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RESEARCH ARTICLE

Effect of HPLC Separated Flavonoids Extracted from *Centraurea Cineraria* to Some Species of Bacterial Pathogenicity

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Abstract

The research included the separation and determination of flavonoids from the extract of *Centraurea Cineraria* gray using the high-performance liquid chromatography technique. Cirsilineol, Jaceosidin, Melitensin, Apigenin and Prunasin were found to be identical to the standard compounds used in chromatography analysis. The flavonoids found in the extract were detected using qualitative chemical analyzes. The effect of the gray catheter extract containing the flavonoids mentioned above was studied in five bacterial species (*Escherichia coli, Streptococcus pneumonia, Klebsiellaspp Staphylococcus aureus, Pseudomonas aeruginosa*) using Agar well diffusion method and then added concentrations (100,75,50,25 mg / Ml of the extract and the measuring sieve. The transparent zone of the bacterial growth inhibitor showed a concentration of 100 mg / ml inhibitory capacity with a zone of 17 mm inhibition for *Klebsella.*

Introduction

The responsible for the color and organoleptic properties in plants are chemical compounds. These compounds occur naturally in plants these are called the secondary metabolites or Phytochemicals. In the past, Phytochemicals have been used as drugs for a long time. Many of the medicinal plant have been characterized for secondary metabolite screening and their possible use in the chemotherapy.

Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from the plants of specific disease [1-4]. Flavonids, Alkaloids, tannis, sterols and triterpenes are bioactive compounds in plants [5-7]. Flavonids are a large family of compounds. It is synthesized by Plants. It has a common Chemical structure. Flavonoids are Poly-phenolic compounds possessing 15 carbon atoms and two benzene rings joined by a linear three carbon chain which may be further divide into Subclasses. In higher plants, Flavonoids considers one of the most significant classes of compound where they can be easily recognized as flower pigments in angiosperms.

This occurrence is not restricted to flowers. It also includes all parts of a plants the screening of those compounds in the plants is the most important task un this paradigm [8]. The chromatographic study of the compounds is a useful and reliable source in the process of bioactive compound screening in plants. The separated compound isolation is made by the preparative HPTLC method.

The final separation was done in the Infrared spectroscopy followed by the ultraviolet-visible spectroscopy [9]. *Centaurea cinerarium is* a small plant in the family Asteraceae. The Matura plants will grow from 15 cm to 60 cm. It will produce small white or yellow flowers in summer. These trimmed because the plant is normally grown as foliage [10].

Herbal plants are commonly used in meals and folk medicine because they add flavor and aroma to foods as well as preservatives of medicinal value [11]. It has been established through research and studies of compound family leaves that these leaves have anti-bacterial effect coli E Bacillus cereus, Pseudomonas aeruginosaureus

Staphylococcus, [12]. The aim of the research is to separate the chemical compounds from the extract *Centaurea cineraria* and calculate their concentrations and study the inhibitory effect of the extract on some bacterial species.

Materials and Methods

Extraction of flavonoids [13]

- Add (85 % methanol and 15% water) (volume / volume) to (100 g) of dry powder of Centaurea cineraria.
- Wait for 12 hours at 4 ° C.
- filtrate solution through glass cotton, preserves the first leachate in (4° C)
- Recycle the residue again in the same way but using 50% methanol and 50% water (size / volume) to get the second leachate.
- Blend the candidates and then leave for several hours; filtration is done using filter paper.
- Evaporating the filter using (Rotary Evaporator) under temperature (40°c), to get a raw extract.
- Taken from the crude extract a certain volume, and then tempered and preserved at a temperature (-20° C)
- Add a volume of hexane to the volume of the extract and produce an organic part (hexane extract) and aqueous fraction.
- Add a volume of chloroform to the water part, producing an organic and aqueous part.
- In addition to the water component, an equal volume of ethylene acetate is produced. Aqueous fraction and organic part. The organic extracts (organic parts) of the evaporation process are dried and stored at a temperature of -20 ° C.

