HPLC ANALYSIS OF *RUBIA TINCTORUM* AND ITS EFFECT OF METHANOL AND AQUEOUS EXTRACT ON BACTERIA ISOLATED FROM BURNS INFECTION

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Abstract

This study provides a scientific information about the aqueous and methanol extracts of *Rubia tinctorum* based on its antimicrobial potential against gram positive and gram negative bacteria isolated from burns infection using the broth dilution and well diffusion method. Results of this study indicate the presence of many phytochemicals which have antimicrobial activity against broad spectrum of bacteria. The methanol extract of *R.tinctorum* showed highest activity than aqueous ones. The minimum inhibitory concentration (MIC) of the aqueous extract on the tested organisms was 25-100mg/ml while in the methanol extract ranged between 25-50mg/ml on the tested organisms and the minimum bacterial concentration (MBC) of the aqueous extract was 25-200 mg/ml while the methanol extract ranged between 25-100 mg/ml. The highest activity of methanol extract demonstrated at 100 C^o, 121 C^o against *S.aureus, K.spp, A.hydrophila*, and *S.marcescens*, while there was low activity against *S.dysenteria* and *E.coli*. The activity of plant extract increased at acidic pH5-3 whearus, there are slightly increased of plant extract at alkaline pH 8. *R.tinctorum* contained. essential element (Pb, Na, K, Ca, Fe, Zn, P, Mn, Co and Cu) at different concertation. The high preformance liquid chromatograph (HPLC) analysis of *R. tinctorum* showed some chemical compounds that have antimicrobial activity against test isolates. The result of this study demonstrate that HPLC analysis of *R.tinctorum* constituent revealed that htis plant have antimicrobial activity against test organism and this may be suggest the use at this extract in treatment of infections disease.

Keywords: Plant extract, *Rubia tinctorum* ,HPLC, Antimicrobial activity.

Introduction

It is well known that infectious diseases are responsible for a high proportion of health problems, especially in developing countries. The situation has created immense clinical problems for infectious disease treatment. More scientists are in search for new antimicrobial substances derived from plants. Historically, plants provide us with a good source of anti-infective agents [1]. However, an emerging problem associated with misuse of antibiotic therapy is the worldwide emergence of higher level tolerance of target organisms against available broad spectrum antibiotics. As a result, and in the light of the rapid spread of multidrug resistance, the development of new antimicrobial or antipathogenic agents that act upon new microbial targets has become a very pressing priority [2]. In the traditional systems of medicine, plants are used in the form of crude extracts, infusions and powders to treat common infections without scientific evidence of efficacy [3]. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils [4]. It is necessary to identify the phytochemical components of local medicinal plants usually employed by herbalists in the treatment of diseases, especially now that there are proposals on the integration of traditional medicine in health care programme in world. In addition, investigations into antimicrobial activities of local medicinal plants will expose the plants as potential sources of therapeutic agents [5]. Madder (*Rubia tinctorum*) is a plant from Rubiaceae family that historically originated from Ghafghaz and Near East. Cultivation of madder is prevailed in the world province for dye industry and extracting the drug components. Nowadays, industrial color is used instead of madder extracted color [6]. The main parts of madder used for mentioned goals are roots and rhizomes, which contain Alizarin, rubestric acid and

pourpourines [7]. The red color of madder is due to the Alizarin component Drugs synthesized from products of madder are used as diuretics, laxatives and also to parry the kidney stones. In India, it has been used to redden lips and cheeks. It has a 2000 year history as a medicinal herb in China, India and ancient Greece for breaking kidney stones (it's a diuretic), to promote the flow of menses, cure jaundice and because of its high tannin content, for various intestinal problems. In Europe, it was used to dye urine and bones for medicinal purposes. It is antibiotic and anti-inflammatory [8].

