

Phytochemical Investigation and Determination of Antibacterial Activities of the Fruit and Leaf Crude Extract of *Ficus palmata*

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Abstract

To cure ailments, the global population makes medicines from plants. *Ficus palmata* can be used to cure a variety of ailments. The purpose of this study was to examine the phytochemical content and antibacterial activity of methanol extract from *Ficus palmata* fruit and leaf crude extract. Maceration was used to make the methanol extracts, which were subsequently fractionated. The crude extracts and fractions were subjected to standard phytochemical screening assays. Phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, tannins, polyphenols, anthraquinones, steroids, coumarins, and terpenoids in the crude methanol fruit extract. Phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, polyphenols, anthraquinones, steroids, and terpenoids in the extract. The determination of trace metals in leaves and fruit samples using a flame atomic absorption spectrophotometer revealed that the fruits contained relatively high quantities of iron and other nutritionally essential elements, such as magnesium, calcium, and copper, whereas the leaves contain a high level of calcium, magnesium, and iron, as well as a small amount of copper.

Keywords: Phytochemical screening, antibacterial activities, *Ficus palmata*, agar well diffusion, flame atomic absorption spectrophotometer, *E. coli*, *K. pneumonia*, *S. typhi*, and *S. aureus*.

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INTRODUCTION

All around the world, medicinal plants are a significant part of indigenous medical systems [1]. The term medicinal plant refers to a variety of plants used in herbalism, some of which have therapeutic properties [2]. These medicinal plants are thought to be abundant in biologically active components that can be employed in medication research and synthesis [3].

Traditional medicine has been used to treat a variety of disorders for a long time around the world [1]. According to a World Health Organization report, most people around the world rely on traditional medicine for their main health care [1, 4].

Ethiopia has one of the world most extensive plant-based medical traditions. Due to the cultural acceptability of healers, the comparatively low cost of traditional medicine, and Ethiopia's poor access to modern health facilities, the majority of the population employs traditional medicine. Phytotherapies are made

from the leaves, roots, barks, and other elements of the plant by traditional healers. In Ethiopia, certain medicinal plants are sold in local markets [4, 5]. *Ficus palmata* is a wound-healing plant.

Ficus palmata Forsk, often known as fig, is a member of the Moraceae [6-8]. Family, which includes over 800 species [8, 9]. It can be found growing wild in Egypt, Eritrea, Ethiopia, Somalia, and Sudan, as well as in Arabia and temperate Asia, including northern India, Nepal, and Pakistan [6, 9]. Plants of the fig family can be found up to 1,000 meters above sea level [6].

Ficus contains alkaloids, flavonoids, tannins, cardiac glycosides [6, 9] sterols, terpenes [6, 8, 9] coumarins, furanocoumarin glycosides, isoflavones, lignans, and chromone[8] according to phytochemical studies. Cerylbehenate, alkaloids, steroids, flavonoids, and tannins have been found in the stem bark. The fruits include carbohydrate-sitosterol, polyphenols psoralene and bergapten[10], while the leaves contain -sitosterol,

triterpene glaucol acetate, gallic acid, rutin and vanillic acid.

The *Ficus palmata* plant has demonstrated antibacterial and antifungal action at various concentrations, as described in the literature [6, 7, 10, 11]. Given the immense potential of plants as sources of antimicrobial medicines, a thorough inquiry was conducted to screen the antibacterial activity of various *Ficus* species [7, 11]. In terms of screening, the diameter of the zone of inhibitions was used to determine antimicrobial (bacterial and fungal) activity. Researchers have recently looked into and identified phytochemicals with an unknown pharmacological activity that has adequate antibacterial and antifungal properties. Many *Ficus* species have been used in traditional medicine for centuries and have a variety of pharmacological effects [12].

There are no reports before about the integrated study of phytochemical screening, antibacterial activity, and wet digestion for mineral analysis of the crude, fractionation of the leaves and fruit of *Ficus palmata*. The goal of this research was to examine the phytochemical composition and antibacterial activity of methanol extract from *Ficus palmata* fruit and leaf crude extract.

MATERIALS AND METHODS

Preparation of the plant material

The fruit and leaves were collected, quickly cleaned with tap water to eliminate dirt or dust, and dried under shade in the chemistry laboratory of the school of chemistry. Using a laboratory pestle and mortar, the fruit and leaves were ground to a coarse powder. The powder sample was weighed and kept in airtight containers until the extraction process began [4].

