CHEMICAL CONSTITUENTS OF FLORA OF YEMEN- PART (I): POTENT ANTIBACTERIAL ACTIVITY OF EXTRACTS AND FRACTIONATIONS OF FLAVARIA TRINERVEA AND ISOLATION OF SOME FLAVONOIDS COMPOUNDS

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ABSTRACT

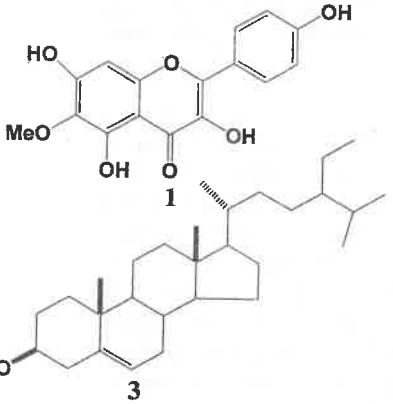
Shoot systems of the yellow tops (*Flaveria trinervia*) were washed with tap water, dried in the shade to a constant weight was obtained, ground into a very fine powder. The assay has shown that the ethanolic extract exhibited activities against *Staphylococcus aureus*, *Escherichia coli*, *kliebsella pneumonia* and failed to give activity against *Pseudomonas aeruginosa*. From this treatment chloroformic fraction, aqueous layer and a solid precipitate were obtained, tested for antimicrobial activity, and fractionations, isolation, and structural determination of two flavonoids from *Flavaria trinervea* was known as Kaempferol (6-methoxy keampferol **1**and keampferol **4**). Previously, the phytochemical study of the extracts of *Flaveria trinervia* showed the presence of flvonoid, sterol, organic acids, glycoside and triterpenoid.

Key words: *Flaveria trinervea,* antibacterial, Asteraceae, flavonoid, keampferol.

1. INTRODUCTION

Davis [1] believed that pathogenic microbes have gradually adapted themselves and developed some kind of resistance against the commonly available antibiotics and this is why patients frequently complain of the negligible effectiveness of such antibiotics. Sierradeski[2] mentioned that the misuse of antibiotics may represent the main reason for that and added also that many bacterial infections now are untreatable using common antibiotics. According to Marchese and Shito[3], the newly developed microbial resistance may be the reason of the appearance of previously uncommon microbial infections. Higher plants, on the other hand, are being used since the prehistory and are being regarded by recent scientists as ideal sources of antimicrobial agents against antibiotic resistant microbial strains[4-11]. The yellow tops which is the plant adopted in this study belongs to one of the most famous plant families, the Asteraceae which represents the largest number of plants of Mexican pharmacopoeia. It may be worthy to mention that antimicrobial activity against a variety of pathogenic bacterial strains was detected in extracts of plants belonging to Asteraceae like *Artemisia obsinthium*[12], *Notania grandiflora*[13], *Epaltes divaricata* and *Spilanthes calva*[14], *Tusilago farfara. L*[15], *Pulicaria wightiana*[16]. The yellow tops (*Flaveria trinervia*, Family: Asteraceae) grow naturally in the mountains of Ibb, Republic of Yemen. It is used to feed rabbits, sheep and even some birds. The aim

of this work is to check if the yellow tops (*Flaveria trinervia*) can be a natural source of antimicrobial agents and study the chemical constituents of this plant such as flavonoids compounds. Flavonoids are compounds that are widely distributed throughout the plant kingdom, [17] and until today more than 4000 flavonoids have been described [18]. The previous phytochemical studies of *Flavaria trinervea*[19] have isolated three constituents 6- methoxy kaempferol **1**, oleanolic acid **2** and β-sitostero-β-D-glucoside **3**.



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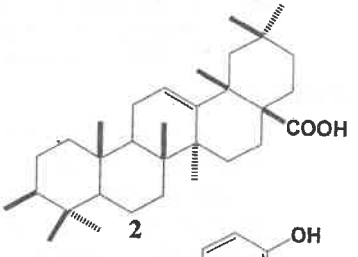

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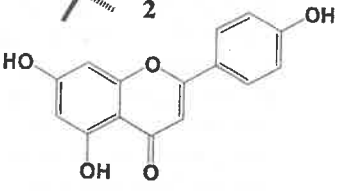

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As part of a program in our work which aims at isolating and identifying the chemical constituents of Yemen medicinal plants, the present study deals with the isolation and identification of constituents of *Flavaria trinervea* with the hope of isolating new compounds, which might have special biological activity. In the present paper we describe the potent antibacterial activity of ethanolic extracts and fractionations, and isolation, structural determination of two flavonoids from solid precipitate of chloroform extract (2) was known as Kaempferol (6-methoxy keampferol **1**and keampferol **4**). Kaempferol and Kaempferol derivatives were found widely in different plants [20-24]. 6-methoxy kaempferol **1** was isolated from the *Flavaria trinervea* previously[19] and kaempferol **4** isolated for first time from the same plant, but previously was isolated from other members *Flaveria genus*[25, 26] and other plants[22, 27]. Kaempferol is one of the most studied flavonoids and is widely distributed among plants[28]. This flavonoid showed a strong antioxidant activity in several models, and a weak cytotoxicity in human cell lines[29].