The extraction process follows the following schema

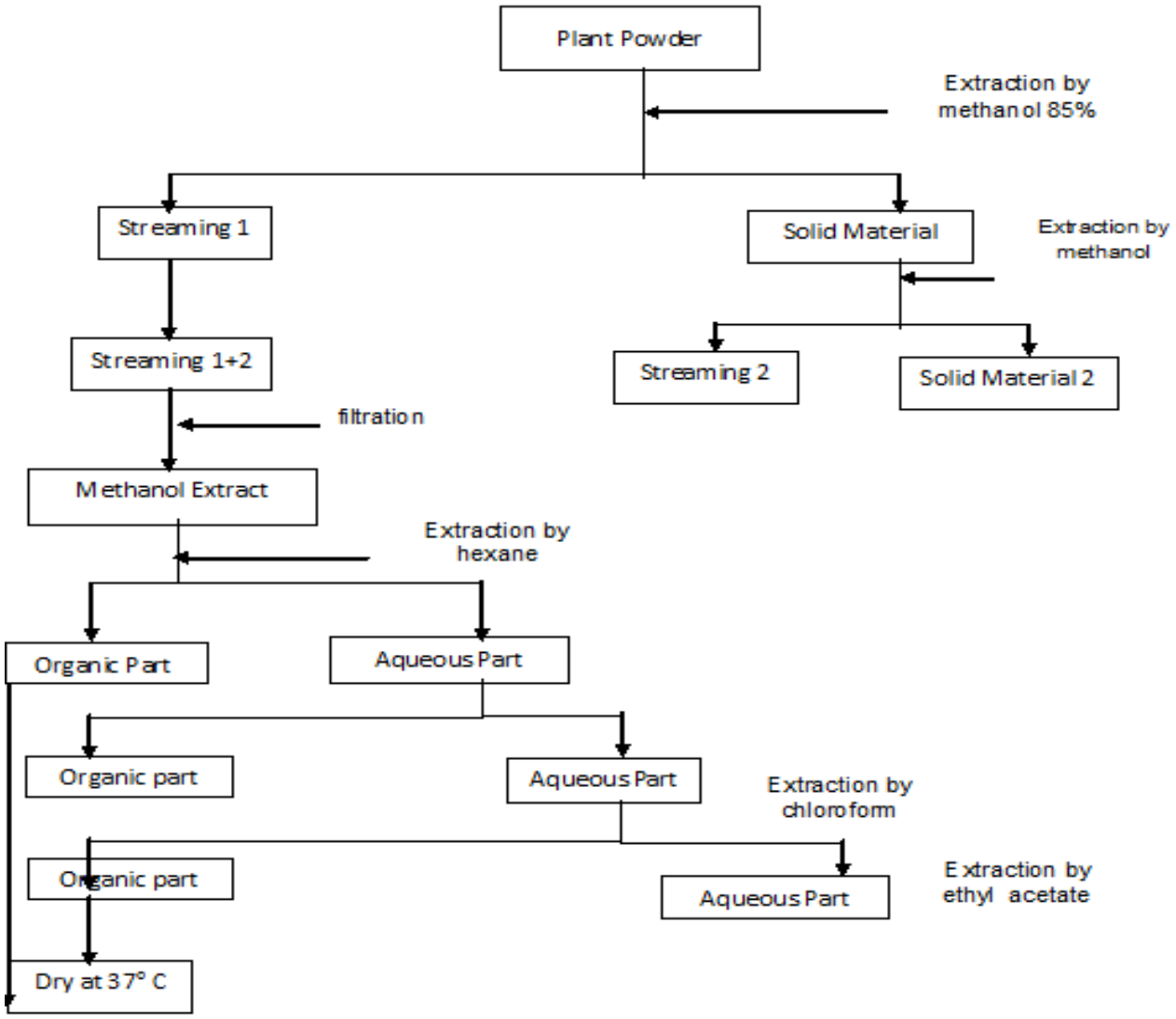


Figure 1: A schema showing the extraction of flavonoids from Centaurea cineraria

High-performance liquid chromatography was used to separate and estimate the flavonoids according to the Phenomenex C-1 column method. The size of the miners is 3 micromel (50 mm x 2.0 mm). Mobile phase: Formic acid 0.1% A: Acetonitrile: methanol Formic acid (6: 2: 1, v / v) respectively Flow rate: 1.5 ml / min. Injection volume: 20µl and dilution factor 10µg / ml Standard solution concentration 25µg / ml Wavelength 280 nm.

The unknown concentrations were calculated according to the following law:

$$\text{Concentration of Sample µg/ml} = \frac{\text{Area of sample}}{\text{Area of Standard}} \times \text{Conc. Of Standard x dilution Factor}$$

Preliminary Qualitative Screening [15, 16]

Ferric Chloride Test

Prepare the reagent by dissolving (7.5 gm) of the ferric chloride in 100 ml with distilled water and then using PH formula with sodium hydroxide, then mixing 1 ml of the extract with a few drops of the 7.5% neutral chloride (PH = 7) The color of the red or black is indicative of the flavonoids.

Lead Acetate Test

The reagent is prepared by dissolving (10 mg) lead acetate in 100 ml with distilled water, taken (1 ml) of the extract and added drops of basal lead acetate solution, with brownish red color with a significant deposit of flavonoids.

Alkaline Test

Adding 1 ml of ammonium hydroxide to 1 ml of the extract. Mix well. The solution is poured on a filter paper. The color of the light is ultraviolet (UV) light, indicating the presence of flavonoids.