Extraction

The powdered fruit and leaves of *Ficus palmata* (200.0 g) were macerated for 3 days at room temperature with 1000.0 mL of methanol using the maceration method. The solution supernatants were gravity filtered with Whatman No. 1 filter paper, concentrated with a rotary evaporator at 40°C, and kept in a refrigerator [4].

Fractionation and purification

The crude extract of *Ficus palmata* fruit and leaves showed the presence of number of spots on its thin layer chromatography profile [7, 8, 10]. This indicates the need for further fractionation and purification. Fractionation and purification of the crude extract of *Ficus palmata* fruit was done using the column chromatography (CC) technique.

Fractionation and purification through column chromatography

A total of 100.0 g of silica gel was measured and mixed with 200.0 mL of Chloroform before being packed into a column (34.0 cm). 15.0 g and 10.0 g of *Ficus palmata* dried fruit and leaves crude methanol extracts were adsorbed using 10.0 g of silica gel and 20.0 mL chloroform, respectively. The adsorbed sample was then applied to the packed column chromatography at the top. As the polarity of the solvent solution increased, the column was eluted with chloroform, acetone, acetone/methanol, and methanol as the eluent.

Phytochemical Screening

The fruit and leaves crude extract were subjected to the following preliminary phytochemical studies. Alkaloids, flavonoids, terpenoids, steroids, saponins, tannins, glycoside, anthraquinones, coumarins and phenol compounds were determined according to [6, 13].

Invitro antibacterial studies

Bacterial test organisms and standard antibacterial disc

Three gram-negative bacterial strains; *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi* and one gram-positive bacterial strain; *Staphylococcus aureus* were obtained from the department of biology, University of Gondar, Ethiopia. The standard antibacterial disc used for the study was gentamicin.

Bacterial Media (Muller Hinton Media)

Muller Hinton Media (36.0 g) was mixed with 500.0 mL distilled water and autoclaved for 15 minutes at 15 lb pressure. Petri dishes were filled with sterile media. A cork borer with a diameter of 6 mm was used to bore the solidified plates. Antibacterial experiments were conducted on plates with wells [14].

Determination of inhibition zone both methanol extract and solvent fractionation

The extracts' antibacterial activity was determined using the agar well diffusion method. The Mueller Hinton Agar (MHA) media was prepared in a sterile environment [15]. Separate sterile cotton buds were used to distribute microorganisms such as *E. coli*, *K. pneumoniae*, *S. typhi*, and *S. aureus* over the agar well plates [16]. Then, using a sterile cork borer tip [4], an equal distance hole with a diameter of 6.0 mm was punched aseptically. To make a 2000 mg/mL stock solution, the extracts (4.0 g) were diluted in 2.0 mL DMSO. The extracts were made at different strengths (10, 50, and 100 mg/mL) by diluting the stock solution in DMSO and pouring it into the well using a micropipette. The addition of gentamicin was used as a positive control disc, while DMSO was used as a

negative control disc in the following phases. The plates were incubated for 24 hours at 37°C. The extracts were evaluated in triplicates, with the diameter of the inhibitory zone measured in millimeters (mm) and the findings reported as mean standard deviation [4].

Determination of some metal in *Ficus palmata* fruit and leaves

Sample preparation

The collected plant samples (fruit and leaves) were dried and grinded into powder. Fruit and leaves of *Ficus palmata* were first digested with acids to release the minerals. The acid digest was then diluted and used for mineral determination by instrumental method [17].

Preparation of acid digest

A powdered sample of *Ficus palmata* fruit and leaves (1.0 g) was obtained and placed into digestive tubes. The hotplate was filled with concentrated HNO₃ (10.0 mL) and maintained at 100°C for 60 minutes. When the temperature was raised to 200°C, nitrogen acid vapors were released, which were red. After cooling the tubes, 10.0 mL of HNO₃: HClO₄ (1:1) mixes were introduced. The tubes were heated to 400 degrees Celsius. As a result, the dense white vapor of perchloric acid (HClO₄) dissipated and turned colorless. The material was transferred to a 100.0 mL volumetric flask when the tubes were cooled. Volume was made up to the mark with distilled water. The digests were put away in a refrigerator and was utilized for mineral determination [18].

