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Phytochemical Screening Study and Anti-Candida Evaluation of *Acacia raddiana* Leaves

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Abstract

Leaves extracts of *Acacia raddiana* (*A. raddiana*) were investigated for its medicinal importance, by valorizing of some chemical characterization and study of the antimicrobial activity. The photochemical screening of the plants constituents were assessed by using qualitative tests were conducted for the presence of the following active components: alkaloid, phenol, flavonoids, glycoside, saponins, carbohydrates. All were present. A quantitative of the flavonoids, isolated from the leaves of *Acacia raddiana* were determined and the percentage of the flavonoids isolated from leaves methanolic extract reach up to 35%. The sensitivity and resistance of *Candida albicans* to the antibiotic, and methanolic leaves extract and the total flavonoids extracted from the leaves of *Acacia raddiana* were tested. The result of this study revealed that there is no effect of plant leaves extracts while the greatest effect was from the antibiotic and the total flavonoids extract. the inhibition percentage of the leaves was 0% while the inhibition percentage of the antibiotics and the total flavonoids extract were 100% and 80% respectively. These findings suggest that the leaves of *Acacia raddiana* are potential sources of flavonoids. Also, it has a great medicinal and nutrition value due to the presence of carbohydrates, alkaloid, phenol, flavonoids, glycoside, saponins. The flavonoid isolated from the leaves has anti-candida activity.

Keywords: *Acacia raddiana; Qualitative and Quantitative Analysis; Anti-Candida Activity; Flavonoids*

Introduction

Medicinal Plant were used subsequently from ancient centuries in traditional healthiness carefulness system and for the treatment of various diseases. The plants consist of various constituents are correlated to organic, biochemistry and may more closely to natural products. Along with their utilities in plant metabolism, these constituents are essentially divided into groups primary constituents which include of amino acid, proteins, chlorophyll and sugars and secondary constituents which it includes saponins, tannin, flavonoids, alkaloids, terpenoids, fatty acids, seed oils and phenolic compounds. These secondary metabolites in plants possibly clarifies the several usages of plants for traditional medicine. Phytochemicals are present in different portions of *Acacia raddiana* which are consumed as vital constituents of both human and animal nutrition. These include fruits, seeds, barks and leaves [1].

Scientists have studied and analyzed the impact of different types of solvents, such as chloroform, hexane, ethyl alcohol and water, for the purpose of antioxidant extraction from various plants parts, such as leaves, seeds and fruits. In order to extract different active compounds from plants with a high degree of accuracy, various solvents of differing polarities must be used [2]. Moreover, scientists have discovered that highly polar solvents, such as methanol, ethyl alcohol have a high effectiveness as antioxidants.

Multiple solvents have been commonly used to extract phytochemicals, and scientists usually employed a dried powder of plants to extract bioactive compounds and eliminate the interference of water at the same time. Solvents used for the extraction of biomolecules

from plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the amount of analogous compounds in the desired yield. Therefore, increasing polarity using multiple mobile phases is useful for highly valued separations [3]. The polarity, from least polar to most polar, of a few common solvents is as follows: Hexane, Chloroform, Ethyl acetate, Acetone, Methanol, Water [4,5].

Purpose of the Study

The main purpose of this study to investigate the presence of active compounds as the possible agent responsible for the medicinal activities of *Acacia raddiana* by carrying out the phytochemical screening of the dried materials of the plant extracts and also to carry out the biological activities of these compounds.

Materials and Methods

Collection and preparation of plant material

In this research, the plant parts of *Acacia raddiana* used were leaves. The plant material was collected from Misurate region, Misurate Libya and was identified at Botany Department, College Sciences-Misurate University. The plant parts were dried in shadow for 7 days then powdered using clean pestle and mortar. The fine powder and sieved in order to obtain a fine green powder stored and sold in labelled bags or glass jars

Extraction of the powdered leaves of the plant

Maceration and alcoholic extraction preparation

The fine powdered of *Acacia raddiana* (leaves) each 50g was weighed and soaked in 150 ml of ethanol (80%) in a conical flask, separately. The flask containing the samples was mixed very well and plugged with cotton wool and then kept on a water bath at 100°C for 30 minutes. After that, the mixture was filtered and the extract was collected and concentrated by evaporation to dryness in a vacuum rotary evaporator stored at 4°C in airtight containers until further use [6].

The separation of ethanol extract in different polarity solvents [6]

Petroleum ether solvent

A 250 ml of distilled water was added to the concentrated solution, ethanol extract, then transferred into a 500 ml separating funnel and extracted twice using 250 ml petroleum ether. The petroleum ether was run off then treated with sodium bicarbonate and filtered then concentrated by evaporation to dryness in a vacuum rotary evaporator stored at 4 °C in airtight containers until further use.

