

Flavonoids And Alkaloids Extracted From marodphali (Helicteresisora) And Their Using Role As Anti-Bacterial, Anti-Fungal And Their Effectiveness As Antioxidants

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Abstract

Natural products are known as raw chemicals that are available in many medicinal plants and characterized as alternatives to medicines and have medicinal and biological applications as anti-bacterial and anti-fungal, antioxidant, immune system boosting, anti-inflammatory and UV-protective effects. Alkaloid and flavonoid fractions were extracted from Marodphali (*Helicteresisora*) plant and tested for their antioxidant and antimicrobial activity. Antioxidant activity for fractions were determined with β -carotene assay and the flavonoid compound showed the highest antioxidant activity compared to the standard BHT. The results of antimicrobial activity of extracts on microbial species (*E. coli*, *P. aeruginosa*, *S. aurea*, *K. pneumoniae* and *Aspergillus flavus*) exhibited the flavonoid have a good activity compared to alkaloid extract.

Keywords: Marodphali, antioxidant, antimicrobial study.

Introduction

Since medicinal plants include many chemicals such as flavonoids, phenolic acids, tannins, and coumarins, they have varied biological impacts on the human health system (1,2,3) in different parts of the plant. Atherosclerosis, arthritis, ischemia, gastritis, cancer, and AIDS are just a few of the frequent disorders that are treated using various plant parts (4).

Because synthetic antioxidants have several adverse effects, the necessity to take antioxidant supplements obtained from natural sources, particularly those generated from plants, such as phenols, has recently gained a lot of attention (5). Studies have proven that consuming plant foods that contain antioxidants are beneficial to health because they control in many degenerative processes. It can reduce the incidence of cancer and cardiovascular disease (6)

The plant Marodphali (*Helicteresisora*) shrub, which reaches a height of 5 cm, is distinguished by its gray bark covered with star hairs. Its flowers are red and the fruit is greenish brown, which is widely cultivated throughout India and Southeast Asia and the southern part of China is in the dry regions (7).

Alpha and beta amyryns, taraxerone, anthoquinones, sterols, lupeol, -sitosterol, and volatile oil are among the therapeutic and antioxidant substances found in the plant's fruits. It's been utilized in traditional medicine for a long time.(8), it used to treat stomach cramping Repellent tapeworm, antipyretic and anti-diarrhea. A treatment for scabies when applied topically and diabetes (9,10,11).

Materials and Methods

Plants 1-1

Marodphali (*Helicteres isora*) fruits were obtained from markets to sell medicinal herbs, and then the plant was classified into herbarium belonging to (College of Science, Department of Life Sciences, University of Basra). The dried fruits were taken and ground with a hand mill and the powder was kept after grinding until the time of use. All of the reagents and solvents used are of the highest purity available and purchased from Sigma-Aldrich

1-2 Flavonoid extraction

The weight of (25gm) Marodphali (*Helicteres isora*) fruit powder mixture with 70% ethanol was taken back for 24 hours, the extract was filtered and diluted with 2% aqueous lead acetate until flocculent and brown sediment formation. Filter paper was used to separate the precipitate, which was then washed with water, methanol, and ethyl acetate.

The product is dissolving in (50 mL acetone and 10 mL 2N HCl) and filtered. The filterate is allowed to dry at room temperature to get (0.1gm) amorphous brown powder (12).

1-3 Alkaloid extraction

Twenty gram of dried fruits powder were heated with 250ml of (10%Acetic acid in EtOH) on water bath for 24 h. The residue was removed by filtration and the filtrate was concentrated under vacuum up to 15ml, and acidified with 2% sulphuric acid. The acidic fraction then basified with ammonium hydroxide to PH 9, and extracted with chloroform(3×25ml).The combined chloroform layer was evaporated under vacuum in rotary evaporated, to afford (0.15) gm (13).

1-4 Determine the ratio of elements:

To find out the ratio of the elements (Mg,Cu,Zn,Fe) were weighed and digested in (1:4) a mixture of nitric acid and perchloric acid (10 ml). After digestion, a few drops of concentrated hydrochloric acid were added and the solution was diluted with deionized distilled water. A dilute filtrate solution was used to analyze the minerals by atomic absorption spectrophotometer (14).

Initial qualitative tests: 1-5

Initial tests were performed on the alkaloid and flavonoid extracts to detect the active groups present in the two extracts

1-6 Thin Layer Chromatography:

(TLC) was performed for both the alkaloid and the flavonoid extracts using the following solvents n-hexane: ethanol (5:3) and n-butanol: acetic acid: water(4:1:5) respectively. The slide was dried and appeared using special reagents. Table (2) illustrates the results.