2. MATERIALS AND METHODS

2.1. Structure Elucidation

The compounds were identified and characterized by nuclear magnetic resonance analysis and MS spectrum and comparison of the spectral data of each of the isolated compounds with the literature. ^{1}H and ^{13}C NMR spectra were recorded in CD_3OD on a Varian Gemini 300 MHz spectrometer. Chemical shift values are given in ppm (δ) with TMS as an internal standard. Mass spectral measurements were carried out by EI method on a Jeol JMC-300 spectrometer at 70 eV. Silica gel

GF254 was used for TLC. Spots were detected on TLC by magnesiumhydrochloric acid as reagent spray.

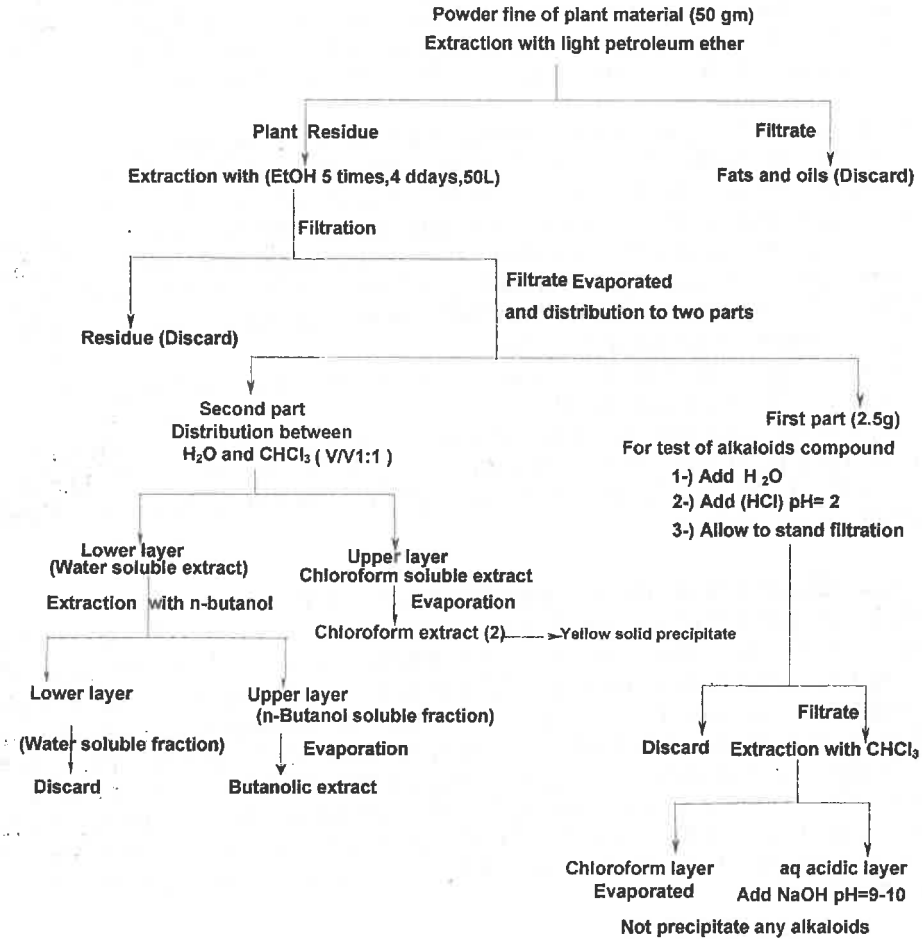
2.2. Plant Material:

The shoot systems of *Flaveria trinervia,* Family: Asteraceae were collected from the mountainous local environment of IBB city, republic of Yemen (February, 2008). The shoots were washed, wiped with towel and air-dried in the shade until a constant weight was obtained and then they were ground into a very fine powder and kept in dark glass bottles at room temperature for further work.

2.3. Defatting, Extraction and Fractionation:

The plants were taken yellow tops (*Flaveria trinervia*) were washed with tap water and then dried to get rid of the existing water and access to the weight of a fixed plant ,it was 50 gm weight. After that we started to draw the chemical components of the chemical plant through the use of various organic solvents as shown in the Scheme (2.3.1).