Bao *et al*. [9] showed certain antibacterial activities compounds were isolated from the roots of Rubia that is alizarin (I),1-hydroxy-2 - methyl- 9, 10-anthraquinone (II), 1, 3, 6-trihydroxy- 2- methyl-9, 10-anthra-quinone - 3-O-(6'-O-acetyl) -alpha- L-rhamnosyl (1-2)-beta-D-glucoside (III),1,3,6- trihydroxy-2-methl-9,10-anthraqueinone-3-O-a lpha- L-rhamnosyl (1-2) - beta - D - glucoside (IV), 1, 3, 6- trihydrozy - 2 - methyl-9, 10-anthraquinone -3-O-(6'-O-acetyl)- beta- D-glucoside (V), 2- carbomethyoxy +++-3-prenyl-1, 4-naphtho-hydroquinonedi -beta-D-glucoside (VI) and rubimallin (VII). A sensitive and reproducible RP-HPLC method was developed for the characterization of madder root and its cell cultures extracts and for the determination of anthraquinone derivatives as glycosides (ruberythric acid, lucidin primveroside) and aglycones (alizarin, lucidin, purpurin) in them. The chief components were identified and quantitatively determined. The natural plant and its cell suspension cultures were compared to each other. The preparative fractionation of the extracts was achieved by gel chromatography, HPLC and selective extractions with solvent series, solid-phase extraction techniques [10].

The aim of this study was to investigate the inhibitory effect of madder secondary metabolites on bacteria isolated from burn infections by using HPLC technique.

Materials and Methods

Collection of plant samples:

The medicinal plant in for the experiment was identified according to various literatures, and including other pertinent taxonomic literature. Collected plant was in January 2009 and the part of the plant used is the root. The plant was washed thoroughly and chopped into small pieces shade dried and grinded into powdered form.

Test microorganisms:

Bacterial species *Shigella dysenteriae; Aeromonas hydrophila; Escherichia coli; Klebsiella spp;Serratia marcescens and Staphylococcus aureus* were all obtained from Al-Kindi Hospital.

Culture medium and inoculum:

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at +4 °C. Cell suspansions were prepared by inoculation of each bacteria into 10 ml of Nutrient broth. Incubation was performed at 37 °C for 24 h. On the next day Mueller-Hinton Agar (MHA) was prepared and cooled to 45 °C. Bacterial suspension was added into MHA to give a final concentration of 10^7 bacteria/ml and plated out.

Phytochemical screening:

The plant extracts were screened for phytochemical constituents using standard procedures of analysis. These were done at College of Science / Department of Biology / Al-Mustansiryah University. The tests were carried out by using a stock concentration of 500mg/ml prepared by dissolving 1gm of the methanol extract (MTE) and aquatic extract into 2 ml of distilled water, [11].

Antibacterial activity:

The plate-hole diffusion assay as described by [12] was used to determine the growth inhibition of bacteria by the plant extract. The isolated bacteria from burn infection were obtained. Nutrient agar was prepared and 25ml each was poured into sterile petri dish. This was allowed to solidify and dry. Using a sterile cock-borer of 9 mm diameter three equi-distant holes per plate were made in the set agar and were inoculated with 0.5 ml over night suspension of the bacteria. Thereafter, the wells (holes) were filled with the extract solution volume 100 µl at varying concentr-ations of 500mg/ml, 400 mg/ml and 300 mg/ml respectively. This was done in triplicate and the plates were incubated at 37 ºC for 18 hours. The antibacterial activities were observed and

measured using a transparent meter rule and recorded if the zone of inhibition was 10 mm [13].

Minimum Inhibitory Concentration (MIC):

Reuben *et al.*, method [14] was employed. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 500 mg/ml (stock concentration) in sterile distilled water and serially diluted (two-fold) into a working concentration ranging from 0.780 mg/ml to 200 mg/ml using nutrient broth and later inoculated with 0.2 ml suspension of the test organisms. After 18 hours of incubation at 37 ºC, the test tubes were observed for turbidity. The least concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value.