Chloroform solvent

The ethanol layer was extracted again by adding 250 ml of chloroform and shaken gently to allow the layer to separate. The lower chloroform layer was run off then concentrated and stored into dark bottle.

Ethyl acetate solvent

A 250 ml of ethyl acetate was added to the ramming ethanol extract into funnel separation shaken then the lower layer drown, filtered and concentrated the stored.

Ethanol solvent

Remaining ethanol extract was evaporated under reduced pressure using Rotatory evaporator. All extracts obtained were selected in the present protocol for further evaluation.

Phytochemical analysis

One of the main goals of the phytochemical screening is to characterize or identify the main chemical groups of the leaves of the plant as alkaloid, sterols**,** tannins, saponin and flavonoids by using following standard methods [7-9].

Quantitative analyses

Identification of alkaloids

A fraction of each extract (Petroleum ether, chloroform, ethyl acetate and Ethanol extract) was treated with 3 - 5 drops of Dragendorff's reagent and observed for the formation of a reddish-brown precipitate was observed to detect the presence of alkaloids.

Identification of flavonoids

10 ml of distilled water was add to 3 ml of each plant extract. The mixture was warmed on a water bath then filtered while hot; the filtrate was allowed to cool and used for the following test.

Sodium hydroxide test: To test tube 2 ml of 10% NaOH solution was added, the yellow solution indicates the presence of flavonoids which on adding dilute hydrochloric acid becomes colourless.

Detection of glycosides

Few drops of HCl were added to the test tube containing 1 ml of the plant extract, and then the tube was transferred to the boiling water bath for 2 minutes and after that, 2 ml of Benedict reagent was added. The appearance of red color indicates of glycosides.

Detection of carbohydrates

Fehling's Test: Equivalent volume of Fehling solution A and Fehling solution B are mixed and few drops of the sample are added and boiled, a brick red precipitate indicates the presence of reducing sugar.

Identification of saponins

Frothing test: The powdered of plant material (1g) was placed in a test tube and 10 ml of warmed distilled water was added and shaken vigorously for 1 minute. It was then allowed to stand for 30 minutes and observed. Formation of honeycomb froth indicates the presence of saponins.

Test for sterols (Salwoski's test)

To test tube 4 - 5 drops of concentrated Sulphuric acid was added to form a lower layer. Reddish-brown colour at the interphase indicates the presence of the steroidal ring.

Identification of tannins

The extract from each solvent was added to 20 ml of 50% alcohol, it was then filtered and the filtrate tested for the presence of tannins using:

Ferric chloride test: 4 - 5 drop of a diluted solution of $FeCl_3$ was added to plant extract; production of a blue or greenish-black colour that changes to olive green as more ferric chloride is added indicates the presence of tannins.

Quantitative analyses

Isolation of crude flavonoids

About 10 grams of dried leaves samples were ground then extracted recurrently with 100 ml of 80% aqueous methanol, at room temperature. The complete solution was filtered through Whatman filter paper No 42. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath; the dry content was weighed to a constant weight [10,11]. The percentage flavonoids content was calculated using the formula below:

% flavonoids = final weight of sample/initial weight of sample × 100

Identification of total flavonoids

For identification of total flavonoids in pure mixture UV-visible spectroscopy, Infrared Spectroscopy and thin layer chromatography were performed.

Ultra violet visible spectroscopy (UV-Vis)

The UV-Vis spectroscopy was used to determine the total flavonoids extract.

UV-vis single beam spectrophotometer Agilent Cary 60 UV-Vis Spectrophotometer was used for the absorbance measurements in the range 200 - 800 nm, Spectra were recorded at 25°C.

Infrared spectroscopy

IR spectroscopy is used to determine the functional group present in the sample.

To determines different functional groups in total flavonoids such-CO, -OH, -NH_2, aromaticity, and so on, present in a molecule.

Diamond attenuated total reflectance (ATR) crystal of the Agilent Cary 630 ATR-FTIR analyzer. The samples were pressed against the diamond crystal using the attached pressure clamp. FTIR spectra were acquired in less than 30 seconds. The spectra of extractions samples were analyzed using an automated output pass/fail or percentage (%) similarity. Pure flavonoids was dissolved in different solvent (petroleum ether, ethyl, chloroform, ethyl acetate, ethanol respectively) and were used for comparison.

Thin layer chromatography (TLC)

TLC analysis Chromatography plates were prepared using silica Gel, 60 F254 TLC Aluminum Sheet 20 x 20 cm Merck- Germany. The samples were spotted on the plates with graduated capillary tubes (5 µL). Methanol: acetic acid: water (4:1:1) were used as mobile phase.

Detection

Total flavonoids band was observed as follows:

Without treatment using UV (254 - 356 nm).