RESULTS AND DISCUSSION

Phytochemical screening result

The presence of flavonoids, alkaloids, saponins, tannins, polyphenols, anthraquinones, steroids, coumarins, and terpenoids was present in a preliminary phytochemical screening of the methanol extract of the fruit of the *Ficus palmata* plant. However, cardiac glycoside was absent in the plant extract. The result is in line with the findings of Nishat Anjum [19], who reported flavonoids, alkaloids, saponins, tannins, phenol compounds, steroids, coumarins and terpenoids. The result is in agreement with the findings of Sarla Saklani *et al.* [20], who reported flavonoids and terpenoids were present. On the contrary, the phytochemical screening results differ from the report by Sarla Saklani *et al.* [20], who reported alkaloids and tannins were absent by using methanol. The differences in results might be related to the geographical distribution of the plant [4] and the extraction techniques previous study was used Soxhlet extracted but in this study was used maceration method.

Alkaloids, flavonoids, terpenoids, steroids, tannins and anthraquinones were found in the methanol extract of *Ficus palmata* leaves. In contrast, saponins,

cardiac glycoside, phenol and coumarins were not detected in the extracts.

Cardiac glycoside was absent in each extract. All the fractions of the methanolic fruit and leaves extracts were noticed to have different secondary metabolites shown in Table 1.

Antibacterial susceptibility assay

The antibacterial activity of fruit and leaf extracts, crude methanol extract, and each solvent fraction were tested using the agar well diffusion method at concentrations of 10, 50, and 100 mg/mL in this study, as shown in Tables 2 and 3. The maximum zone of inhibition for the Fruit and leaves crude methanol extracts was achieved for *S. aureus* at 14.0±0.8 mm and 16.7±0.5 mm at the concentration of 100 mg/mL, while the minimum antibacterial activity was obtained at 6.3±0.1 mm and 7.3±0.1 mm at a concentration of 10 mg/mL respectively. On the contrary, no zones of inhibition were observed in 10 mg/mL of acetone for leaves against *S. aureus*.

On the other hand, Fruit crude methanol showed a high maximum average zone of inhibitions at 100 mg/mL concentration were determined to be 15.7±0.5 mm for *E. coli*, 15.3±0.5 mm for *K. pneumoniae* and 16.3±0.1 mm for *S. typhi*. On the opposite, no zones of inhibition were observed in all concentrations of acetone for leaves against *K. Pneumoniae*.

The result is in line with the findings of Sarla Saklani *et al.* [20], who reported *S. aureus*, *E. coli*, and *K. pneumoniae* were inhibited by using methanol at a concentration of 50 mg/mL. On the other side, the present finding differs from that of Sarla Saklani *et al.* [20], who reported *S. aureus* and *E. coil* was not detected by using methanol at a concentration of 10 mg/mL.

Fruit crude methanol extract has stronger zone inhibition than leaves crude methanol extract in gram positive bacteria than gram negative bacteria at equal doses in the majority of test microorganisms. The number of bioactive metabolites and their synergetic effects may be linked to the increased activity of certain extracts and fractions.

METAL ANALYSIS

The metal contents in the samples were found at different levels which play a vital role in the cure of diseases. These results can give importance to the wild edible fruits and harvesting of the fruit.

Copper concentrations were tested in *Ficus palmata* fruit and leaves, with concentrations of 0.42 ± 0.07 and 0.80 ± 0.00 mg/Kg respectively. The results showed that the concentration of copper in medicinal

plants was less than the FAO/WHO permissible limit and the permitted limits for copper in medicinal plants set by China and Singapore, which were 20 mg/kg and 150 mg/kg, respectively [21].

Fruit *Ficus palmata* (10.92 ± 1.44 mg/Kg) had the highest iron concentration, followed by leaves *Ficus palmata* 9.53 ± 0.48 mg/ kg. Although there are no specified limitations for iron in medicinal plants [22]. In edible plants, the FAO/WHO defined an acceptable level of 20 ppm [23]. When compared to the above values, the iron content in this study was significantly lower than the permitted limit in edible plants.

Magnesium concentrations in plant species were higher than other metal concentrations. Magnesium levels were found in leaves *Ficus palmata* (15.78 ± 0.15 mg/kg) and fruit *Ficus palmata* (10.09 ± 0.14 mg/kg). Mg levels in various medicinal plants ranged from 2241.88 ppm to 6350.63 ppm in a study

conducted in Pakistan 23. However, the Mg content in this study was lower than literature values and lower than the suggested limit for plants as a macronutrient.

The concentration of Calcium was measured in fruit and leaves *Ficus palmata* with the concentration of 6.44 ± 0.13 and 15.19 ± 0.44 mg/Kg respectively. According to the study conducted in Ethiopia, it was reported 170- 320 $\mu\text{g/g}$ in medicinal plants [25]. However, the result of this study showed that the concentration of calcium was below the above literature values.