Bacterial Isolates: samples were planted on the following media.

- Blood agar
- MacConkey's agar
- Nutrient agar
- Mannitol Salt Agar to diagnose bacteria S.aureus.

All Medias attended and dissolved in distilled water and then sterilized by autoclave at a temperature 121 C0 and pressure 15 pound for 15 minutes, the dishes were incubated aerobically and at a temperature 37 C0 for 24 hour.

Diagnosis of Bacterial Isolates

The morphological and chemical properties of developing colonies were observed.

Microscopy and agricultural characteristics: The bacteria were first identified by observation agricultural characteristics of the developing colonies on the media used form where size, height and colonial color and attended thin swabs and a pigment with a Gram stain to observe cell shapes, arrange and their susceptibility to pigmentation [17].

B-**Bacteriological Test**

IMVIC test were conducted includes (Indol test, Methyl red, Vog'ousproskauer, Cimmon citrate) [17,18] As well as tests Oxidase, Catalase, Coagulase to confirm isolated bacterial species [19].

Results and Discussion

Quantitative screening which shown in materials and methods is made to reagent on flavonoids extract. The results showed that the centaurea cineraria extract contain these Combination.

Identification Quantitative determination of centaurea cineraria flavonides by HPLC

A number of standard flavonoids were used in HPLC analysis as shown in Fig. 2 and Table 1, which shows the retention time and time of standard flavonoids.