Universal detection reagents: using Ammonium chloride (5%) then comparing its rate of flow (Rf) value with references [12].

Anti-candidal activity assay

The anti-candida activity of ethanolic plant extract and total flavonoids extract were confirmed using *Candida albicans*. Where was determined by adding 1ml of leaves extract and total flavonoids extract (individually) on Sabouraud dextrose agar medium.

Candida albicans was identified microscopically and by microbiological specialist at microbiology department, Misurate university, Misurate, Libya.

Culture media and inoculum

Candida albicans was maintained on Sabouraud dextrose agar, the concentration of microbial inoculums was within the range of 10^8 $CFU.ml^{-1}$.

The antimicrobial effect of the leaves extract and total flavonoids extract was tested by adding 1 ml of leaves extract and total flavonoids extract (individually) on Sabouraud dextrose agar medium. Overnight microbial cultures were used for surface inoculation of Petri dishes containing 15 ml of Sabouraud dextrose agar (SDA). Petri dish was spread on with 0.5 ml of *Candida albicans* inoculum streaked thoroughly all over the surface. Subsequently, 1 ml of leaves extract and total flavonoids extract (individually) were punched into the inoculated medium. All plates were incubated at 35 ± 2°C and inhibition zones were measured after 24h. The experiment was repeated twice, including control. After incubation the zones of inhibition of the growth of the fungi around the disks were measured. The mean values of three replicates were calculated.

Results and Discussion

Quantitative phytochemical analyses

Plant name	Percentage Yield	Leaves (%)
Acacia raddiana	Flavonoids	35%

Table 1: *Results of Quantitative phytochemical analyses of the leaves of Acacia raddiana extracts.*

As shown in table 1, the percentage yield of total flavonoids from plant leaves reached 35% this result in agreement with the study done by [2].

Qualitative phytochemical analyses

Chemical Constituent	Tests	Leaves solvent			
		Petroleum ether	Chloroform	Ethyl acetate	Ethanol
	Wagner's	-	-	-	+
Alkloids	Dragendorff's test	-	-	-	-
Flavonoids	Ferric chloride test	-	+	+++	++
Glycosides	Baljet test	-	-	+	+++
Carbohydrates	Fehling's Test	-	+++	+	+++
Phenols	Ferric chloride test	-	-	-	+++
Saponins	Frothing test	-	-	-	+++
Sterols	Lieberman-Burchard's test	-	-	-	-

Table 2: *Results of qualitative phytochemical analyses of the leaves of Acacia raddiana extracts.*

Positive reaction: +++; Moderately Positive reaction: ++; Trace: +; Negative test: -.

As revealed in table 2 the qualitative phytochemical analyses of the leaves of *Acacia raddiana* extracts were presence of 6 chemical constituents such as alkaloid, tannins and phenols, saponin, flavonoids, glycosides and carbohydrates, while not presence of each of Sterols. Similarly, to the previous studies of [10,13] on *Acacia raddiana*, their results found that the leaves extracts contain phenolic compounds such as flavonoids, tannins, alkaloids and saponins.

The result of this study revealed that ethanol solvent has most tested chemical constituents and lower chemical constituent were presented in Petroleum ether, Chloroform and Ethyl acetate solvents (respectively).

Total flavonoids

Flavonoids were isolated from the leaves with total percentage 35% of the leaves. Flavonoids were identified using following technique.

UV-Vis spectroscopy

In this study detection by UV-Vis spectroscopy have revealed that total flavonoids have two major absorption bands: Band II (250 - 285 nm) corresponds to the A ring absorption. Band II appears as two peaks (258 nm) with a shoulder (272 nm) thus that is clarify the presence a di-, tri-, or o-substituted B ring (Figure 1).

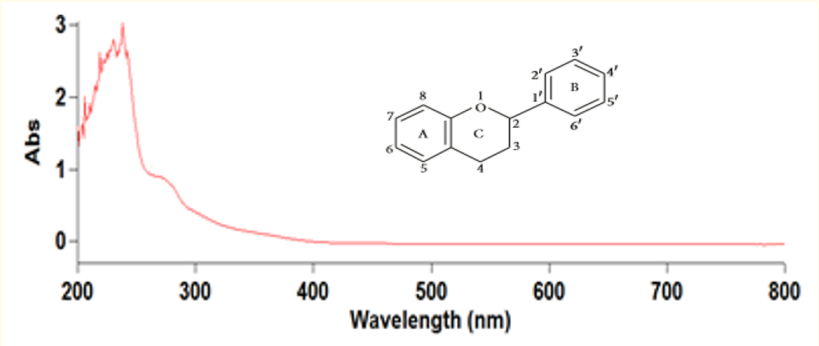


Figure 1: *UV-Vis spectroscopy of total flavonoids with Basic flavonoid structure.*

Wollenweber and Dietz [14] explains that B ring and B and II peaks in the 240 - 280 nm region due to the benzoyl system of the A ring. Flavanones have a saturated heterocyclic C ring, with no conjugation between the A and B rings, as determined by their UV spectral characteristics [15].