Scheme (2.3.1). The ethanolic extract and fractions



The fine dry powder was defatted with petroleum ether (b.p.40-60 C°) by placing it in a bag of cloth and in the petroleum ether. Defatting was carried out for 5 times, each time the powder was soaked in one liter of the solvent and the whole process was carried out at room temperature, aliquots were combined and evaporated to dryness and subjected to antimicrobial activity test. The defatted plant material was cold extracted with ethanol (96%) several times until the plant material was exhausted.

2.4. Isolation and Identification:

Ethanolic extracts were combined, evaporated to dryness, weighed and distributed to two parts, the second part was distributed between H_2O and $CHCl_3$ (1:1,v/v) to give the two layers. The lower layer chloroform extract (2) was evaporated and obtained as a yellow solid precipitate that gave a positive magnesiumhydrochloric acid reaction for flavonoids compounds scheme (**2.3.1**). Separation of the yellow solid precipitate from chloroform extract (2) using several chromatographic techniques resulted in the isolation of two flavonids derived compounds, including 6- methoxy kaempferol **1** and kaempferol **4**. The structure determinations of these chemical constituents were done by analysing ^1H and ^{13}C NMR, and MS spectra.

2.4.1.3,5,7,4'-tetrahydroxy-6-methoxyflavone (6-methoxy kaempferol) 1:

Yellowish crystal R_f 0.79; isolated from the chloroform fraction (2) by TLC and eluted by methanol, the attempts to purify this solid through crystallization from hot and cold methanol were failed but recrystallization by another mixture solvents (EtOH: Water) to give a flavonoidal compound corresponding to that of 6- methoxy kaempferol **1** (65 mg) which identified by MS m/z and ^1H -^{13}C NMR. MS m/z: [316[M] $^+$]. ^1H-NMR and ^{13}C NMR in CD_3OD showed that signals corresponding to 3,5,7,4'-tetrahydroxy-6-methoxyflavone **1**

^1H- NMR (300 MHz, CD_3OD) □ ppm: 11.59 (1H, bro s, HO-C5), 7.94 (1H, d, *J*= 8.4, H-C6'), 7.74 (1H, d, *J* =8.9,H-C2'), 6.83 (1H, d, *J* =8.4, H-C5'), 6.44 (1H, s, H-C8), 3.95 (3H, s,OCH_3). ^{13}C- NMR (75 MHz, CD_3OD) □ ppm: 175.8 (C-4), 159.0 (C-7),130.6 (C-5), 153.1 (C-9), 158.7 (C-2), 117.3 (C-3'),156.5 (C-4'), 135.8 (C-3), 128.8 (C-6'), 121.7 (C-1'), 128.5 (C-5'), 128.5 (C-2'), 102.9 (C-10), 130.2 (C-6), 93.6 (C-8), 58.7 (OCH_3).

2.4.2. 3,5,7,4'-tetrahydroxyflavone (Kaempferol) 4:

Yellow powder R_f 0.82; isolated from the chloroform fraction (2) by TLC and eluted by ethanol, gave after purification by recrystallization a single flavonoidal compound corresponding to that of 3,5,7,4'-tetrahydroxyflavone **4** (80 mg) which identified by Ms and ^1H - ^{13}C NMR . [M- 286 and fragments at m/z 285, 258, 229,121 and 93]. ^1H- NMR (300 MHz, CD_3OD) □ppm: 12.19 (1H, bro s, HO-C5), 7.74 (2H, d, *J*= 9.0, H-C6'), 8.11 (2H, d, *J*= 9.0, H-C2'), 6.95 (2H, m, H-C3', H-C5'), 6.33 (1H, s, H-C6), 6.46 (1H, s,H-C8). ^{13}C- NMR (75 MHz, CD_3OD) □ ppm: 175.8 (C-4), 159.0 (C-7), 130.6 (C-5), 153.1 (C-9), 158.7 (C-2), 117.3 (C-3'),156.5 (C-4'), 135.8 (C-3), 98.8 (C-6'), 121.7 (C-1'), 128.5 (C-5'), 128.5 (C-2'), 102.9 (C-10), 130.2 (C-6), 93.7 (C-8).

All physical and spectral data for the two compounds were comparable with respective published data[19, 27].

2.5. Test microorganisms:

The pathogenic microbes used in this study were gifted by the Department of Medical Microbiology, faculty of science, Ibb University. These microbes are: *Staphylococcus aureus* NCTC 7447, *Escherichia coli* BPP 01, *Kliebsella pneumonia* and *Pseudomonas aeruginosa* CS25, all microbes were activated on nutrient broth and assayed on nutrient agar containing Petri-dishes.