The fruits of the *Ficus palmata* contained relatively high quantities of iron and considerable amounts of other nutritionally essential elements, including magnesium, calcium and copper whereas the leaves contain a high level of calcium, magnesium, iron and a small amount of copper as shown in Table 4.

Table-1: Result of Phytochemical Screening tests of fruit and leaves crude and their fractions

Metabolites	Fruit crude extract	Fractionation of solvents		
		Leaves crude extract	Chloroform for fruit	Acetone for leaves
Alkaloids	+	+	+	-
Flavonoids	+	+	-	-
Terpenoids	+	+	-	+
Steroids	+	+	-	+
Saponins	+	-	+	-
Tannins	+	+	+	-
Cardiac glycoside	-	-	-	-
Anthraquinones	+	+	+	-
Phenol	+	+	-	-
Coumarins	+	-	-	-

Observed + = Present - = Absent

Table-2: The inhibition zone of the Fruit and leaves crude and their fractions against gram positive bacteria

Test bacteria	Solvent and fraction	Concentration of extracts Zone of inhibition in diameter (mm)				
		10.0 mg/mL	50.0 mg/mL	100.0 mg/mL	(-) control	(+) control
<i>S. aureus</i>	Fruit crude methanol	6.3 \pm 0.1	10.0 \pm 0.0	14.0 \pm 0.8	NA	19.0 \pm 0.0
	Leaves crude methanol	7.3 \pm 0.1	10.3 \pm 0.5	16.7 \pm 0.5	NA	19.0 \pm 0.0
	Chloroform for fruit	6.1 \pm 0.1	8.1 \pm 0.1	9.3 \pm 0.9	NA	19.0 \pm 0.0
	Acetone for leaves	NA	6.7 \pm 0.9	7.3 \pm 0.3	NA	19.0 \pm 0.0

Values are expressed as mean \pm SD (n=3).NA= no activity (+) = positive control (gentamicin) and (-) control = negative control (DMSO).

Table-3: The inhibition zone of the Fruit and leaves crude and their fractions against gram negative bacteria

Test bacteria	Solvent and fraction	Concentration of extracts Zone of inhibition in diameter (mm)				
		10.0 mg/mL	50.0 mg/mL	100.0 mg/mL	(-) control	(+) control
<i>E. coli</i>	Fruit crude methanol	7.3 \pm 0.1	11.7 \pm 0.5	15.7 \pm 0.5	NA	20.0
	Leaves crude methanol	6.3 \pm 0.1	9.3 \pm 0.9	11.7 \pm 0.5	NA	20.0
	Chloroform for fruit	6.7 \pm 0.9	8.1 \pm 0.1	9.7 \pm 0.5	NA	20.0
	Acetone for leaves	6.0 \pm 0.1	6.2 \pm 0.2	7.3 \pm 0.9	NA	20.0
<i>K. pneumoniae</i>	Fruit crude methanol	6.3 \pm 0.1	10.3 \pm 0.9	15.3 \pm 0.5	NA	22.0
	Leaves crude methanol	7.3 \pm 0.9	9.7 \pm 0.5	12.7 \pm 0.5	NA	22.0
	Chloroform for fruit	6.0 \pm 0.2	7.3 \pm 0.5	10.7 \pm 0.9	NA	22.0
	Acetone for leaves	NA	NA	NA	NA	23.0
	Fruit crude methanol	6.1 \pm 0.1	10.1 \pm 0.1	16.3 \pm 0.1	NA	21.0

<i>S. typhi</i>	Leaves crude methanol	7.3±0.9	9.7±0.5	14.0±0.8	NA	21.0
	Chloroform for fruit	6.7±0.9	8.3±0.9	10.3±0.5	NA	21.0
	Acetone for leaves	NA	6.0±0.2	7.3±0.5	NA	21.0

Values are expressed as mean ± SD (n=3).NA= no activity (+) = positive control (gentamicin) and (-) control = negative control (DMSO).

Table-4: The calculated concentration of fruit and leaves of *Ficus palmata*

Metal	Concentration (mg/ Kg)	
	Fruit	Leaves
Mg	10.09±0.14	15.78±0.15
Ca	6.44±0.13	15.19±0.44
Fe	10.92±1.44	9.53±0.48
Cu	0.42±0.07	0.80±0.00

Values are expressed as mean ± SD (n=3).

CONCLUSION

The findings of this investigation showed that crude methanol extracts of the fruit and leaves of the traditional medicinal plant *Ficus palmata* had good antibacterial activity against pathogen bacteria. Furthermore, important nutrients such as magnesium, calcium, iron, and copper are abundant in the fruit and leaves.

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