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Screening for Bioactive Compounds in Extracts from Leaves, Leafstalks, and Fruits of Ficus Carica

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Abstract

Aqueous, Ethanol extracts and Fig's milk were preliminary phytochemically screened and tested against some pathogenic bacteria. Qualitatively analysis revealed Tannin, Saponin, Flavonoids and Terpenoids and phlobatannins and Steroids gave positive results. Quantitative analysis showed that the highest percentage of alkaloid was 41% in fig milk and 39% in plant leaves. In addition, the crude extracts of fig's milk as well as of the raw plant extracts were tested (using the Disc Diffusion Method) for their antimicrobial activity against the bacterial pathogens. The influences of aqueous and ethanol extracts on some pathogenic: two strains of gram-positive bacteria include Staphylococcus aureus and Streptococcus pneumonia.., and two strains of gram-negative bacteria including Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Salmonella SPP and Klebsiella aerogenes. , and the sensitivity of the microorganisms to the extracts of plant's species were compared with each other and with designated antibiotics by measuring the diameter of the inhibition zones, After incubation, the zone of inhibition was measured in mm, a good inhibition of more than 6 mm were observed indicating the effective antibacterial activity of the bioactive compounds in both of the plants leaves extracts and fig's milk. Moreover, the including results of the antibacterial activities of plants leaves extracts and fig's milk were discussed regarding their phytochemical components which exhibited that have a good inhibition zone against six tested bacterial strains.

Key words: Fig's Milk, Bioactive Constituents, Qualitative and Quantitative Analysis, Antibacterial Activities.

1. Introduction

As the Etymology the word fig, first documented in English in the 13th century, originates from ancient French figue, the aforementioned from Occitan Provençal figa. And as of Romance fica, and from Traditional Latin ficus which is fig or fig-tree [1]. As well as by the Italian has fico, directly resulting from the Latin ficus. The name of the caprifig, Ficus caprificus Risso, is derived both from the Latin capro which is billygoat and the English fig [2]. The fig tree is considered one of the oldest trees known to man. Its seniority may reach a thousand years ago, and therefore it may have been known before even agricultural crops such as wheat and barley. Furthermore, Decondole noted that Syria and Anatolia are the natural habitats of the fig tree and from there it was transferred to North Africa, Spain, Mexico, Chile, Peru, and California and to rest of world [3]. Several dissimilar species are grown in Libya. The fig fruit was well-known by ancient Libyan. It is called "Karmoos" and the origin in Arabic is "Teen". While *Ficus carica* in Libya is mainly found in the Western Mountain, covering several varieties of fig trees. And may the city of Zliten (located 150 Km east of Tripoli the Capital), be called this name because in the past travellers passed through this location and take a rest under the shadow of fig trees then called this place with Fig trees shadow (Del alteen in Arabic) and then released this name in that time until it becomes Zliten now. The fig trees are well known in the Mediterranean countries and Libya. In coastal and mountainous regions Libya has a wide range of cultivated genotypes, including cultivars and strains with different characteristics. The fruits may eaten fresh or dried, and due to their high and rich nutritional value, they are perfectly dried, preserved and stored for more than a year to be used later. Dried figs contain great nutritional value, such as sugars, fibre and minerals. These nutrients include groups of health-promoting bioactive constituents. The fig tree is significant because it has a high ability to withstand extreme climatic circumstances, mainly in semi-arid regions, where the amounts of rainfall are low, which leads to an increase in the soil content of calcium carbonate. It is also possible to profit from the fruits of the fig tree as fresh fruit, eat or prepare jam, make rolls from it, prepare biscuits, or use them in making sweets of all kinds.

1. Scientific Classification of the Fig Tree

The scientific classification of the fig tree, commonly known as *Ficus carica* or also known as Ordinary fig. In addition, *Ficus carica* is a gynodioecious, deciduous tree that may reach a height of 10 meters, with fragrant leaves up to 25 cm in size and 18 cm in width, lobed from 3 to 5 lobes, and its stump and branches have smooth white bark.

Scientific Classification	
Kingdom	Plantae
Family	Moraceae
Order	Rosales
Genus	Ficus
Subgenus	F. subg. Ficus
Species	F. carica
Taxon	Tracheophytes, Angiosperms, Rosids, Eudicots
Synonymy	*Caprificus insectifera, Caprificus leucocarpa and Caprificus oblongata Gasp.*
Binomial name	Ficus caricaa

Table 1: The Scientific Classification of the Fig Tree

2. Methodology

2.1 Plant Material Collection

2.2 Collection of Leaves

During the ripened season of fig fruits (During July Month). Samples were collected for this research study, where the healthy leaves were collected and washed with tap water, and distilled water, and dried without exposing them to sunlight, that is, in the shade for 5 days, and then completed drying in the oven at a temperature of 40 degrees Celsius. After that, the dry leaves were ground using an electric grinder until a fine powder was obtained, which was kept in opaque plastic vials, and the vials were closed and preserved until use.