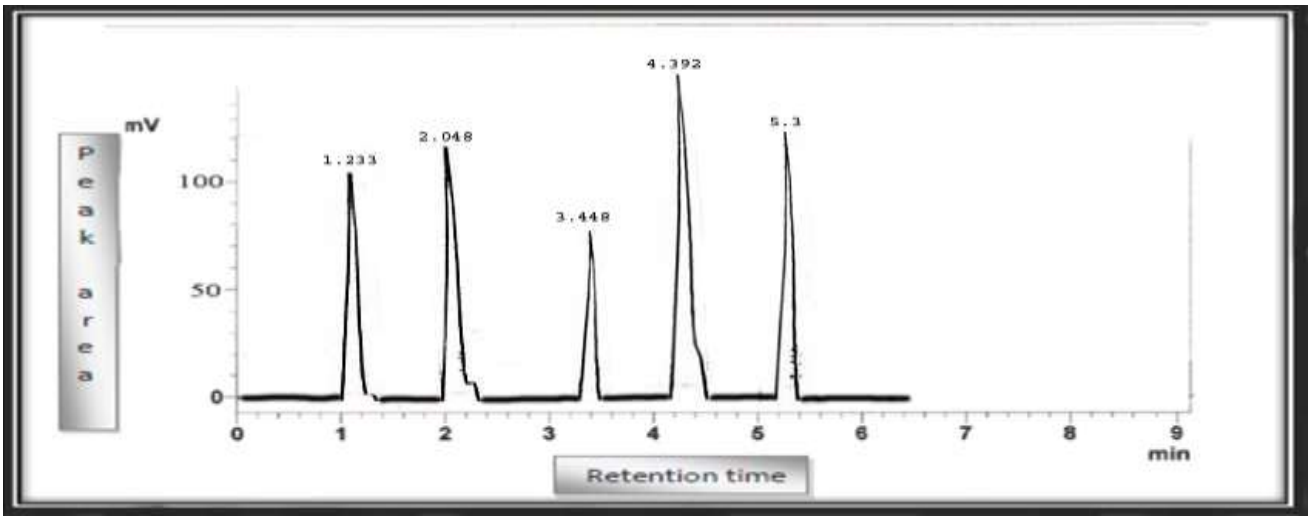


Figure 2: Analysis of HPLC for standard flavonoids

Table 1: Retention time and Peak area for standard flavonoids

Standard Flavonoids	Retention time(mint)	Peak Area
Cirsilineol	1.233	108891
Jaceosidin	2.048	116933
Melitensin	3.448	70284
Apigenin	4.392	143861
Prunasin	5.300	74374

The results of the HPLC analysis indicated that the flavonoids extracted from the *Centaurea cineraria* were identical to all the standard compounds used in the chromatographic analysis, which showed five identical packages for the standard compounds as shown in Fig. 3 and the concentrations of the separated compounds were calculated as shown in the method of operation.

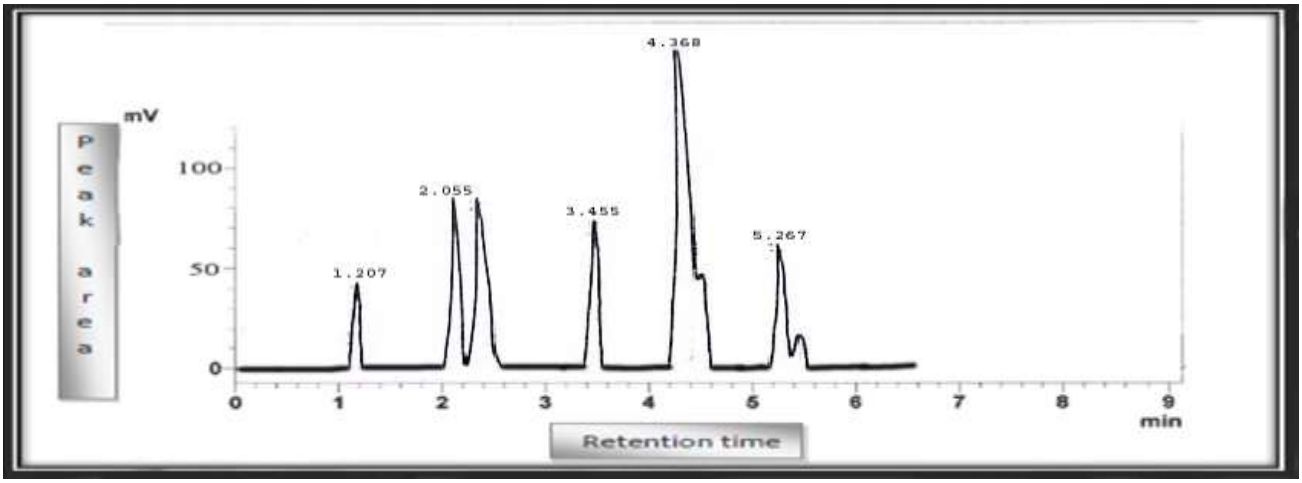


Figure 3: Analysis of HPLC to separated flavonoids from extracts Centaurea cineraria