2.6. Assay of Antimicrobial Activity:

This was carried out by the disk-diffusion method described by Salie *et al*[30]. Five mm (diameter) filter paper disks were impregnated with 50µl of the extract (equivelant to 10 mg of dry extract). After the organic solvent was completely evaporated, the discs were put on the surface of solid agar seeded with test microbe in 9 cm diameter Petri-dishes. All the plates were incubated at 37°C for 24 h. The experiment was performed 3 times under strict aseptic conditions. Microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values were calculated.

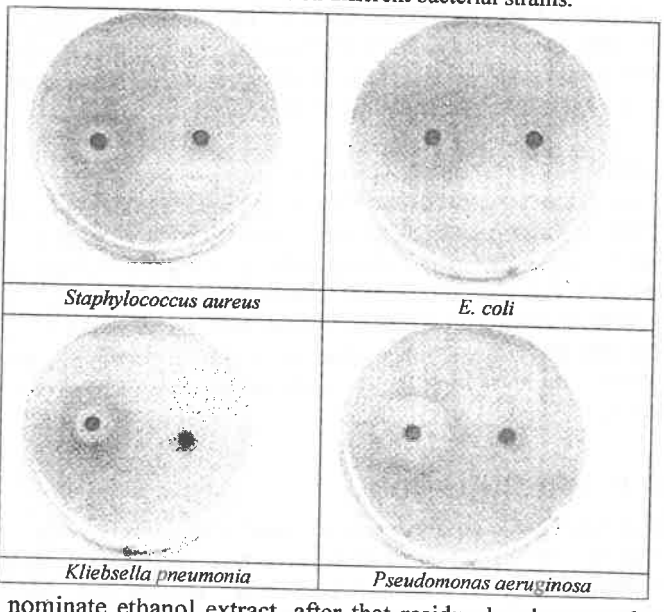
3. RESULTS AND DISCUSSION

Ethanolic extracts were combined, evaporated to dryness, weighed and tested for antibacterial activity for a number of types of bacteria compared to both petroleum ether and ethanol extract raw materials are shown in table **(3.1)** and plate **(3.1)** and that positive outcome had appeared in the ethanol extract. Emphasis has been placed with neglecting petroleum ether extract of a negative result which has been the trend towards the use of ethanol extract fragmentation with other organic solvents.

Type of extract	Tested bacteria			
	Staphylococcus aureus	*E. coli*	*Kliebsella pneumonia*	*Pseudomonas aeruginosa*
Petrolium ether	zero	zero	zero	zero
Crude ethanolic	18	19	16	22

Table (3.1): Antibacterial activity of Petrolium ether and ethanolic extracts of *Flaveria trinervia*. Each value is a mean of 3 determinations: Diameter of inhibition zones is measured in (mm).

Plate (3.1): Antimicrobial activity of petroleum ether (right) and ethanolic extract (left) of *Flaveria trinervea* on different bacterial strains.



We have to nominate ethanol extract, after that residue has been neglected over a nomination paper. soluble filtrate was taken and a chemical was dealt with , and divided into two main parts: The first part was subjected to chemical methods used to detect vehicles alkaloids, in this process we found that it was not alkaloids compounds in the plant, as is evident in the scheme **(2.3.1)**. A mixture of water and chloroform equal volume exported [$CHCl_3$: H_2O (1:1, v/v)] was added to the second part, and then left for a week and then extracted and separated into two layers. the upper layer containing water and class compounds learned with water and an organic layer down containing compounds with chloroform learned, were the work of antimicrobial testing of these extracts and may result has been positive, as shown in the table **(3.2)** and in the photographs in plate **(3.2)**. Results of the present study, as illustrated in table **(3.1)** and plate **(3.1)** have shown that the petroleum ether extract has not exhibited any antibacterial effect on any of the tested bacterial strains. In contrast the crude ethanolic extract showed marked antibacterial activity against the four tested bacterial strains. The effect was much more pronounced with *Pseudomonas aeruginosa.* Different fractions of the ethanolic extract, as illustrated in table **(3.2)** and plate **(3.2)** showed various effects on the tested bacteria. Fraction "A" which represents the chloroformic layer after dissolving the ethanolic extract in acidic water (pH = 2) inhibited the growth of the tested bacteria. The inhibition hallo of *Staphylococcus aureus* reached 25 mm and that of *E. coli* 26 mm. *Kliebsella pneumonia* was the most sensitive one and showed the wider inhibition zone (28 mm). *Pseudomonas aeruginosa* inhibition zone was 24 mm. Results of this study