Minimum Bacterial Concentration (MBC):

This was determined from the broth dilution resulting from the MIC tubes by sub culturing to antimicrobial free agar as described by Usman *et al.*, [15]. In this technique, the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37 ºC for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC.

The effect of heat and pH on the antimicrobial activity of medicinal plant extract:

The samples of plant extract (one vial of 100 ml) were provided to determine the effect of heat on it, test samples were heated 45 ºC, 70 ºC, 100 ºC and 121 ºC for 15 min. [16]. To determine the effect of pH, extracts were treated at pH ranges of 3 to 8 using 1 N HCl and 1 N NaOH solutions respectively in series of test tubes for 1 h and then tested for antibacterial activity [17].

Determination of Essential elements:

This experiment was carried out in the central laboratory, College of Science/ Department of Biology / University of Baghdad. Three grams of dried plant were taken and mixed with 8ml of concentrated H_2SO_4 (98%) and 2ml of $HClO_3$ (60%) in aconical flask for 24 hours which covered by watch class. Then this mixture was left for 6 hours on the sand bath at 80 C°, until the digestion material converted into white powder. Then 8ml of deionized water were added to this powder and the trace elements were determined by flame atomic absorption spectrophotometer, [18].

HPLC analysis:

The analysis of the sample was performed according to the method of Shalini and Rachana. [19]. This work was carried out in Ibn-sina company/Ministry of Industrial and Minerals. The HPLC system (Shimadzu LC-10 A, Japan) Reverse-phase chromato-graphic analysis was carried out in isociratic conditions using a C-18 reverse phase column (250 x 4.6 mm) particle size 5 µm, Luna 5 µ C-18 at 30 ^{o}C. Running conditions included: injection volume, 5 µl; mobile phase, acetic acid 25%,water 75%, flow rate, 0.4 ml/min; and detection at 350 nm. Samples were filtered through an ultra membrane filter (pore size 0.45 µm) prior to injection in the sample loop. Kaempferol and Chalcon (2^{-}, 3^{-}, 4^{-}-trihydroxy-4-methoxychalcone), Antho-cyanidin (3^{-},4^{-}, dihydroxy-3,5,7-trihydroxy flavylium chloride) were used as Flavonoids standards. The sample of plant and standards determined according to retention times obtained from authentic standards run at identical condition.

Results and Discussion

The results of phytochemical screening for *R. tinctorum* are shown in Table (1) which reveals the presence of Alkaloids, Phenol,Cardiac glycosides, Flavonoids, Terpenes, Tanins, Ratenges, Coumarines, and Essensial oil which were secondary metabolites have been used in traditional Chinese medicine for its antibacterial, antioxidant and anti-inflammatary activities [20,21]. In other research phytocemical constituents in the roots of Rubia was identified with the aid of high-preformance liquid chromatography and liquid chromatography mass spectrometry and by comparsion with authentic standards [22]. Controlled studies indicate the great potential of phytochemicals to be the richest reservoir of new and novel therapeutics [23]. Although the antimicrobial activities of plant extracts are

beyond doubt, in many instances their exact mechanism of antimicrobial functionality is not well understood [24, 25].

Table (1)

Phytochemical screening of Methanol, Hot and Cold water extract of R. tinctorum.

Number	*Constituents*	*Methanol extract*	*Hot water extract*	*Cold water extract*
1	**Alkaloids**			
	i.Dragendorff's test ii.Meyer's test	+	+	+
2	**Pheno l**			
		+	+	+
3	**Cardiac glycosides**			
	Killer-killanis test	+	+	+
4	**Flavonoids**			
	i.Shinoda's test ii.$FeCl_3$ test	+	+	+
5	**Saponins**			
	Frothing test	–	–	–
6	**Terpenes**			
	Salkowski test	+	+	+
7	**Steroids**			
	Libarman-Burchard's test	–	–	–
8	**Tanins**			
	i.$FeCl_3$ test ii.Lead acetate test	+	+	+
9	**Ratenges**			
		+		
10	**Coumarines**			
		+		
11	**Essensial oil**			
		+		