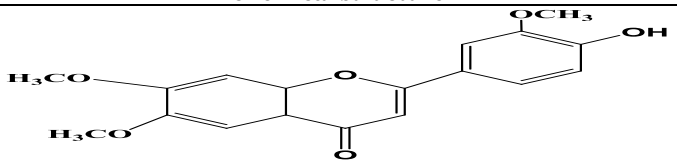
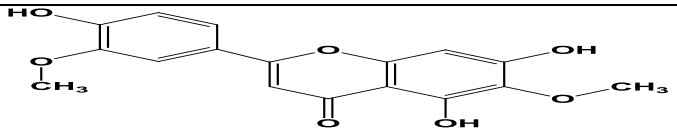
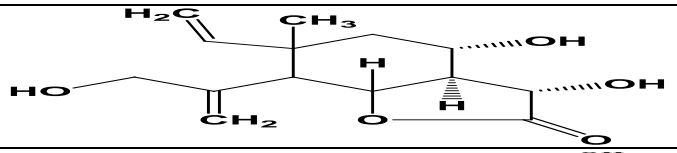
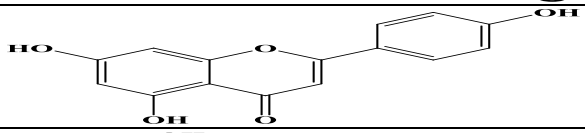
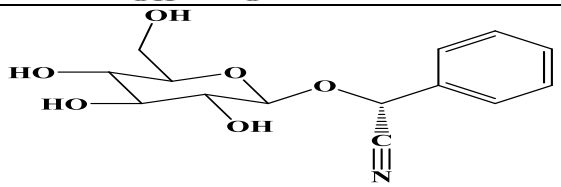
Table 2: Retention time and Peak area and Concentrations of separated flavonoids from extract *Centaurea cineraria*

Separated Flavonoids	Peak Area	Retention time(mint)	Concentration(µg/ml)
Cirsilineol	46876	1.207	10.76213
Jaceosidin	84537	2.055	10.87381
Melitensin	77029	3.455	27.39919
Apigenin	186363	4.368	32.38594
Prunasin	62188	5.267	20.90409

Figure (3) and Table (2) show the possibility of separating and quantifying the chemical compounds of plants quantitatively and qualitatively through the use of separation

techniques such as HPLC and other techniques such as TLC [9].

Tabal 3: Chemical structure of separated flavonoids

Compounds	Molecular formula	Chemical structure
Cirsilineol	$C_{18}H_{16}O_7$	
Jaceosidin	$C_{17}H_{14}O_7$	
Melitensin	$C_{15}H_{22}O_4$	
Apigenin	$C_{15}H_{10}O_5$	
Prunasin	$C_{14}H_{17}O_6$	

The effect of flavonoids extracted from *Centaurea cineraria* was tested on five bacterial species (*Staphylococcus aureus*, *Pseudomonous aeruginosa*, *Eschericea coli, Klebsella.spp* and *Streptococcus pneumoniae*) to determine the effect of the plant on the bacterial isolates under study. The results showed that the plant had an effect against all bacterial strains and all concentrations. Of the extract at 100% concentration in inhibiting all bacterial species with the highest rate of inhibition of *Klebsella. Spp* was 14 ± 0.577 while the concentration was 75% less inhibited with a concentration of 25 ± 8%.

