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Flavonoids And Alkaloids Extracted Frommarodphali (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 wereextracted fromMarodphali (Helicteresisora) plant and tested for their antioxidant and antimicrobial activity. Antioxidant activity for fractions were determined with β-carotene assay andthe flavonoid compound showed the highest antioxidant activity compared to thestandard BHT.The results of antimicrobial activity of extracts on microbial species (E. coli, P. aeuroginosa, 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 plantMarodphali (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 amyrins, 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 for24 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 layerwas 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 flavonide 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 with3.8 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).

$$\%AA = 1 - [(Ai - At) / (*Ai - *At)] \times 100$$

Where Ai: sample first absorbance

At: sample lastabsorbance after (105)min

*Ai: control first absorbance

*At: 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 aurogenosa, 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 andplaced in incubator at 30^{o}C for 24h. after that a dilution of bacterial solution with Physiological Normal saline compared with the standard test tube McFarland for 108 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 at37°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 exhibitedone 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 plantMarodphali (Helicteresisora)

Table (1): Initial qualitative tests foralkaloid and flavonoid extracts

Phytochemical	Alkaloidextract	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 amountfound in plantMarodphali (Helicteresisora)

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 DMSOsolvent fig. 1. The electronic spectra of compound displayed threebands the first one at (275 nm) belong to л-л* transition, the second and third band at (510 and 665nm)respectively indicating n - л* transitions.

The infrared spectrum of flavonoid compound fig.2appearancesstretching vibration of the hydroxyl group (-OH) at (3417.9) $cm^{-1}$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 υ (C=C) stretching vibrations of aromatic ring, the appearance of band at 1284 cm^{-1}can conforming the phenolic C-O stretching vibration (17).