2.3 Collection of Fig's Milk (F. M.)

The Fig's milk was collected from each of the juice secreted or produced when picking the fruits, as well as when picking the leaves. During the ripened season of fig fruits (During July-August Months). Samples were collected for this research study, where the juice produced by the stem of leaves during pick up, or also that when the immature fruits were picked up a juice will released from it which is in the form of a white emulsion like regular milk. This is done by squeezing and pressing gently on the leafstalk (Leave is attached to the stem by a leafstalk (short stalk) or the fruit to extract the juice from them (Fig's Milk). The juice is collected in small, sterile, opaque containers that are suitable and easy to use in terms of closing and opening, as well as transportation. Then the samples were kept in the freezer until use.

2.4 Extraction

The biochemical components of the fine powder of *Ficus carica* leaves were extracted using Soxhlet. To extract the sample, 10 grams were packed into the Soxhlet apparatus and boiled with 200 ml of the suitable solvent (water re-distilled three times, ethyl alcohol 70%, each separately) for a duration of 4 hours. Where the aqueous extract was obtained in a dark brown colour, while the alcoholic extract was in a dark green colour. Then, the solvents were separated with the help of a rotary evaporator at a temperature of 40 °C and 90 rpm.

2.5 Getting the Powdered of the Fig's Milk (F. M.): (The collected Milk from Fruits and Leafstalk).

The Fig's Milk samples were collected and then transferred into 500 ml flasks. Afterwards, Fig's milk was heated in order to produce a highly concentrated mixture. and dried in an oven (In an oven with a powerful fan to help dry as quickly as possible) for 48 hours at 43 o C Afterward, the drying process was completed in a water bath at 90-95 o C for 5 hrs., after obtaining powder flakes of a snow-white colour, almost like powdered milk. Samples were collected in opaque glass vials, tightly closed, and kept in a refrigerator at 4 °C until use.

2.6 Phytochemical Screening

The dried leaves powder, extracts, and samples of dried Fig's milk (FM) were subjected to both preliminary and quantitative testing using approved standard methodologies.

2.7 Phytochemical Screening (Qualitatively)

The qualitative analysis for each of was carried out according to the approved standard methods [4-8].

2.8 Tannins Test

• Catechic Tannins

2 mL of concentrated Hydrochloric acid (HCl) was added to 4 ml of the plant sample and heating at 100o C for 5 minutes. Appearance of a red coloration confirmed presence of Catechic Tannins.

• Catechic or Gallic Tannins

Tannins were examined in powdered case of Ficus carica, extracts and F M. Where 3 g of the plant sample was homogenized in 20 ml of methyl Alcohol, then stirred for 15 min formerly filtered. Afterthought 5 drops of Ferric Chloride 1% were added. Brownish Green or Blue Black colors indicated the presence of Catechic or Gallic tannins, respectively.

2.9 Alkaloids Test

5 ml of the plant sample was mixed with 4 ml 0f 1% Hydrochloric Acid, and then 3-5 drops of Dragendroff reagent were added. The observation of brown or orange as indicating the presence of Alkaloids.

• Cardiotonic Glycosides Test: (Keller Kiliani test)

1 ml of the plant sample was mixed with 1 ml of Ferric Chloride

solution 5%, (1 ml of glacial acetic acid containing one drop of Ferric Chloride solution), then underlayered with 1 ml of concentrated Sulphuric Acid. The appearance Green-Blue color indicated presence of Cardiotonic Glycosides.

2. 10 Saponin Test

10 ml of plant sample in a test tube and mixed with 2 ml of water and vigorous shaken for 2 minutes. The presence of a froth was observed and persisted indicating the presence of saponnins.

2.11 Terpenoids Test

5 ml of the plant sample was mixed well with 1 ml of chloroform in a test tube, and then on the tube wall very carefully, 3 ml of concentrated sulfuric acid was added to it. A reddish-brown at the interface indicated the presence of terpenoids.

2.12 Phlobatannins Test

2 ml of plant sample was mixed with 2 ml of 1% Hydrochloric Acid and warmed. The appearance of Red precipitate indicates the presence of phlobatannins.

