R., BRANCHERIAU L., MARCHAL R., BOUTAHAR N., LOTTE S., DUMARÇAY S., GÉRARDIN P., and CANDELIER K. Comparison of extractive chemical signatures among branch, knot and bark wood fractions from forestry and agroforestry walnut trees (*Juglans regia* × *J. nigra*) by NIR spectroscopy and LC-MS analyses. *iForest - Biogeosciences and Forestry*, 2022, 15(1): 56-62. <https://doi.org/10.3832/ifer3973-014>

[31] KADHIM M. S. Antifungal Activity of Derum (*Juglans Regia* L. Bark) Extracts Against *Candida Albicans* Isolates (In Vitro Study). *Mustansiria Dental Journal*, 2018, 15(1): 49-57. [https://mdj.iraqjournals.com/article\\_145014.html](https://mdj.iraqjournals.com/article_145014.html)

#### 参考文献:

[1] ARADHYA M., VELASCO D., IBRAHIMOV Z., TOKTORALIEV B., MAGHRADZE D., MUSAYEV M., BOBOKASHVILI Z. 和 PREECE J.E. 核桃冰川避难所的遗传和生态学见解 (核桃核桃.). *公共科学图书馆一号*, 2017, 12(10): e0185974. <https://doi.org/10.1371/journal.pone.0185974>

[2] BERNARD A., LHEUREUX F. 和 DIRLEWANGER E. 核桃: 遗传改良的过去和未来。 *树遗传学和基因组*, 2018, 14(1): 1-28. <https://doi.org/10.1007/s11295-017-1214-0>

[3] SAVILL P. S. 英国林业中使用的树木造林。 *卡比*, 2019.

[4] EBRAHIMI S., JAMEI R., NOJOOMI F. 和 ZAMANIAN Z. 波斯核桃组合物及其对人类健康的重要性。 *国际肠道病原体杂志*, 2018, 6: 3-9. <https://doi.org/10.15171/ijep.2018.02>

[5] JAHAN N., & MAKBUL S. A. 乌纳尼药用植物树皮的植物化学和药理学评估。 在: SWAMY M. (编辑) *植物衍生的生物活性物质*。施普林格, 新加坡, 2020: 523-548. [https://doi.org/10.1007/978-981-15-1761-7\\_22](https://doi.org/10.1007/978-981-15-1761-7_22)

[6] BAKHT J., KHAN S., SHAFI M., 和 IQBAL A. 从受控干燥和市售的胡桃茎皮中粗提物的分馏及其抗菌作用。 *巴基斯坦药物科学杂志*, 2017, 30(5): 1581-1588. <http://www.pjps.pk/wp-content/uploads/pdfs/30/5/Paper-9.pdf>

[7] MENSAH M. L., KOMLAGA G., FORKUO A.D., FIREMPONG C., ANNING A.K. 和 DICKSON R.A.

非洲使用的草药的毒性和安全影响。 在: BUILDERS P. F. (ed.)

草药。 *IntechOpen*, 伦敦, 2019年. <https://dx.doi.org/10.5772/intechopen.72437>

[8] DAR R. A., SHAHNAWAZ M. 和 QAZI P.H. 药用植物概述: 综述。 *植物药理学杂志*, 2017, 6(6): 349-351. <https://doi.org/10.31254/phyto.2017.6608>

[9] JAMSHIDI-KIA F., LORIGOINI Z. 和 AMINI-KHOEI H.

药用植物: 过去的历史和未来的展望。 *草药药理学杂志*, 2018, 7(1): 1-7. <https://doi.org/10.15171/jhp.2018.01>

[10] SANTINI A., TENORE G. C. 和 NOVELLINO E. 保健品: 主动医学的范例。 *欧洲药物科学杂志*, 2017, 96: 53-61. <https://doi.org/10.1016/j.ejps.2016.09.003>

[11] LIU C., GUO D.-A., 和 LIU L. 基于质量标志的草药产品质量传递与追溯体系。 *植物医学*, 2018, 44: 247-257. <https://doi.org/10.3389/fphar.2021.705498>

[12] 黄丽清. 赵玉. 袁 Y. 新兴分子生药理学。 在: 黄 L. (编辑) *分子生药理学*。施普林格, 新加坡, 2019: 1-12. <https://doi.org/10.1007/978-981-32-9034-1>

[13] VILLA F., QUARTO R. 和 TASSO R. 细胞外囊泡作为天然、安全和高效的药物输送系统。 *药剂学*, 2019, 11(11): 557. <https://doi.org/10.3390/pharmaceutics11110557>

[14] KAMRAN R. M., KHALIQ H. A. 和 UZAIR M. 鸡蛋花的生药学和植物化学研究。 *植物药理学杂志*, 2020, 9(2): 120-124. <https://doi.org/10.31254/phyto.2020.9208>

[15] ZHOU X., LI C.-G., CHANG D., 和 BENSOUSSAN A. 中草药安全性和有效性的现状和主要挑战。 *药物*, 2019, 6(1): 14. <https://doi.org/10.3390/medicines6010014>

[16] KESKIN D., CEYHAN N. 和 UGUR A. 来自西安纳托利亚的核桃 (核桃) 绿皮和叶子的化学成分和体外抗菌活性。 *纯粹与应用微生物学杂志*, 2012, 6(2): 583-588.