IR Spectra

The IR spectrum of total flavonoids isolated from *Acacia raddiana* leaves were shown in figure 2. the peaks were recorded in the range of 1000 to 3500 cm^{-1}. The characteristic bands occurring in the spectra were listed in table 3.

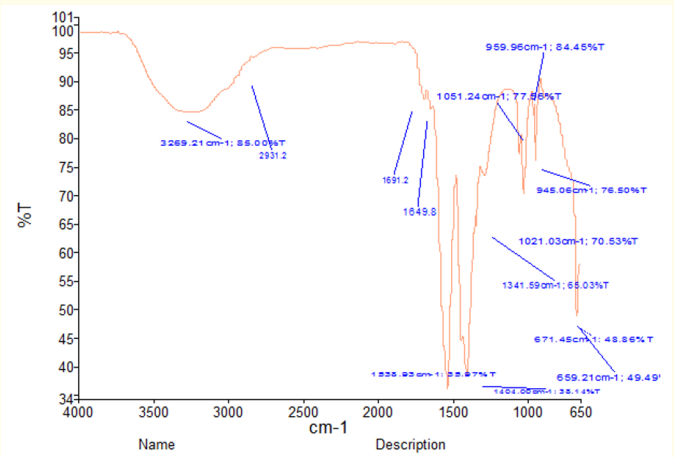


Figure 2: *IR spectra of total flavonoids isolated from Acacia raddiana leaves.*

Description	Frequency (cm^{-1}) of leaves extract
3269.21	0-H stretch
2931. 2	Aliphatic C-H stretch
1649.8-1691.2	C=O stretch
1021.03	C-O-C bending
959.95	C=CH

Table 3: *Peak of total flavonoids in IR Spectra.*

Regarding to the IR spectra descripted by Bimla [10] and the result of this study, the major functional group presented in the total flavonoids of the leaves of *Acacia raddiana* were hydroxyl and carbonyl group at frequency 3269.21 and 1649. These functional groups were found in flavone compound at the same frequency.

Thin layer chromatography

The total flavonoids of *Acacia raddiana* leaves was spread by Thin layer chromatography in order to identify compounds number in the flavonoids group. The result of this study shown the presence of 6 spots, with different R_f value, after separation of total flavonoids of methanolic leaves extract, see figure 3 and table 4.

Figure 3: *TLC of detected on TLC detected by ammonium chloride.*

R_f	Spots number
11.9	1
11	2
10	3
8.1	4
6.3	5
1.2	6

Table 4: *R_f value of leaves total flavonoid.*

The spots detected on TLC under UV light appears light fluoresce and red in their colors, after spraying with aluminum chloride their light fluoresce were red, yellow and brown.

According to the results presented in figure 3 and UV and IR spectra analysis, it can be emphasized that total flavonoids contain flavones as an active compound which appeared in red color.

Anti-bacterial activity assay

Table 5 shows the sensitivity and resistance of pathogenic fungi to the leaves methanolic extract and the total flavonoids of leaves extract, where there is no effect of plant leaves extracts while the greatest effect was from the antibiotics and the total flavonoids extract,

Where the inhibition percentage of Nystatin (Antibiotic) reach to 100% while the inhibition percentage of total flavonoids against *Candida albicans* was 70%

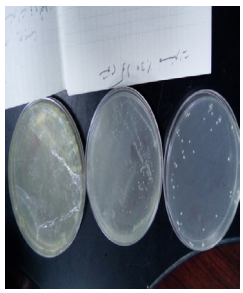
Fungi Name	Inhibition percentage (%)		
	Leaves extract	Total flavonoids extract	Antibiotic Nystatin
Candida albicans	0%	70%	100
			

Table 5: *Results of Anti-bacterial activity assay of the leaves extract and the total flavonoids isolated from the leaves of Acacia raddiana.*

Conclusions

This paper describes the phytochemical screening of *Acacia raddiana* along with the evaluation of its antimicrobial activity. The phytochemical tests performed on the leaves ethanolic extracts of *Acacia raddiana* shows the presence of alkaloids, phenol, saponins, glycosides, flavonoids and carbohydrate. The present study revealed the presence of flavonoids in *Acacia raddiana* leaves extracts which were confirmed by various techniques studies. Since flavonoids contains a wide range of medicine and pharmacological properties, they can be exploited more active compounds in future, moreover clinical researchers are required to investigate its medicinal benefit in order to establish it as a regular drug.

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