2.13 Steroids Test

10 ml of the plant sample was mixed well with 2 ml of acetic anhydride in a test tube. Then 2 ml sulphuric acid, was added. The observation of the change in colour from violet to blue or green is indicating the presence of steroids.

2.14 Amino Acids Test

1 ml of a Ninhydrine solution was added to the plant sample extracts. Appearance of a Red colour indicated presence of amino acids.

2.15 Free Quinones Test

To 5 ml of Petroleum Ether was mixed with the plant sample, then 1-2 drops of Sodium Hydroxide 0.1 N was added. The appearance of a Yellow coloration indicated the presence of free quinone.

2.16 Phenolic Derivatives Test

5 ml of the plant sample were added 1-2 drops of FeCl3 5%. Appearance of Blue or Green color indicated the presence of the phenolic derivatives.

2.17 Glycosides Test

5 ml of plant sample was hydrolyzed with HCl followed by neutralized with NaOH solution then added 3-4 drops of Fehling's solutions A and B. Appearance of Red precipitation which indicated the existence of Glycosides.

3. Phytochemical Screening (Quantitively)

Consistent with the results of the qualitative analysis of the secondary metabolites products obtained for each of the saponnins, alkaloids and flavonoids, these components were quantitively evaluated as the following:

4. Determination of Total Cardiac Glycosides

Precisely, weighed 1 g of the plant sample and transferred it into an Erlenmeyer flask 250 ml. To extract its full essence, 10 mL of 70% ethanol to each was added to it. The flask was covered and put in a shaker and shaken at 200 rpm for 7 hours at 25 °C (room temperature). The muddle mixture was filtered off (Whatman No. 42 filter paper). To precipitate pigments, resins, and tannins, 1 mL of 12.5% lead acetate was added after 5 mL of distilled water. Distilled water was used to adjust the volume to 8mL, followed by shaking with a shaker set at 300rpm for 10 minutes. Next, 2mL of a solution containing 4.77 % disodium hydrogen phosphate (Na_2HPO_4) was added to induce precipitation of any excess phosphorus ions. To obtain a clear filtrate, the resultant solution was filtered with filtered-off (Whatman No. 42 filter paper). And for obtaining filtrate dryness, the solution was evaporated at 40 °C in an oven. Finally, the percentage of cardiac glycoside content was calculated as follows [9].

$$Percentage\ of\ cardiac\ glycoside\ (\%) = Weight\ of\ residue\ Weight\ of\ the\ taken\ sample \times 100 \quad (1)$$

5. Determination of Total Terpenoid

5 g precisely of the plant sample was soaked with 150 ml of Ethyl alcohol 95% for 24 hours. The muddy mixture was collected by filtering by using Whatman No. 42 filter paper. Afterward, the filtrate was extracted with 50 ml of petroleum ether with heat in a water bath at 65 °C and then dried using a water bath at a temperature of 80 °C. The percentage of total terpenoids content was calculated as followed [10].

$$Percentage\ of\ total\ terpenoid\ (\%) = Weight\ of\ residue\ Weight\ of\ the\ taken\ sample \times 100 \quad (2)$$

6. Determination of Total Flavonoid

20 g of the plant sample was extracted with 200 ml of methanol 80%, at room temperature. The extraction process was repeated three times. Formerly the solution was filtered off with filter paper 125 mm. Then the filtrate was heated at 65°C to dry condition in a water bath till constant weight was gained. The percentage of total flavonoid content was calculated as follows [65].

$$Percentage\ of\ Total\ \text{Flavonoid}\ (\%) = Weight\ of\ residue\ /\ Weight\ of\ the\ taken\ sample \times 100 \quad (3)$$

7. Determination of Total Saponin

8 g of plant sample was mixed with 100 ml of 20 % ethanol and then heated over a water bath with continuous stirring for 4 hours at 55 0C. Then the mixture was filtered. Formerly residue was re-extracted via the same method as the previous one. The merged extracts were concentrated to about 20 ml over a water bath at 90 0C. The concentrate was transferred into a 250 ml separator funnel and 10 ml of diethyl ether was added and shaken vigorously. The ether layer was discarded while the aqueous layer was recovered. Subsequently, the purification process was replicated, and 25 ml of 1-butanol was added. The mixed 1-butanol extracts were washed two times with 10 ml of 5% aqueous sodium chloride solution. The remaining solution was heated in a water bath, and after evaporation, the samples were dried out and weighed then content was calculated as follows.