[https://microbiologyjournal.org/archive\\_mg/jmabsread.php?snoid=830&month=&year=](https://microbiologyjournal.org/archive_mg/jmabsread.php?snoid=830&month=&year=)

[17] KHADHRI A., AOUADHI C., HUSEIN N., MASSON E. 和 PIZZI A. 核桃树皮精油成分的特征、次生代谢物的比较及其提取物的生物活性。 *开放获取生物学和药学研究杂志*, 2022, 4(1): 60-68. <https://doi.org/10.53022/oarjbp.2022.4.1.0025>

[18] AYADI HASSAN S., GHADAM P. 和 ABDI ALI A. 核桃绿壳水提取物一步绿色合成铜纳米颗粒: 评估其物理化学、环境和生物活性。 *生物过程与生物系统工程*, 2022, 45(3): 605-618. <https://doi.org/10.1007/s00449-022-02691-2>

[19] RAJA V., AHMAD S. I., IRSHAD M., WANI W. A., SIDDIQI W. A. 和 SHREAZ S. 胡桃(大号)乙醇根提取物的抗念珠菌活性: 对生长、细胞形态和关键毒力因子的影响。 *医学真菌学杂志*, 2017, 27(4): 476-486. <https://doi.org/10.1016/j.mycmed.2017.07.002>

[20] OLIVEIRA I., SOUSA A., FERREIRA I.C., BENTO A., ESTEVINHO L. 和 PEREIRA J.A. 核桃 (核桃核桃) 绿壳的总酚、抗氧化潜力和抗菌活性。

- 食品和化学毒理学, 2008, 46(7): 2326-2331. <https://doi.org/10.1016/j.fct.2008.03.017>
- [21] ROHILLA S., BHATT D. C. 和 GUPTA A. 植物药物的治疗潜力和用于有效治疗念珠菌病的新型聚合物策略。当前药物设计, 2018, 24(16): 1748-1765. <https://doi.org/10.2174/1381612824666180524102933>
- [22] IKE C. O., ORJOKE N. M., OKORO U. C., OGBUANU C. C., CHUKWUEMEKA-OKORIE H. O., AGUOMA C. C., NWELOKE C. R., NSUDE O. P., UGWU M. C. 和 BENJAMIN P. A. 核桃种子和树皮抗菌性能的比较研究。药理学在线, 2021, 1: 262-269. [https://pharmacologyonline.silae.it/files/archives/2021/vol1/PhOL\\_2021\\_1\\_A033\\_Ike.pdf](https://pharmacologyonline.silae.it/files/archives/2021/vol1/PhOL_2021_1_A033_Ike.pdf)
- [23] DEVI T. N., APRAJ V., BHAGWAT A., MALLYA R., SAWANT L. 和 PANDITA N. 核桃核桃的生药学和植物化学研究。吠。生药学杂志, 2011, 3(25): 39-43. <https://doi.org/10.5530/pj.2011.25.7>
- [24] BENNACER A., SAHIR-HALOUANE F., AITSLIMANE-AITKAKI S., OUKALI Z., OLIVEIRA I. V., RAHMOUNI N. 和 AISSAOUI M. 核桃核桃的植物化学成分、抗菌和抗真菌活性的结构表征。阿尔及利亚种植叶子。生物催化与农业生物技术, 2022, 40: 102304. <https://doi.org/10.1016/j.bcab.2022.102304>
- [25] NISTANE N. T., CHAURIYA C. B. 和 GAJBHIY V. R. 针对牙齿相关问题的连翘不同部分的比较生药学和抗微生物评估。生药学与植物化学杂志, 2019, 8(4): 772-779. <https://www.phytojournal.com/archives/2019/vol8issue4/PartN/8-3-651-110.pdf>
- [26] PURI A. V. 柿属蒙大拿(罗克斯)叶子的显微镜评估、植物化学筛选和高性能薄层色谱指纹图谱。国际绿色药学杂志, 2018, 12(3): 205-211. <https://doi.org/10.22377/IJGP.V12I03.1953>
- [27] 阿育吠陀药典委员会。印度的阿育吠陀药典。印度政府 AYUSH 部, 卫生和福利部, 新德里, 2001年。
- [28] SINGH T. G. 草药的植物化学活性和药理学: 毛竹。植物档案, 2020, 20(1): 3842-3848. [http://www.plantarchives.org/SPECIAL%20ISSUE%2020-1/241\\_3842-3848\\_.pdf](http://www.plantarchives.org/SPECIAL%20ISSUE%2020-1/241_3842-3848_.pdf)
- [29] JAFER F. N. 和 NASER L. A. 胡桃的水和甲醇提取物对酵母和病原菌的生物活性。临床微生物学档案, 2020, 11(3): 2405-2410. <https://doi.org/10.36648/1989-8436.11.3.113>
- [30] HEIM L., DODELER R., BRANCHERIAU L., MARCHAL R., BOUTAHAR N., LOTTE S., DUMARÇAY S., GÉRARDIN P. 和 CANDELIER K. 分支、结和树皮之间提取化学特征的比较通过近红外光谱和液质联用分析, 来自林业和农林业核桃树(核桃×J. 黑人)的木材部分。艾森林-生物地球科学与林业, 2022, 15(1): 56-62. <https://doi.org/10.3832/ifer3973-014>
- [31] KADHIM M. S. 德鲁姆(核桃核桃)提取物对白色念珠菌分离物的抗真菌活性(体外研究)。穆斯坦西里亚牙科杂志, 2018年, 15(1): 49-57. [https://mdj.iraqjournals.com/article\\_145014.html](https://mdj.iraqjournals.com/article_145014.html)