The results of antibacterial activity of plant extract against test organism are list in Table (2). In this study a positive correlation was found between concentration of test plant extract and the inhibition zone of pathogenic isolates. As is shown, the methanol extract of *R. tinctorum* was more effective than two aqueous extract (hot and cold) for the same plant,and the hot aqueous extract of plant was more effective than cold extract. *S.aureus* showed zone of inhibition for aqueous and methanol extract. while all gram negative bacteria; *K.spp, A..hydrophila,S.marcesence, S.dysenteriae* and *E.coli* exhibit variation in zone of inhibition respectively. The results of this study in agreed with other researches which showed that plant extracts with well documented antimicrobial activities could possess antipathogenic as well as antivirulent activities, which may not be linked to the growth and inhibition of the microorganism [26]. Al-Fatimi, *et al*. [27] studied *In vitro* antimicrobial activity of crude dichloromethane, methanol and aqueous extracts of medicinal plants in Yemeni ethnomedicine and showed good activity against gram positive and negative bacteria. Aysegul, *et al*. [28] reported that the hydro alcoholic extract of *R. tinctorum* has an antioxidant activity and an antimicrobial effect on bacilli, escirichiae and staphylococci. The magnitude of activity varied in terms of the type and number of bacteria and fungi tested and the part of the plant extracted. In addition

it is well established that the polarity of these extracts where in our case ethanol is highly polar which probably means getting different profiles in the activity if other extracts of different polarity were used [29].

Table (2)
Antibacterial Activity of R. tinctorum Extracts against Test Organisms.

Extract/concentration Mg/ml	*Zone of inhibition (mm)*						
	Cone.	*K.spp*	*S.marcescens*	*A.hydrophila.*	*S.dysenteriae*	*E.coli*	*S.aureus*
Methanol Extract of *R.tinctorum*	500	25	17	22	10	13	40
	400	22	15	20	9	11	35
	300	18	14	17	7	10	32
Hot aqueous Extract of *R.tinctorum*	500	19	15	20	10	11	38
	400	16	14	19	8	10	35
	300	15	12	17	7	8	30
Cold aqueous Extrac of *R.tinctorum*	500	16	14	14	8	9	35
	400	15	13	12	7	7	28
	300	13	13	10	7	6	20
Control (water)	–	–	–	–	–	–	–
Control (Methanol)	–	–	–	–	–	–	–

The minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) results are shown in Table (3 and 4), respectively. The highest MIC and MBC values is an indication that either the plant extracts are less effective on some bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC and MBC values for other bacteria is an indication to the efficacy of the plant extract. The result of this study was in agreement with other researches which showed antimicrobial activity of ethanol, methanol, ethyl acetate and water extract of *R. tinctorum* by disc diffusion method, from this study it was found that *R. tinctorum* revealed antimicrobial activity against some gram positive and gram negative bacteria, yeast, filamentous fungi and actinomycetes [30]. These MIC values for the different bacteria though relatively high, are definitely demonstrative of the potential clinical use [31]. The microorganisms were least sensitive to the aqueous crude extracts due to negligible secondary metabolites in it [1]. Biswas, *et al*. [32] pointed out to the use of this extract in the treatment of wounds and Injuries in the traditional medicine of India.

Table (3)
Minimum Inhibitory Concentration (MIC) values for Bacterial Isolates Against R.tinctorum extracts.

