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Research Article

DETERMINATION OF THREE SUDAN DYES AND ANTIBACTERIAL ACTIVITY OF NATURAL BEET ROOT FROM SULAIMANI CITY KURDISTAN REGION, IRAQ

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Abstract

A rapid high-performance liquid chromatography (HPLC) system consisting of an ultraviolet-visible (UV–VIS) detector was developed for the separation and determination of Sudan dyes (B,G,7B) in reed beet root .The chromatographic separation was achieved on a reverse phase C_{18} column with isocratic elution, using a mobile phase of acetonitrile/methanol (80:20, v/v); detector was set at 254 nm. All three Sudan dyes Red was separated in less than 1.5 min contained 22.96, 14.54 and 9.74 µg/ml Sudans B,G and 7B,respectively determination of Sudan dyes was demonstrated. This method has potential to be used for Sudan dyes in beet root and some foodstuffs due to its simple, reliable, rapid, and excellent precision .other study was antibacterial activity of red boot root was tested against five Gram-positive, three Gram-negative bacteria and two fungi by disc diffusion method for different extracts and determining their minimum inhibitory concentration (MIC) values.

Keywords: Sudan dyes, Beta vulgaris L.,HPLC-UV-VIS, Antibacterial activity

Introduction

Beta vulgaris (Beet root) is a herbaceous biennial or, rarely, perennial plant with leafy stems growing to 1–2 meters tall. It comes under the family Chenopodiaceae commonly called as table bet, garden bet, red or golden bet, or informally as bet [1]. The leaves are heart-shaped, 5–20 cm long on wild plants. The flowers are produced in dense spikes; each flower is very small, 3–5 mm diameter, green or tinged reddish, with five petals; they are wind pollinated. The fruit is a cluster of hard nutlets [2] . They are mainly grown for their edible taproots. However, other cultivated varieties such as the leaf vegetable chard, as well as the rot vegetable sugar bet is used for production of table sugar, and mangelwurzel, as a fodder crop [3]. Three recognized subspecies include Beta vulgaris subsp. vulgaris, Beta vulgaris subsp. Maritime , known as the sea beet found throughout the Mediterranean, the Atlantic coast of Europe, and Kashmir and Beta vulgaris subsp. adanensis, occurs from Greece to Syria [4] . The rots are most commonly deep red-purple in color, but come in a wide variety of other shades, including golden yellow and red-and-white striped (Figure 1)

Fig(1) Beta vulgaris (Red Beet root)

Sudan dyes (Sudan I, II, III, IV ,Red G ,Black B and red 7B) are a family of synthetic azo dyes that are extensively used in industries such as car wax, petrol, shoe polish etc [5]. They have been banned for food usage in most countries due to their carcinogenicity [6]. However, illicit use of Sudan dyes can still be found in various food products, especially in ketchup and chili sauce. Therefore, development of specific and convenient analytical methods for these Sudan dyes in food products is of great interests for many researchers. Many methods have been developed for the determination of Sudan II in food products, including HPLC [7, 8], HPLC-DAD [9], HPLC/MS [10, 11], HPLC/UV-vis [12, 13], HPLC-PAD [14], UPLC [15], HPLC-electrochemical detection [16], GC/MS [17], resonance light scattering [18], capillary electrophoresis [19], enzyme-linked immunosorbent assay [20]and voltammetric method [21, 22]. Several methods based on liquid chromatography with UV or MS detection [23-25] or on gas chromatography mass spectrometry have been developed for analysis of Sudan I-IV. Although these methods have been success-fully applied to analysis of the dyes at trace levels in food products.

Beta vulgaris L. (beetroot) is ranked among the ten most powerful vegetables with respect to antioxidant capacity ascribed to a total phenolic content of 50–60 mmol/g dry weight . It contains a significant amount of phenolics: catechin hydrate, epica- techin, ferulic, protocatechuic, vanillic, *p*-coumaric, *p*-hydroxybenzoic, caffeic and syrin-gic acids. Beetroot is a potential source of valuable water-soluble nitrogenous pig- ments, called betalains, which are composed of two main groups, the red betacyanins and the yellow betaxanthins. In addition to their red color, betalains possess several desirable biological activities, including antioxi‘dant, anti-inflammatory, hepatoprotective, and anti- tumor properties [26].

Materials and Methods

Red Beet root, (Beta Vugaris)plant was harvested by hand in its optimum state for two consecutive seasons in 2013 in sulaimani city –Kurdistan Region –Iraq. After, a morphological and chemical characterization, the samples was prepared for determined Sudan Dyes compounds and antibacterial activity.