$$Percentage\ of\ Total\ \text{Saponin}\ (\%) = Weight\ of\ residue\ /\ Weight\ of\ the\ taken\ sample \times 100 \quad (4)$$

8. Determination of Total Alkaloid

10g of the plant sample was mixed with 200 ml of a mixture of ethanol acetic acid (10%) (Ethanol 180 ml and 20 ml of acetic acid). The mixture was left for 4 hours at room temperature. The mixture was then filtered using Whatman No. 42 - 125 mm filter paper. The filtrate was concentrated on a quarter of its initial volume utilizing a water bath. Then a 5mL of concentrated ammonium hydroxide solution (NH_4OH) was added to the reduced mixture drop-wise until precipitation occurred. After filtration and drying in an oven at 40 °C, the precipitate was collected and weighed. The percentage of the total alkaloid content was calculated as follows [12].

$$Percentage\ of\ the\ total\ alkaloid\ (\%) = \frac{Weight\ of\ residue}{Weight\ of\ the\ taken\ sample} \times 100 \quad (5)$$

9. Antibacterial Activity

The antimicrobial properties of the plant extracts were tested against several strains of bacteria. Gram-negative pathogenic bacteria, such as Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Salmonella SPP and Klebsiella aerogenes, were tested for bioactivity of the study plant samples in the presence of antibiotics (Tetc. = Tetracyclines, Penicillin = Pen, and cefazolin = cef.). The experimental assay also included Gram-positive bacteria such as Staphylococcus aureus and Streptococcus pneumoniae.

10. Disc Diffusion Method

The biological activity of the Fig's Milk samples, as well as the extracts obtained from the leaves plant materials, was assessed independently. The evaluation was conducted through the disc diffusion technique, with certain adjustments implemented to enhance the precision and dependability of the outcomes. [13-14]. the disc diffusion method was employed to determine antibacterial activity. Muller Hinton agar plates were utilized, and 0.2 ml bacterial suspension was inoculated for 24 hours. Each bacterium culture was adjusted to 0.5 McFarland turbidity (108 colony-forming units [CFU/ml]) and evenly distributed. The culture plates were placed in an incubator set at 37 °C and were allowed to incubate for a period of 24 hours [14]. For each type of solvent used in the extraction, negative controls were present in all experiments in the form of discs. Likewise, positive controls in the form of antibiotic discs suitable for each type of pathogenic bacteria were also included. After the incubation period, the inhibition zone around the discs was measured and documented. All exams were successfully approved in triplicate. Three measurements were taken in different directions to determine the average diameter of growth inhibition.

11. Results and Discussion

11.1 Phytochemical Screening (Qualitatively)

Phytochemical	Extracts			
	Aqus.	EtOH	F. M.	Reagents used
Alkaloid	+++	+++	+++	Dragendroff's
Flavonoid	+++	+++	+++	HCl + Mg turnings
Saponin	+++	+++	+++	H2O
Tannin	+++	+++	+++	Acidic FeCl3
Phlobatannin	+	+	+	1% HCl
Cardiotonic Glycosides	+++	+++	+++	Keller Kiliani
Steroid	++	++	++	Salkowski
Terpenoid	++	++	++	Chloroform and Conc. Sulphuric Acid
Amino Acid	+	+	+	Ninhydrine Solution
Glycosides	+++	+++	+++	Fehling's solutions A and B
Phenol	+++	+++	+++	Aqus. FeCl3
Quinones	+++	+++	+++	Sodium Hydroxide Solution

Table 2: Qualitative Phytochemical Screening of Ficus Carica

Key: + ++ = Abundant, ++ = Moderate, + = Low, – = absent, Aqus. = Aqueous extract of Ficus carica leaves, EtOH = Ethanolic extract of *Ficus carica* leaves and F. M. = Fig's milk of leaves stem and fruits of *Ficus carica*, Ac_2O = $\{(CH_3CO)_2\ O\}$ = Acetic anhydride

As showed in Table 2 Figs include considerable vitamins such as vitamin A, vitamin B, and vitamin C and as well as nutrients that are essential in healing numerous diseases. Also, dextrose sugar, which is about 50% of its components. Likewise, salts such as iron, calcium, copper and potassium exist at 50 % approximately. When the fig fruit or fig leaves is harvested before its ripeness, it secretes a milk-like liquid (An emulsion) that is known locally in Libya as fig milk which is considered the strongest thing in the fig tree, Which is medicine for all intractable skin diseases. May surpass its potency in several medical chemical drugs, particularly the green raw fig fruits. Correspondingly, is present in its stem when it is broken from the leaves.