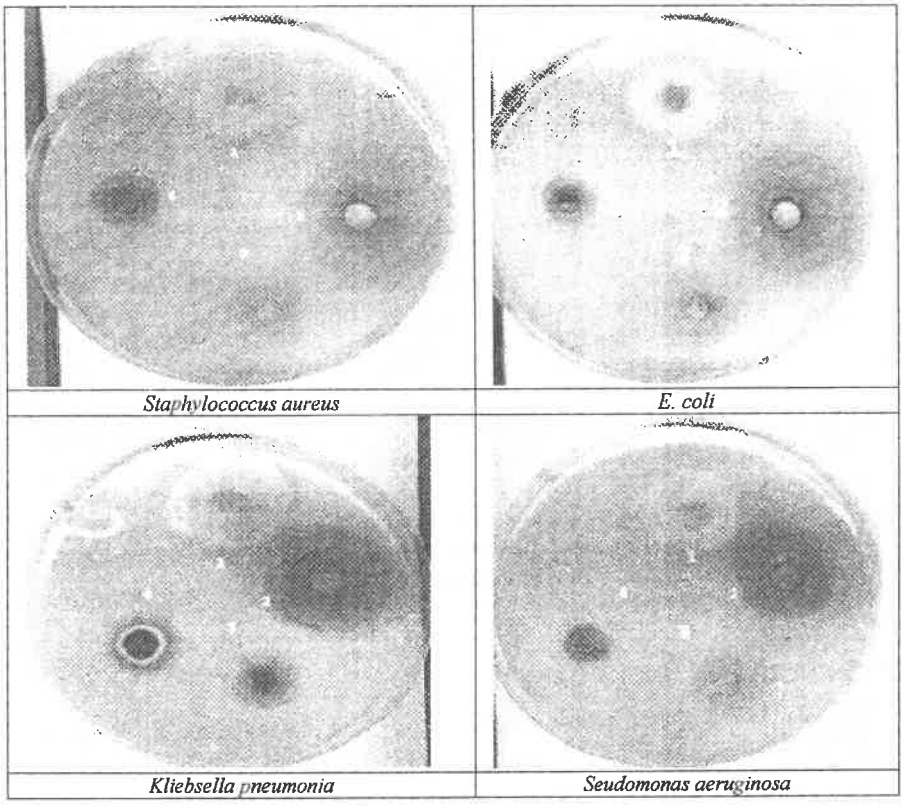
also agree and confirm the opinion that higher plants are ideal sources of antimicrobial agents against antibiotic resistant bacterial strains [4-11]. The results of the present study are similar to the results given in the literature on other plants belonging to the family (Asteraceae) [12-16].

Table (3.2): Antibacterial activity of fractions of ethanolic extract of *Flaveria trinervia.*

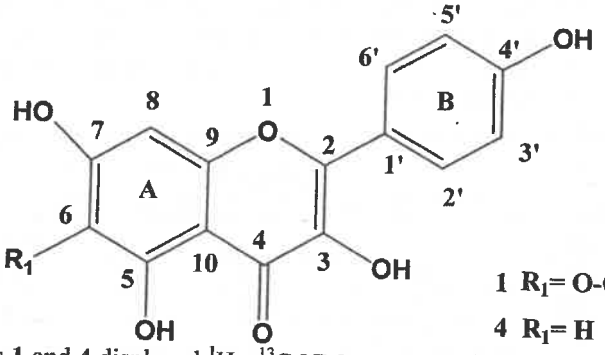
No of fraction	Tested bacteria			
	Staphylococcus aureus	*E. coli*	*Kliebsella pneumonia*	*Pseudomonas aeruginosa*
1	25	26	28	24
2	12	11	20	0
3	0	0	0	0
4	11	0	12	0

Each value is a mean of 3 determinations: Diameter of inhibition zones is measured in (mm).

Plate (3.2): Antimicrobial activity of different fractions obtained from ethanolic extract.



Extraction of the *Flavaria trinervea* followed by an extensive chromatographic techniques resulted in the isolation of 6-methoxy kaempferol **1** and kaempferol **4**. two compounds type flavonoids were isolated from the chloroform extract (2) of *Flavaria trinervea*:



1 R_1= O-CH_3

4 R_1= H

Compounds **1** and **4** displayed ^{1}H-, ^{13}C-NMR and MS spectra. The ^{1}H-NMR spectra of the 6-methoxy kaempferol **1** showed a typical kaempferol **4** type with the presence of singlet signals in ^{1}H-NMR at 3.95 ppm due to 3 protons (CH_3O-) and an additional signal in ^{13}C-NMR at 59.0 ppm due to (CH_3O-) group. The fundamental difference between the two flavonoids is at C-6 of the A-ring. The spectra data suggested a kaempferol substituted with one methoxyl group. Therefore, the structure of flavones **1** was established to be 3,5,7,4'-tetrahydroxy-6-methoxyflavone [6-methoxy kaempferol **1**] . Further confirmation for compound **4** was performed by ^{1}H- and ^{13}C-NMR in CD_3OD and the results were in agreement with the reported data of kaempferol [27]. The ^{1}H-NMR spectrum of kaempferol **4** displayed signals for two protons at □ 6.33 (1H, s, H-6) and 6.46 (1H, s,H-8). The chemical shifts were assigned for two protons in the A- ring and indicated the absence of the metoxyl group at C-6 position.