1-7 Determine the Antioxidant activity

The radical's scavenger ability of the two extract were performed by using β -carotene assay. (1 ml) of β -carotene (0.2 mg / ml in chloroform) was added to mixture of 0.02 ml and 0.2 ml of linoleic acid and Tween 20 and 50 ml of distilled water was added to the mixture after evaporating the chloroform. 0.2 ml of tested extracts and reference (butylated hydroxy toluene BHT) then mixed with 3.8 ml of the combinations. The absorbance was set at 470 nm, and samples were thermally autoxidated for 2 hours at 45 °C in a water bath. Every 15 minutes, the absorbance was measured (15). The following equation was used to compute antioxidant activity (AA).

$$\%AA = 1 - [(A_i - A_t) / (*A_i - *A_t)] \times 100$$

Where A_i : sample first absorbance

A_t : sample last absorbance after (105)min

* A_i : control first absorbance

* A_t : control last absorbance after (105)min.

1-8 Antimicrobial Activity

Antimicrobial properties of the extracts against Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and the fungus *Aspergillus flavus* were tested in vitro using the agar well diffusion method. 100 ml of nutrient broth was mixed with 1ml microbe inoculum and placed in incubator at 30°C for 24h. after that a dilution of bacterial solution with Physiological Normal saline compared with the standard test tube McFarland for 10^8 cells / ml of stuck bacterial and inoculated into nutrient agar, using L-shape to spread bacteria on Muller Hinton media, the two extracts (100mg /ml in DMSO) placed in dishes which incubated in the incubator at 37°C. Each isolate's inhibitory zones were measured in millimeter units (16).

Results and Discussion

Table 1 lists the results of qualitative chemical analysis of the alkaloid and flavonoid extracts and table 2 shows the results of thin layer chromatography which exhibited one spot for flavonoid extract with Rf value 0.61 and three spots to alkaloid extract (0.76, 0.55 ,0.37). Table (3) shows the elements and its amount found in plant Marodphali (*Helicteres isora*)

Table (1): Initial qualitative tests for alkaloid and flavonoid extracts

Phytochemical	Alkaloid extract	Flavonoid extract
Glycoside	-	-
Phenols	-	+
Flavonoids	-	+
Tannins	-	-
Saponins	-	-
Alkaloids	+	-
Terpenoids	-	-
Sterols	-	-
Carbohydrate	-	-

Table (2): preliminary qualitative tests on TLC for alkaloid and flavonoid extract

Test extract	P- ansaldehyde& Phosphoric acid	Ninhydrin	Folin reagent	Drangdroff	40% H2SO4	H2SO4 2ml+ Chloroform	visible
Alkaloidextract	-	-	-	0.76 0.55 0.37	-	-	-
Flavonoid extract	0.61	-	0.61	-	0.61	0.61	0.61

Table (3) The elements and its amount found in plant Marodphali (Helicteres isora)

Quantity of elements measured mg/g	Elements
10.38	Magnesium
7.56	Copper
15.87	Iron
6.15	Zinc

Characterization of flavonoid

At room temperature, the flavonoid compound's UV-visible spectrum was recorded in DMSO solvent fig. 1. The electronic spectra of compound displayed three bands the first one at (275 nm) belong to π - π^* transition, the second and third band at (510 and 665nm) respectively indicating n - π^* transitions.

The infrared spectrum of flavonoid compound fig.2 appearance stretching vibration of the hydroxyl group (-OH) at $(3417.9) \text{ cm}^{-1}$ and band at 1724.4 cm^{-1} that attributed to the C=O stretching vibration. Also IR spectrum showed the band at 1612 cm^{-1} which point to ν (C=C) stretching vibrations of aromatic ring, the appearance of band at 1284 cm^{-1} can conforming the phenolic C-O stretching vibration (17).