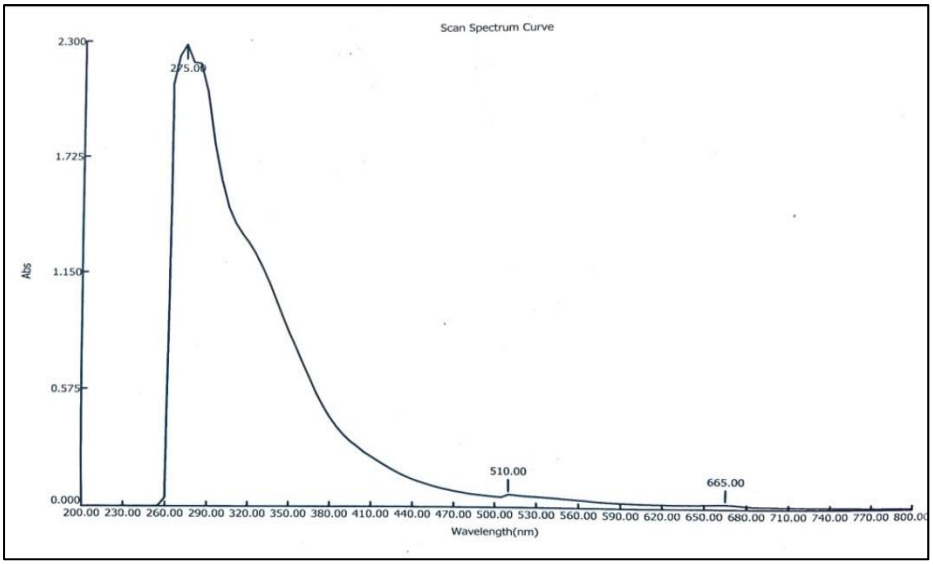

Fig-1-flavonoid UV. Visible spectrum

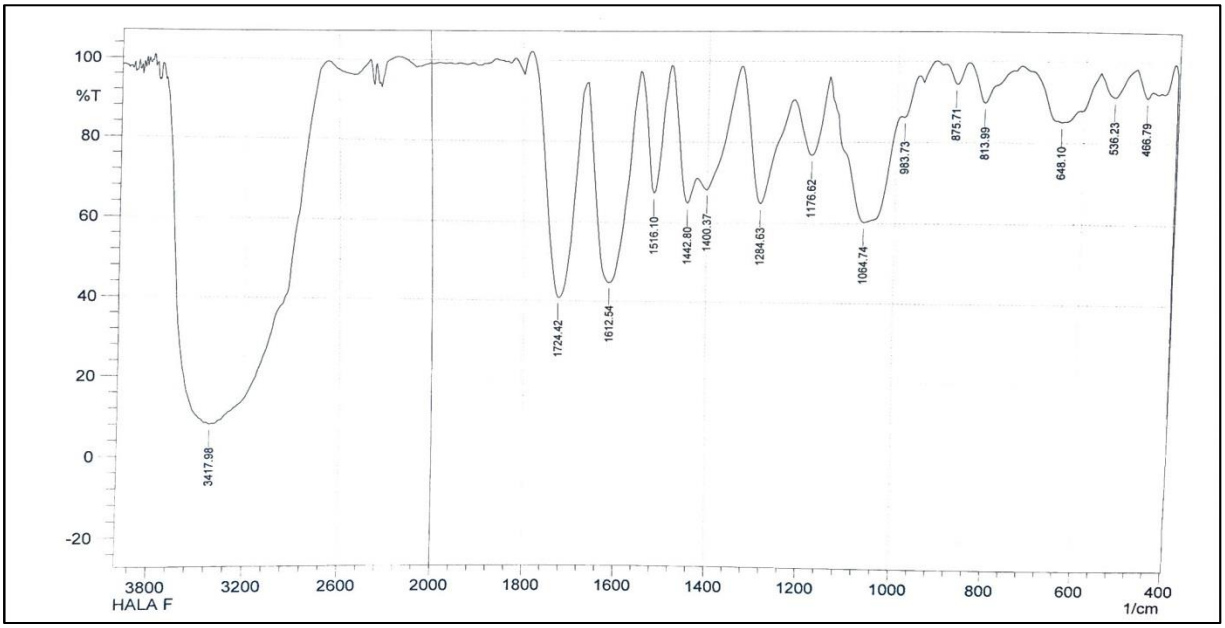

Fig -2- flavonoid Infrared spectrum

Antioxidant Activity

The radical scavenger activityfor flavonoid and alkaloid was performed byβ –carotene assaycorresponding to the relationship betweenabsorbance and time as viewing in Table -4- and figure-3- andcomparable those with the standardphenolic compound BHT which used in various food systems. with useof previous mathematicalequationthe results elucidate theflavonoid compound have the highest percent inhibitionof lipid peroxidation was 80% morethan the standard BHT (72%), this can beattributed to the existence phenolic groups which canenhance 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 flavonoidsby scavenging of reactive oxygen species which are produced by exogenous injury orduring usual oxygen metabolism andexposed body cells and tissues to damage.

the radicalsstabilize 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:

Flavonoid(OH) + R• = flavonoid(O•) + RH (19)

Also, Alkaloids are compounds which contain OH and NH functional group, and they are behaves 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

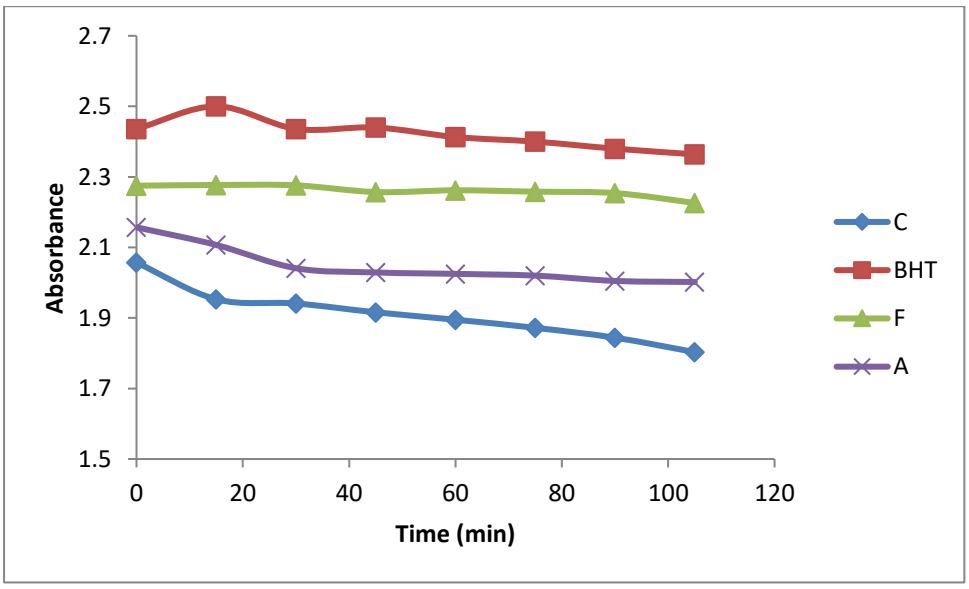

Fig. 3 Antioxidant activity of flavonoid (F) and alkaloid (A)

Antimicrobial Activity

The Resultsof the effectiveness of extracts on Microbial species (table 5) exhibited the flavonoid have a good activity onEscherichia coli, Pseudomonas aeuroginosa, Staphylococcus aurea and Aspergillus flavus with inhibition zones ranged between 9-18 mm and have no activity on Klebsiellapneumoniae.

The alkaloid extract showednoticeable activity on Escherichia coli and Pseudomonasaeuroginosa 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) onselected microbes withconcentration (100mg/ml)

Microbes	A	F
Control	0	0
Escherichia coli	14	18
Pseudomonas aeuroginosa	10	9
Klebsiella pneumoniae	0	0
Staphylococcus aureas	0	10
Aspergillus flavus	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 showeda high significant difference in inhibition to the two extracts.Gram negative bacteria a sensitive to chemical compound effect compared togram positive bacteria due to thin layer of peptidoglycanlocated between two lipid layers (21).

The alkaloid showed noinhibition toAspergillus flavus due to its cell wall proteinsare 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 canattributed 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

studiesilluminatedthe 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 discoveredand thefungal growth inhibitionbynumerous mechanisms, including rupture of the plasma membrane, mitochondrial dysfunctionstimulation, cell wall formation inhibition , cell division inhibition, RNA and protein synthesis inhibition, and the efflux mediated pumping systeminhibition (25).

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