4. CONCLUSION

The shoot systems of the yellow tops (*Flaveria trinervia*) which grow well in Yemen may, in future, play a role in the treatment of some of the infectious diseases caused by common antibiotic resistant bacteria. Screening the air dried plant powder for antibacterial activity after defatting showed marked inhibitory effects on *Staphylococcus aureus, E. coli, Kliebsella pneumonia* and *Pseudomonas aeruginosa*. Screening different fractions of the ethanolic extract has shown that the chloroformic fraction is the most potent one against the tested bacteria. The results obtained, thus, may suggest a possible future role of using *Flaveria trinervea* as a source of antibacterially active agents.

REFERENCES

1. **Davis, J. (1994).** Inactivation of antibiotics and the dissemination of resistance genes. *Science,* **264**: 375-382.

2. **Sierradeski, K., Roberts, R.B. and Haber, S.W (1999).** The development of vancomycin resistance in a patient with methicillin-resistant *Staphylococcus aureus* infection.N. *Engl. J. Med,* **340**: 517–523.

3. **Marchese, A. and Shito, G.C. (2001).** Resistance patterns of lower respiratory tract pathogens in Europe. *Int. J. Antimicrobial Agents*, **16**: 25–29.

4. **Murier-Grimes, B., Mcbeth, D.L., Hallihan, B. and Delph, .S (1996).** Antimicrobial activity in medicinal plants of the families Scrophulariaceae and Acanthaceae. *Int. J. Pharmacog*, **34**: 243-248.

5. **Rabe, T. and Van, Staden, J. (1997).** Antibacterial activity of South African plants used for medicinal purposes. *J. Ethnopharmacol*, **56**: 81-87.

6. **Cowan, M.M.(1999).** Plant products as antimicrobial agents. *Clin Microbio Rev* **12**: 564-582.

7. **Bradford, P.A. (2001).** Extended-spectrum â-lactamases in the 21st Century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* **14**: 2871-2889.

8. **Shariff, Z. U. (2001).** Modern Herbal Therapy for Common Ailments. *Nature Pharmacy Series* (Volume 1), Spectrum Books Limited, Ibadan Nigeria in Association with Safari Books. (Export) Limited, United Kingdom, pp. 9 – 84.

9. **Afolayan, A.J. (2003).** Extracts from the shoots of *Arctotis artotoides* inhibit the growth of bacteria and fungi. *Pharm. Biol.* **41**: 22-25.

10. **Karamann, I., Sahin F., Güllüce M., Ögütçü H., Sengul M. and Adigüzel A (2003).** Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *J. Ethnopharmacol,* **2837**: 1–5

11. **Babayi, H., Kolo, I., Okogun, J. and Ijah U. (2004).** The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. *Biokemistri*, **2**: 106-111.

12. **Marta, G.O; Dinorah, T.I. and Leticio, M. (2001).** Validacion del uso tradecional de plantas medicinales cultivadus en Cuba. *Rev. Cubana Plant Med*, **2**: 48-51.

13. **Vasanth, S.; Govindarajan, S. and Raj, M.K. (2001).** Antimicrobial activity of *Notania grandi* flora (Asteraceae). *Indian Journal of Pharmaceutical Science*, **63**:235-244.

14. **Nishanta, R. ., Cory, S.H. and Towers, G.H. (2002).** Antimicrobial activity in plants collected from serpentine outcrops in Sri Lanka. *Pharmaceutical Biology*, **40**: 235-244.

15. **Anovsk, D. ., Kubikova, K. and Kokoska, L. (2003).** Screening for antimicrobial activity of some medicinal plant species in traditional Chinese medicine. *Czeckh J. Food Sci*, **3**: 107-110.

16. **Nair, R., Kalaryia, T. and Sumitra, C. (2005).** Antibacterial activity of some selected Indian medicinal flora. *Turk. J. Biol,* **29**: 41-47.

17. **Bohm, B. A. (1998).** Introduction to Flavonoids. Harwood Academic Publishers, Amsterdam.

18. **Bravo, L. (1998).** Polyphenols: Chemistry, dietary sources, metabolism and nutritional significances. *Nutr.Rev*, **56 (11)**: 317-33.