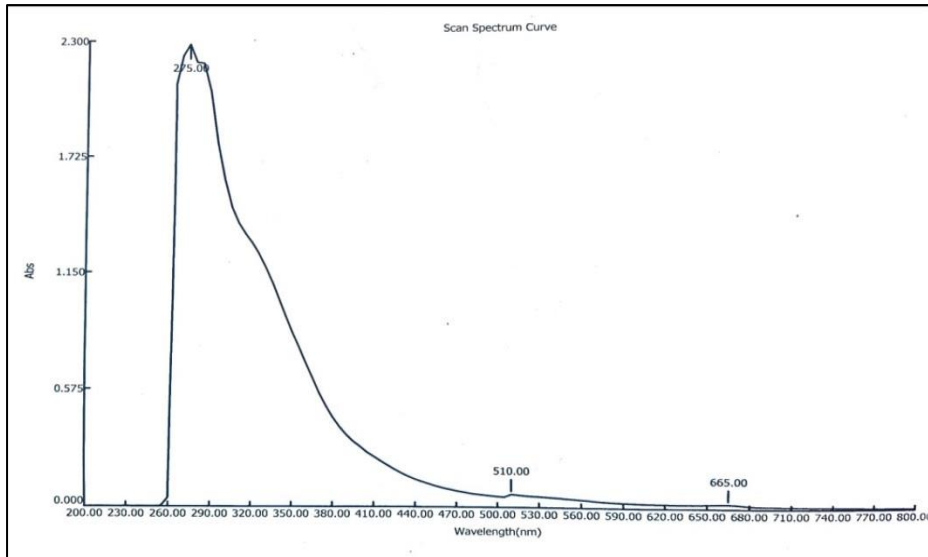


Fig-1-flavonoid UV. Visible spectrum

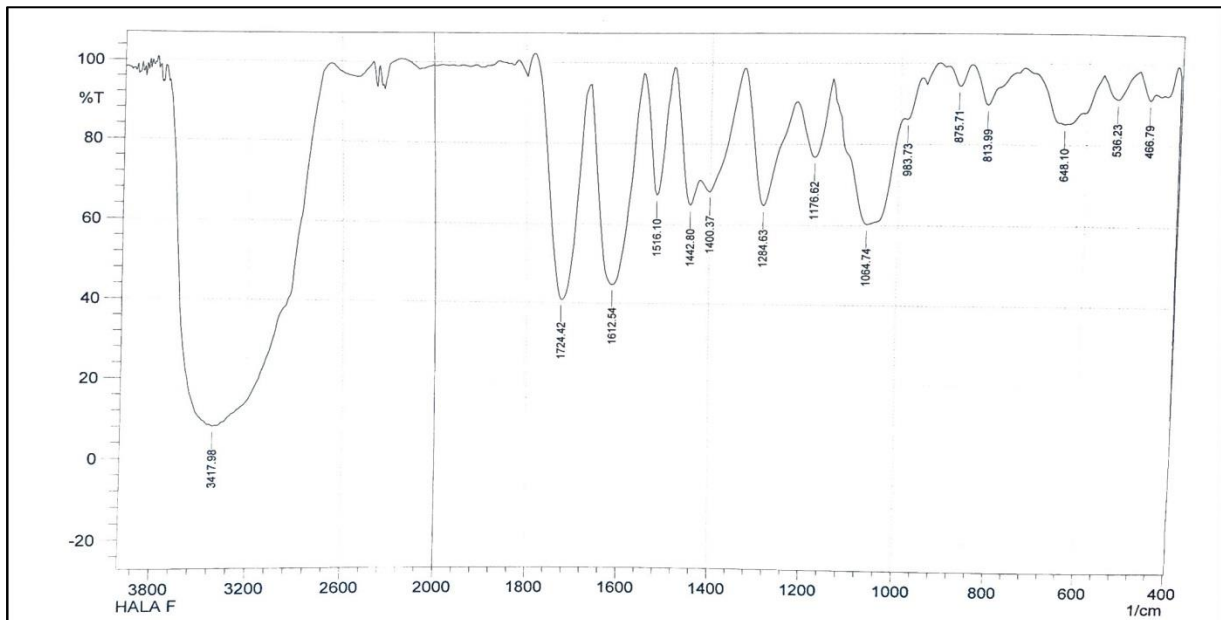


Fig -2- flavonoid Infrared spectrum

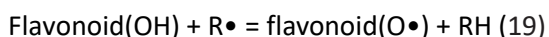
Antioxidant Activity

The radical scavenger activity for flavonoid and alkaloid was performed by β -carotene assay corresponding to the relationship between absorbance and time as viewing in Table -4- and figure-3- and comparable those with the standard phenolic compound BHT which used in various food systems. with use of previous mathematical equation the results elucidate the flavonoid compound have the highest percent inhibition of lipid peroxidation was 80% more than the standard BHT (72%), this can be attributed to the existence of phenolic groups which can enhance the antioxidant activity (18).

As well the alkaloid compound has a moderate activity as antioxidant with (39%) compared to BHT.

The antioxidantability is the most important property of flavonoids by scavenging of reactive oxygen species which are produced by exogenous injury or during usual oxygen metabolism and exposed body cells and tissues to damage.

the radicals stabilize by flavonoids to produce a more stable, less-reactive radicals. According to below equation, the hydroxyl group of flavonoids donates hydrogen to render radicals inactive:



Also, Alkaloids are compounds which contain OH and NH functional group, and they behave as antioxidant by donating their hydrogen to radicals (20).