Bacterial Isolates	*Extract concentration (mg/ml)*																										
	0.780			*1.560*			*3.125*			*6.25*			*12.5*			*25*			*50*			*100*			*200*		
	M	*H*	*C*	*M*	*H*	*C*	*M*	*H*	*C*	*M*	*H*	*C*	*M*	*H*	*C*	*M*	*H*	*C*	*M*	*H*	*C*	*M*	*H*	*C*	*M*	*H*	*C*
K.spp	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I	I	+	I	I	+
S.marcescens	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I	I	+
A.hydrophila	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I	I	+
S.dysenteriae	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I	I	–
E.coli	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I	–	–
S.aureus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	I	–	–	+	I	I	I	+	+	+	+	+

– = Resistance (growth of bacteria).
+ = Concentrations show no turbidity (inhibition of bacterial growth).
I = least concentration showing no turbidity (MIC).
M = Methanol extract.
H = Hot aqueous extract.
C = Cold aqueous extract.

Table (4)
Minimum Bactericidal Concentration (MBC) values for Bacterial Isolates Against R. tinctorum extracts.

Bacteria Isolates	Extract concentration (mg/ml)																										
	0.780			1.560			3.125			6.25			12.5			25			50			100			200		
	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M	H	C
K.spp	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	B	B	B	+	+	+	+	+	+
S.marcescens	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	B	B	B	+	+	+
A.hydrophila	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	B	+	+	B	B	+	+	+	+
S.dysenteriae	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E.coli	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S.aureus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	B	B	B	+	+	+	+	+	+	+	+

– = Resistance (growth of bacteria).
+ = Concentrations show no turbidity (inhibition of bacterial growth).
B = Minimum Bactericidal (MBC).
M = Methanol extract.
H = Hot aqueous extract.
C = Cold aqueous extract.

Results of the effect of temperature on the plant extracts showed that various temperatures; 45 C°, 70 C°, 100 C° Controllability and 121 C° had various effects on the antimicrobial activity of the extracts (Fig. (1,2,3)) The highest activity of methanol extract at 100 C°, 121 C° *against S.aureus, K.spp., A.hydrophila*, and *S.marcesencs* respectively; while there was low activity against, *S.dyesnteria* and *E.coli* (no zone of inhibition). Whereas aqueous extract of *R. tinctorum* revealed low activity than methanol extract and hot aqueous extract are more effective than cold aqueous extract.

Result of the effect of pH on the plant extract showed that plant extract was action in pH between (6-7), but the activity of plant extracts increased at acidic pH(5-3), i.e. increased in zone of inhibition of isolates at acidic pH. While at pH 8 there was slightly increase in activity. The methanol extract was more effective than hot and cold aqueous extract as shown in (Fig. (4,5,6)). Suleyman and Huseyin. [33] showed that *R. tinctorum* generally grow on loam and clayey-loam, neutral to slightly alkaline soils and the activity of phytoconstiuents of this plant increased in the presence of acidic medium has earlier been reported. The treatment of plant with high temperature could commence to release simple sugars that could be readily utilized in protein synthesis. Release of hormones such as auxins and ethylene, which could increase nucleic acid metabolism and protein synthesis [34].

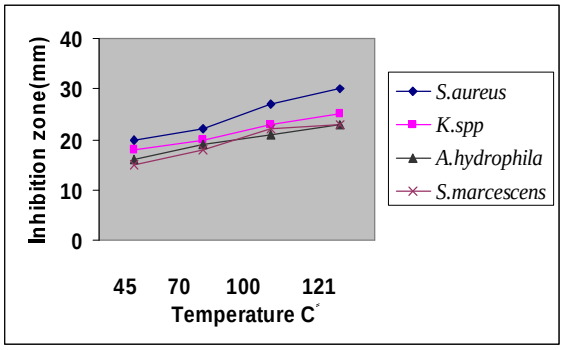

Fig. (1) : Effects of temperature on antimicrobial activity of Methanol extract R. tinctorum.

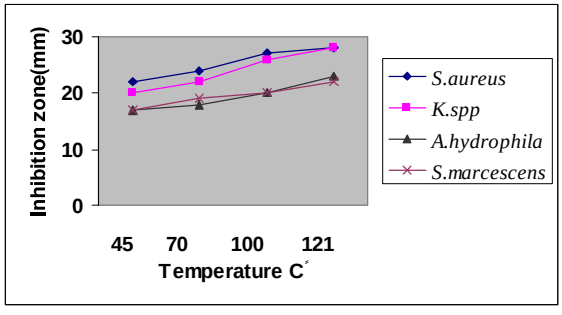

Fig. (2): Effect of temperature on antimicrobial activity of Hot aqueous extract R. tinctorum.