19. **Umadevi, S., Mohanta, G. P., Balakrishna, K. and Manavalan, R. (2005).** Phytochemical Investigation of the Leaves of *Flaveria trinervia*. *Natural product Sciences*, **11(1)**:13-15

20. **Fathy, M. Soliman., Afaf, H. Shehata., Amal, E. Khaleel and Shahera, M. Ezzat. (2002).** An Acylated Kaempferol Glycoside from Flowers of Foeniculum vulgare and F. Dulce. *Molecules*, **7**: 245.251

21. **Crowden, R.K., Harborne, J.B., Heywood, V.H. (1969).** Chemosystematics of the Umbelliferae -- A General Survey. *Phytochemistry*, **8**: 1963-84.

22. **Young-Hee Lim., In-Hwan Kim and Jung-Ju Seo.(October 2007).** In vitro Activity of Kaempferol Isolated from the Impatiens balsamina alone and in Combination with Erythromycin or Clindamycin against Propionibacterium acnes. *The Journal of Microbiology*, **45 (5)**: 473-477.

23. **Nakaoki, T., Morita,Y., Nagata, Y., Oguri, H. (1961).** Flavonoids of the Leaves of Nelumbo nucifera, Cosmos bipinnatus and Foeniculum vulgare. *Yakugaku Zasshi*, **81**:1158-59. [Chem. Abstr.,1962, 56,1527d].

24. **Ali El Antri., Nadya Lachkar., Hanane El Hajjaji., Farah Gaamoussi., Badiaa Lyoussi., Brahim El Bali., Nicole Morel., Hassan Allouchi and Mohammed Lachkar. (2010).** Structure elucidation and vasodilator activity of methoxy flavonols from Calycotome villosa subsp. Intermedia. *Arabian Journal of Chemistry*, 3:173-178.

25. **Abdelali, Hannoufa., Luc Varin and Ragai K. Ibrahim. (1991).** Spatial Distribution of Flavonoid Conjugates in Relation to Glucosyltransferase and Sulfotransferase Activities in Flaveria bidentis. *Plant Physiol*, **97**: 259-263.

26. **Luc, V and Ragai K . Ibrahim.(1992).** Novel Flavonol 3-Sulfotransferase Purification, Kinetic Properties, and Partial Amino Acid Sequence. *The Journal of Biological Chemistry*, **267(3) January 25**:1858-1863.

27. **Mabry, T.J., Markham, K .R., Thomas, M . B. 1970.** The Systematic Identification of Flavonoids, *Springer-Verlag*, New York.

28. **Middleton, Jr. E., Kandaswami, C. and Theoharides, T. C. (2000).** The effects of plant flavonoids on mammaliancells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev*, **52**: 673D751.

29. **Cos P., Calomme M., Sindambiwe J. B., Bruyne T., Ci- Rmanga K., Pieters L., Vlietinck A. J., and Berghe D. V. (2001).** Cytotoxicity and lipid peroxidation-inhibiting activityof flavonoids. *Planta Med*, 67: 515-519.

30. **Salie, .F., Eagles, P.F.K and Leng H.M.J. (1996).** Preliminary antimicrobial screening of four South African Asteraceae species. *J. Ethonopharmacol*, **52**: 27-33.

Chemical Constituents of Flora of Yemen -

Part (I): Potent antibacterial activity of extracts and fractionation of Flavaria trinervea and isolation of some flavonoid compounds

المكونات الكيميائية لنباتات اليمن

: التأثير الضد ميكروبي للمستخلصات والقطفات الكيميائية (1)الجزء لنبتة القمم الصفراء وعزل بعض مركبات الفلافونويد