Consequently, fig milk is medicine for most skin diseases, and from its potency, the result comes fast and occasionally even from once, and the thoroughgoing is delayed as an action for one week, and these substantial properties are what is required to be an effective medication. Likewise, The Libyans utilised it as a traditional medication for especially for warts by drip fig milk on it, or it is ironed with fig wood. In addition, used for treatment such as the removal of excess flesh, itching, melasma, allergies, Hemorrhoids, sinuses, vitiligo, elephantiasis, mouldy and eroding sores, gangrene, impetigo, alopecia, psoriasis, nails, and weevils. And it is a strong exfoliant for the skin and a purifier for various traces and coloured spots after diluting or being made into drops for use. And it must be avoided from contact with the eyes, as it harms them. It must be taken with caution or under the supervision of a doctor or an expert. Furthermore, Libyans utilised it as a conventional medication by dripping it into the corroded tooth to kill the worms, and if more, the tooth crumbles and falls off. When used in the mouth, the tongue must be isolated with chalk so as not to ulcerate it. As well as to avoid tongue ulceration when it is used in the mouth, the tongue must be isolated by covering it with chalk or anything preventing in touch with it.

11.2 Phytochemical Screening (Quantitatively)

Ficus carica part's	Constituent's Yields %				
	Cardiac Glycosides	Terpenoid	Flavonoid	Saponin	Alkaloid
Leaves*	19	33	16	23	39
F. M.	22	29	18	26	41

Table 3: Results of Constituents yield % of Leaves (finely powdered leaves) and Leafstalk milk

Leaves* = (fine powdered leaves)

As Showed in Table 3 Phytochemical Screening (Quantitatively) for each of Terpenoid, Cardiac Glycosides, Flavonoid, Saponin, and Alkaloid. For the powdered leaves 19, 33, 16, 23% and while F M 22, 29, 18, 26 and 41%. Usually, a quantitative evaluation is carried out to determine the standards for primary standards for crude medicines, in order to have preliminary information that is very essential for obtaining a high-quality drug.

12. Antibacterial Activities

Bacterial Types	Inhibitions Zones (mm)					
	Plant's Samples			Antibiotics		
	Aqus.	EtOH	F. M.	Tetc.	Pen.	Cef.
Pseudomonas aeruginosa	26	29	27	31	22	31
Klebsiella pneumoniae	25	22	23	26	29	18
Escherichia coli	21	23	19	25	27	24
Proteus vulgaris	18	15	19	24	23	21
Klebsiella aerogenes	19	22	21	30	25	22
Salmonella spp	19	23	22	28	24	19

Table 4: Results of the Bioactivities of the Plant Samples against Pathogenic Bacteria
Tetc. = Tetracyclines, Penicillin = Pen. And cefazolin = cef.

Table4 shows the antibacterial activity extracts, which were tested against specific pathogenic bacteria. The antibacterial activity was found to be highest in the ethanol extract, exhibiting 29 mm against *Pseudomonas aeruginosa*, while the Fig's milk showed 31 mm against *Pseudomonas aeruginosa*. The aqueous extract exhibited 25 mm against Klebsiella pneumonia, and the ethanol extract showed 23 mm against both *Escherichia coli* and *Salmonella*. In addition, the F M exhibited a significant inhibition zone of 22 mm towards Salmonella and Klebsiella aerogenes. The ethanol extract showed 22 mm and 22 mm against *Klebsiella pneumoniae* and Klebsiella aerogenes, respectively. And where the aqueous extract was 26, 21 and 19 mm against each of *Pseudomonas aeruginosa* and *Escherichia coli* and *salmonella*. As compared to the results of antibiotics, the results of the resistance of the extract for the species of bacteria were very good. As a result, the Fig's milk can be commonly used as a traditional medicinal tool, as it is used for the treatment of such as the removal of excess flesh, itching, melasma, allergies, Hemorrhoids, sinuses, vitiligo, elephantiasis, mouldy and

eroding sores, gangrene, impetigo, alopecia, psoriasis, nails, and weevils. . This reason can also play a vital role at present as a useful raw material for medicines.

13. Conclusion

The aqueous, ethanolic, and Fig's milk solutions are rich in phytochemical constituents, which provide numerous highly potent bioactive components. These phytochemicals encompass a range of compounds, including tannins, quinones, phenols, and terpenoids. These natural chemical components had an effective role in combating and killing the bacteria used in this research.

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