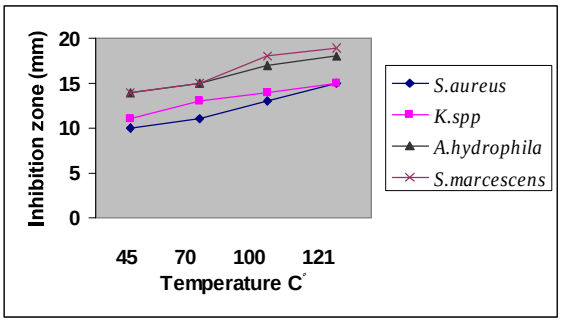

Fig. (3) : Effect of temperature on antimicrobial activity of Cold aqueous extract R. tinctorum.

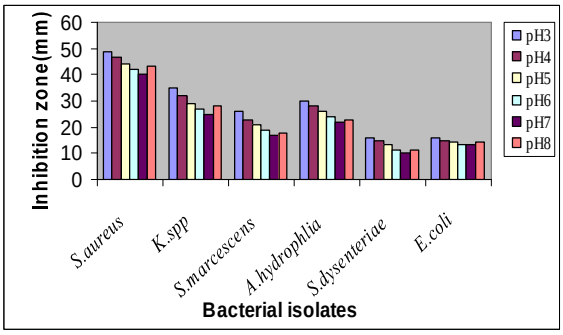

Fig. (4) : Effects of pH on antimicrobial activity of Methanol extract R. tinctorum.

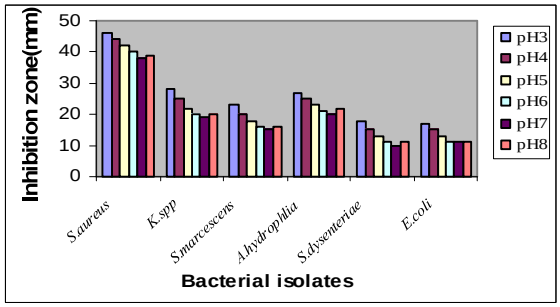

Fig.(5): Effect of pH on antimicrobial activity of Hot aqueous extract R. tinctorum.

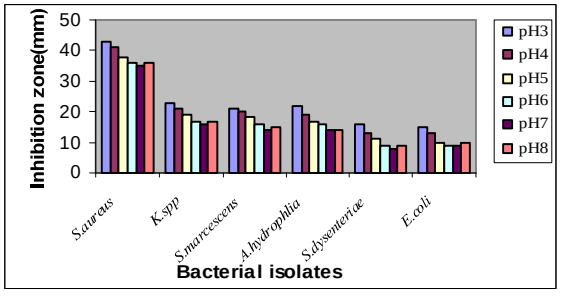

Fig.(6) : Effect of pH on antimicrobial activity of Cold aqueous extract R. tinctorum.

The results of essential elements determination (Pb, Na, K, Ca, Fe, Zn, P, Mn, Co,and Cu) in *R. tinctorum* (Table (5)) releavel the prescence of these elements at different concentration,these results are in agrrement with the results of Suleyman and Huseyin. [33] who showed higer concentration of Na, Mn, Zn, and K in *R.tinctorum* whereas N, P,and Ca were at lower concentration.

Table (5)
Essential elements concentration of R. tinctorum.