Table 4: Effectiveness of Alkaloid extract and flavonoid compound as radical's scavengers

Comp. symbol	Ai	At	*Ai	*At	AA%
BHT	2.436	2.364	2.057	1.803	72
F	2.275	2.226	2.057	1.803	80
A	2.157	2.002	2.057	1.803	39

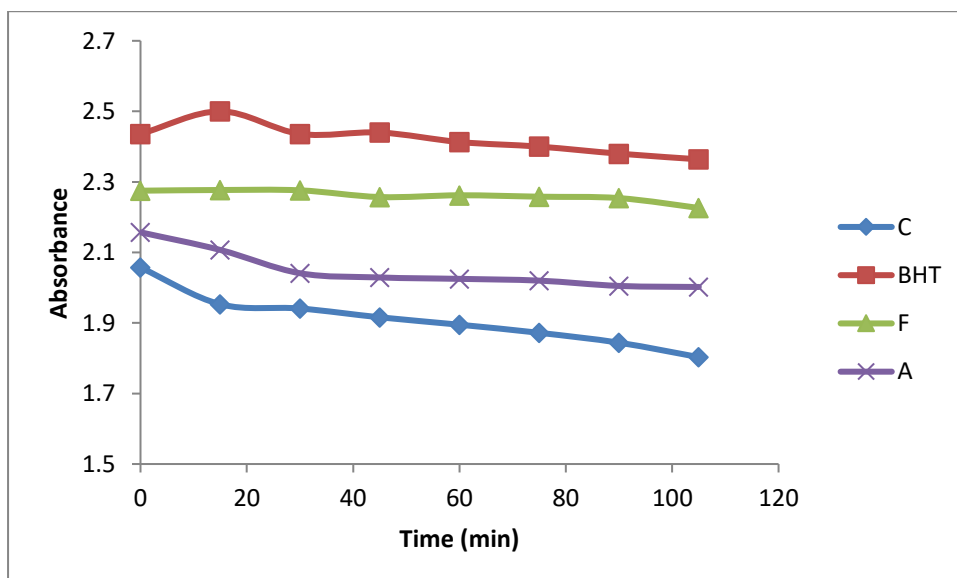


Fig. 3 Antioxidant activity of flavonoid (F) and alkaloid (A)

Antimicrobial Activity

The Results of the effectiveness of extracts on Microbial species (table 5) exhibited the flavonoid have a good activity on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aurea* and *Aspergillus flavus* with inhibition zones ranged between 9-18 mm and have no activity on *Klebsiella pneumoniae*.

The alkaloid extract showed noticeable activity on *Escherichia coli* and *Pseudomonas aeruginosa* with inhibition zones 14 and 10 mm respectively with no activity on other microbes.

Table 5: Inhibition zones (mm) of alkaloid (A) and flavonoid (F) on selected microbes with concentration (100mg/ml)

Microbes	A	F
Control	0	0
<i>Escherichia coli</i>	14	18
<i>Pseudomonas aeruginosa</i>	10	9
<i>Klebsiella pneumoniae</i>	0	0
<i>Staphylococcus aureas</i>	0	10
<i>Aspergillus flavus</i>	0	10

Because of the differences between gram positive and gram negative bacteria, such as the thickness of the cell wall, which is approximately 20 to 30 nm thick in (+ve) bacteria and 8 to 12 nm thick in (-ve) bacteria; lipid amount in the cell walls; and the content of lipoprotein, which is low in (+ve) bacteria and high in (-ve) bacteria so the results showed a high significant difference in inhibition to the two extracts. Gram negative bacteria are sensitive to chemical compound effect compared to gram positive bacteria due to thin layer of peptidoglycan located between two lipid layers (21).

The alkaloid showed no inhibition to *Aspergillus flavus* due to its cell wall proteins are adhesions and receptors. Since, some components have a high immunogenic capacity, certain wall components can drive the host's immune response to promote fungus growth and dissemination. The cell wall of fungus is made up primarily of glucans, chitin, and glycoproteins, and is a distinctive feature of the organism. Because the components of the fungal cell wall are not found in humans, this structure is a good target for antifungal treatment (22).

Antimicrobial properties can be attributed to many plant compounds, including alkaloids, phenolics, flavonoids, carotenoids, coumarins, terpenes, tannins, and several primary metabolites (amino acids, peptides, organic acids) and the flavonoids are a favorable substances with low systemic toxicity. Several

studies illuminated the antibacterial mechanisms of flavonoids that included mainly: nucleic acid synthesis inhibition, influence the biofilm formation, porins, permeability can lead to cytoplasmic membrane function inhibition, and the interaction with a number of important enzymes (23,24).

Antifungal properties of flavonoids have been discovered and the fungal growth inhibition by numerous mechanisms, including rupture of the plasma membrane, mitochondrial dysfunction stimulation, cell wall formation inhibition, cell division inhibition, RNA and protein synthesis inhibition, and the efflux mediated pumping system inhibition (25).

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