The concentration was 25%. The lowest concentration was 8 ± 0.577. The Gram negative bacteria were found to be the most affected by the *Centaurea cineraria*. The ability of the plant to inhibit bacterial strains was attributed to some phenolic compounds such as thymol, Against bacteria and fungi, which has a direct effect on the wall of the bacterial cell through the dissolution of cell wall fat and the formation of the HID With the water molecules and nitrogen amino acids inside the cell that have antimicrobial efficacy by disrupting the cytoplasmic membrane mechanism of microorganisms [20] there by inhibiting the growth of microorganisms.

Table 5: Effect of *centaurea Cineraria* extract on the isolated bacterial species

bacterial species					%Conc.
Ps. *aeruginos*	*Streptococcus pneumoniae*	*Klebsella.spp*	*Staphylococcousaureus*	*Escherichia coli*	
0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00b	0.00± 0.00c	Con.
0.00±0.00c	0.00±0.00c	8± 0.577c	0.00±0.00b	0.00±0.00c	25
0.00±0.00c	0.00±0.00c	9± 0.577b	0.00±0.00b	0.00±0.00c	50
0.00±0.00c	10 ±0.57b	8±1.155c	0 33± 0.57b	10± 0.577b	75
9±0.57a	11±1.15a	14± 0.577a	1.33±1.527a	11± 0.577a	100

Whereas:
A represents The High Value
B represents The Medium Value
C represents The Less Value

Figure A: illustrated *E. coli*

Figure B: illustrated *Klebsella.*

Figure C: illustrated *S. aureus*

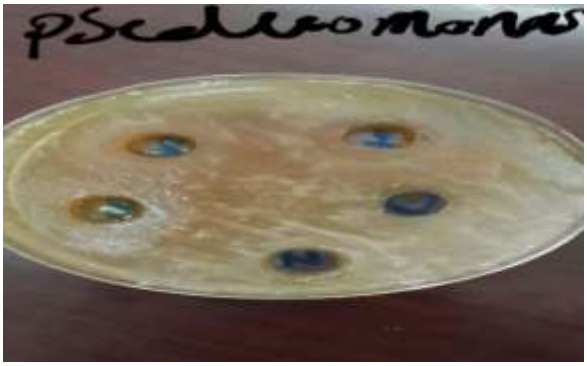


Figure D: illustrated *Psedoumonous .aeruginosa*

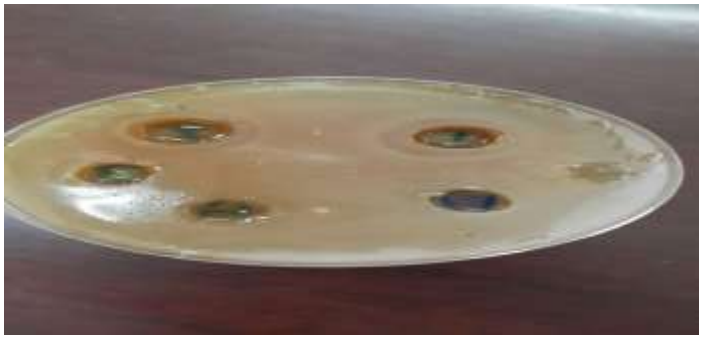


Figure E: illustrated *Streptococcous. Pneumonia*

A: Esherichia coli

B: Ps.aeruginosa

C: St.pneumonia

D: Escherichia coli

E: Klebsiella

It has been shown that the leaves of the wormwood (composite family) have anti-bacterial effect (*E.coli*,. *Bacillus cereus*, *Pseudomonas* a*eruginosa*, *Staphylococcus aureus*).

Conclusion

The method of separation of *Centaurea cineraria* extracted from *Asteraceae* and quantitatively assessed by qualitative and spectral method and HPLC technique of the best methods of separation to determine the concentration of a chemical compound in a plant or biological model. The activity extracts of *Centaurea cineraria* can be studied on types of bacteria.

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