Elements	*Concentration*	*Rubia tinctorum*
Pb	ppm	0.3
Na	ppm	613
K	%	1.8
Ca	%	0.85
Fe	ppm	620
Zn	ppm	94.2
P	%	0.21
Mn	ppm	6.4
Co	ppm	1.9
Cu	ppm	5.2

The HPLC analysis (Fig. (7)) of *R. tinctorum* showed major peaks at the retention time(min).at a wavelength of 350 nm one of these retention time of this peaks (6.657min) compatibile with two retention time of standards flavonoids with (Chalcon and Kaempferol). But one of these standards (Anthocyanidin) which has peak at the retention time (2.451 min) was incompatibile with any of peak at the retention time. This compatibility indicate that the presence of phytochemical (flavonoids) in this plant which has antimicrobial activity against broad spectrum of microorganism and this agreement with the uses of *R.tinctorum* in traditional medicine. HPLC analysis of the plant sample revealed wide variability in their flavonoids content. The constituents of *R. tinctorum* called also madder root (pseudopurpurin, purpurin, alizarin, lucidin, munjistin and nordamnacanthal) was identified using gas chromatography (GC), high-performanace liqud chromatography (HPLC) [35]. Yizhong, cai *et al.* [22] identified the phenolic constituents in the root of Rubia by (HPLC) and by comparison with authentic standards.A

total of 17 hydroxyanthraquinones, gallic acid tannins were separated, and 14 of them were identified. Hydroxyanthraquinones, tannins and gallic acid were the predominant antioxidant phenolic constituents in the roots of Rubia.