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تعتبر الجمهورية اليمنية من أغنى مناطق الشرق الأوسط بتنوع غطائها النباتي فالغطاء النباتي اليمني ينفرد بتعدد أنواع النباتات وخاصة النباتات الطبية حيث تنتشر هذه النباتات في مساحات شاسعة من اليمن وتتنوع هذه النباتات لتنوع البيئة المناخية المختلفة في اليمن فهذه النباتات تنتشر في الجبال والهضاب والسهول والوديان والسواحل والجزر. إلا إن أكثر هذه النباتات تعتبر نباتات موسمية ولذلك سوف نركز هنا على احد النباتات الطبية الموجودة في اليمن وخاصة في محافظة إب وهي نبتة القمم الصفراء (Flaveria trinervia) وتم دراسة تأثير المستخلصات الكيميائية المختلفة لها على أنواع مختلفة من البكتريا. حيث قمنا بجمع النبتة في موسم نموها من البيئة المحلية لمدينة إب وتم غسلها مع مياه الصنابير ثم تجفيفها في الظل للتخلص من المياه الموجودة فيها والحصول على وزن ثابت للنبتة وتم طحنها للحصول على مسحوق ناعم جدا من النبتة. وكان وزنه خمسين غراما. وبعد ذلك بدأنا بالعملية الكيمائية وهي عملية استخلاص المكونات الكيميائية من النبتة عن طريق استخدام المذيبات العضوية المختلفة كما هو موضح في المخطط رقم (2.3.1) عن طريق وضعها في كيس من القماش و نقعها في البترولويوم أيثر ($40 - 60\ C^0$) لاستخلاص الأحماض الدهنية حوالي خمس مرات (لتر / كل ثلاثة أيام) لمدة أسبوعين وبعد ذلك تم تجفيف النبتة وتكرر عملية نقع النبتة في الإيثانول (96٪) خمس مرات لمدة أربعين يوم بنفس الطريقة السابقة وتجميع المستخلص في كل مرة وعمل الفحص الضد ميكروبي لعدد من أنواع البكتريا مقابل كلا من مستخلصي البترولويوم أيثر ومستخلص الايثانول الخام كما هو موضح في جدول رقم (3.1) ولأن النتيجة الايجابية قد ظهرت في مستخلص الايثانول فقد تم التركيز عليه مع إهمال مستخلص البترولويوم ايثر لوجود نتيجة سلبية وبالتالي جزيء مستخلص الايثانول باستخدام مذيبات عضوية أخرى. قمنا بترشيح المستخلص وتم إهمال البقايا على ورقة الترشيح واخذ الراشح والتعامل معه كيميائيا حيث تم تجفيفه من الايثانول وتقسيمه إلى جزئيين رئيسيين الجزء الأول تم استخدم الطرق الكيميائية للكشف عن مركبات الألكلويدات

(alkaloids compounds) كما هو واضح في مخطط رقم (2.3.1) حيث لم يتكون أي راسب وبالتالي لا توجد الكلويدات في النبتة ثم اخذ الجزء الثاني و أضيف اليه مزيج من الماء و الكلوروفورم بنسبه حجميه متساوية (1 : 1) : ($CHCl_3$ H_2O) وبعد ذلك ترك لمدة أسبوع لنقوم بعد ذلك باستخلاصه وفصله إلى طبقتين الطبقة المائية وتحتوي على المركبات المستخلصة مع الماء والطبقة العضوية وتحتوي على المركبات المستخلصة مع الكلوروفورم حيث تم عمل الاختبارات الضد ميكروبية لهذه المستخلصات و قد كانت النتيجة ايجابية كما هو موضح في الجدول رقم (3.2) وكما هو موضح في الصور الفوتوغرافية في اللوحتين رقم (3.1 و3.2) وكما نلاحظ من الجداول المذكورة سابقا أن التأثير الضد ميكروبي قد ظهر في المستخلص الخام

للايثانول ولا يظهر أي تأثير لمستخلص البترواليوم ايثر جدول رقم (3.1) لوحه رقم (3.1) بينما جدول رقم (3.2) لوحه رقم (3.2) فإنهما يظهران لنا التأثير الناتج عن تجزيء مستخلص الأيثانول إلى أجزاء كل جزء يحتوي على مركبات كيميائية مختلفة عن الأخر لاختلاف المذيب المستخدم.

وكجزء هام من برنامج عملنا في المعمل هو التعرف على المكونات الكيميائية للنباتات الطبية اليمنية و دراسة التأثير البيولوجي للمستخلصات والقطفات الكيميائية ، الدراسة الحالية تناولت بجانب دراسة التأثير الضد ميكروبي للمستخلصات والقطفات الكيميائية للنبته جانب اخر هو محاولة التعرف على المكونات الكيميائية للنبته وذلك عن طريق فصل وعزل هذه المكونات على أمل عزل مركبات الجديدة ، التي قد يكون لها نشاط بيولوجي خاص بها . مستندين الى دراسات سابقه تم فيها عزل أنواع مختلفة من المركبات و تم التعرف عليها مثل :

فلافونويدات (6-methoxy keampferol) ، والأحماض العضوية (oleanolic acid)، ستيرول ، و التربينات و جليكوسيدات (β-sitostero-β-D-glucoside) وفي بحثنا هذا تم عزل مركبان مختلفان من الفلافونويدات من نبتة القمم اصفراء *Flavaria trinervea* وهذه المركبات معروفه بإسم كايمبفيرول Kaempferol وهي :

6-methoxy keampferol 1 وكذلك keampferol 4 . وسابقا تم العثور على هذا المركب كايمبفيرول Kaempferol ومشتقاته على نطاق واسع في النباتات المختلفة. كما أنه تم عزلها سابقا من هذه النبته ومن النباتات المنتميه لنفس الجنس.