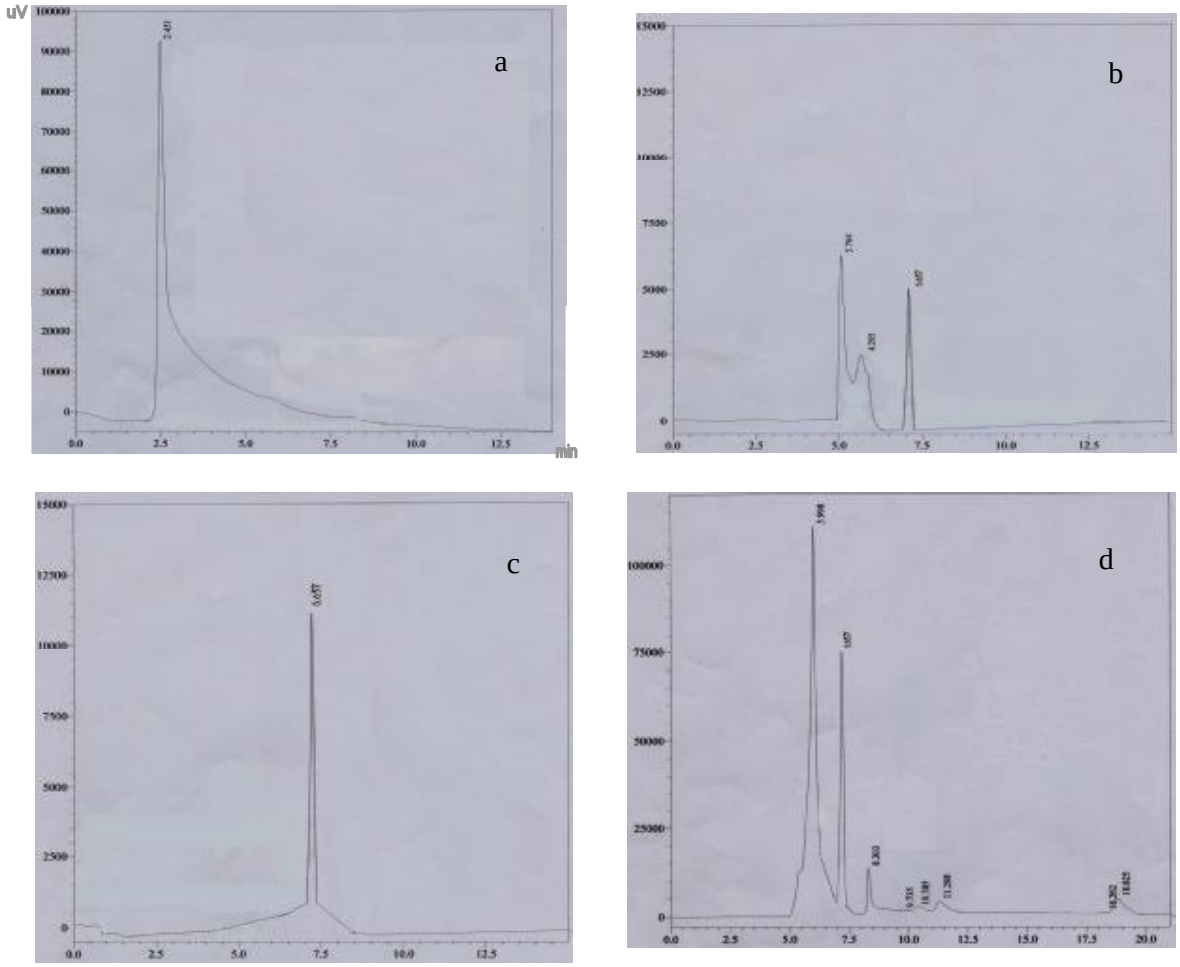

Fig. (7) : HPLC analysis of R. tictorum constituents and the standards a. Anthocyanidin b. Kaempferol c. Chalcon d. Sample plant.

Conclusion

Results of this study demonstrated by the aid of HPLC analysis of *R. tinctorum* extracts revealed that this plant has antimicrobial activity against test organisms and this may be suggest the use of this extract in treatment of infectious diseases.

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الخلاصة

أظهرت هذه الدراسة معلومات علمية حول المستخلص المائي والكحولي لنبات الفوة وذلك اعتماداً على الفعالية المضادة للميكروبات تجاه البكتريا الموجبة والسالبة لصبغة كرام المعزولة من اخماج الحروق بأستعمال طريقة الانتشار بالحفر.

أظهرت نتائج الدراسة وجود العديد من المركبات الكيميائية والتي تمتلك فعالية مضادة للمايكروبات ضد مدى واسع من البكتريا. كما لوحظت فعالية عالية للمستخلص الكحولي مقارنة بالمستخلص المائي وبلغ التركيز المثبط الأدنى للمستخلص المائي تجاه الأحياء المجهرية قيد الأختبار 25 – 100 ملغم/ مل في حين تراوح المستخلص الكحولي 25 – 50 ملغم /مل اما التركيز القاتل الادنى للمستخلص المائي 25- 200 ملغم/مل في حين تراوح المستخلص الكحولي 25- 100 ملغم/مل.وقد اظهر المستخلص الكحولي فعالية عالية عند الدرجات الحراري 100 م^{o} و 121م^{o} تجاه بكتريا *K. spp, S. aureus*، *A. hydrophila, S. marcescens* بينما لوحظت فعالية واطئة للمستخلص ضد البكتريا *S. dysenteria*، *E.coli*. كما لوحظت في هذه الدراسة ازدياد فعالية مستخلص نبات الفوة عند الدالة الحامضية (pH 5 – 3) في حين لوحظت هنالك زيادة طفيفة لفعالية المستخلص عند الدالة القاعدية (pH 8). كما لوحظ احتواء نبات الفوة على العناصر الأساسية (P, Mn) Co, Ca, Pb, Na, K, Cu, Fe, Zn, بتراكيز مختلفة.

وقد أظهرت نتائج تحليل نبات الفوة بالتقنية العالية للكروماتوغرافي السائل (HPLC) وجود بعض المركبات الكيمياوية الفعالة.وقد أوضحت نتائج الدراسة بأن تحليل مكونات نبات الفوه باستخدام التقنية العالية للكروماتوكرافي السائل إن هذا النبات يمتلك فعالية مضادة للبكتريا ضد الأحياء المجهرية قيد الاختبار وهذا يوضح انه ربما بألا مكان استخدام هذا المستخلص في الاصابات المرضية.

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