HPLC Methodology

The alcoholic extract of sample were separated on FLC 9fast liquid chromatographic) coloumn,3um particle size (50 4.5mm I.D)c-18 column, Mobile phase were 0.1% formic acid in deionized water: acetonitrile (20:80 v/v) detection UV set at 250 nm, flow rate 1.5 ml/min.The separation occurred on liquid chromatography shimadzu 10AV-LC equipped with binary delivery pump model LC- 10A shimadzu ,the eluted peaks were monitored by UV –Vis 10A- SPD spectrophotometer

Preparation of sample

1.0 gram of sample was weighed ,then homogenized and dissolved in 10 ml HPLC methanol , the sample shaking and agitated in Ultrasonic bath for 15 minutes, left settled for 1 h. ,then decanted the supernatant, and flittered ,then concentrated by evaporation the solvent with a stream of liquid N_2 until reach 1 ml. The extract were filtered on disposable minister filter 0.2 mm (supelco company cat No 16534k) then 20 µl were injected on HPLC column. The concentration for each compound were quantitatively determined by comparison the peak area of the standard with that of the samples, each standard was 25µg/ml.

Calculation: Concentration of sample µg/ml = [area of sample/area of standard] x conc. of standard x dilution factor

Antimicrobial activity

Preparation of the Extracts

The dried and powdered root of the plant were extracted with methanol for 48 hours. The methanol extract was dissolved in water and fractionated with chloroform and ethyl acetate, respectively. The extracts were filtered using Whatman filter paper (no. 1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were stored in a freezer until further tests.

Antimicrobial Activity

The extracts were tested individually against a range of 11 microorganisms, including 9 bacteria and 2 fungi species.

Disk Diffusion Assay

The antimicrobial activity of essential oil and extracts was determined by the disk diffusion method (NCCLS 1997). Briefly, 0.1 ml of a suspension of the test microorganism (10^8 cells ml^{-1}) was spread on Mueller–Hinton Agar plates for bacteria and Sabouraud Dextrose Agar for the fungi. Sterile 6 mm disks, each containing 10µl of samples were placed on the microbial lawns. The plates were incubated at 37 °C for 24 h for the bacteria and at 30 °C for 48 h for the fungi. The diameters of the zones of inhibition were

measured and are reported in mm. Triplicate tests were performed in all experiments.

Determination of minimum inhibitory concentration (MIC)

The MIC values were determined by the broth micro dilution assay (NCCLS 1997). Serial two-fold dilutions of the samples were made in Mueller–Hinton Broth containing 0.5 % Tween 80 for the bacteria and Sabouraud Dextrose Broth with 0.5 % Tween 80 for the fungi in 96-well micro titer plates. Fresh microbial suspensions prepared from overnight-grown cultures in the same media were added to give a final concentration of 5×10^5 organisms ml^{-1}. Controls of medium with microorganisms or the samples alone were included. The micro plates were incubated at 37°C for 24 h for the bacteria and 30 °C for 48 h for the fungi. The first dilution with no microbial growth was recorded as the *MIC*.

Results and Discussion

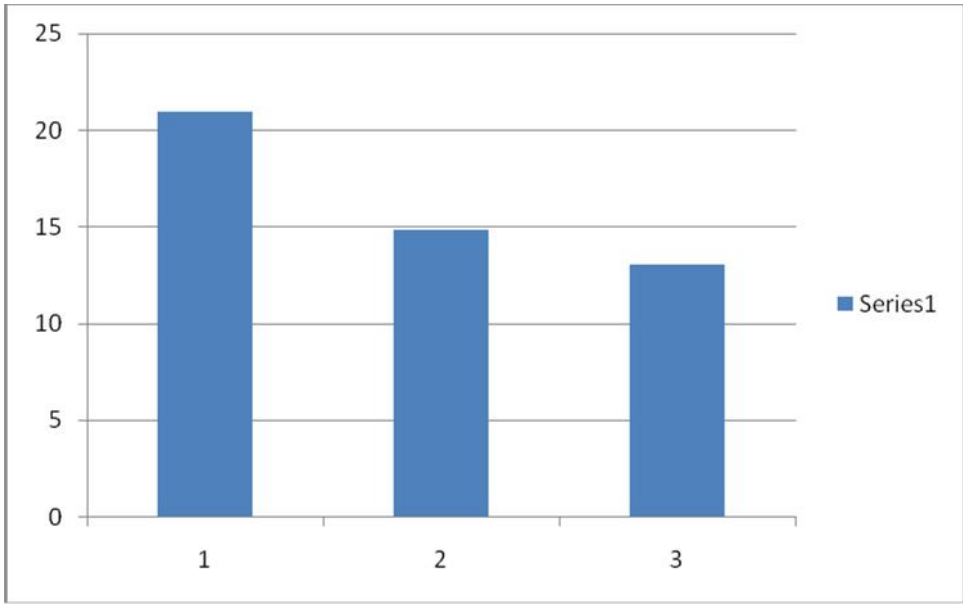
The HPLC system was used for qualitative and quantitative of three sudan dyes in natural beet root this analysis that shows in table(1,2)contained 22.96, 14.54 and 9.74 µg/ml sudan B,G and 7B,respectively fig(6,7,8).

Table (1) Standard Sudan compounds

No.	subject	Retention time minute	Area	Con. µg/ml.	%
1	Sudan B	2.037	196336	25	26.0479
2	Sudan G	2.858	220337	25	29.2321
3	Sudan 7B	3.938	298237	25	39.5671

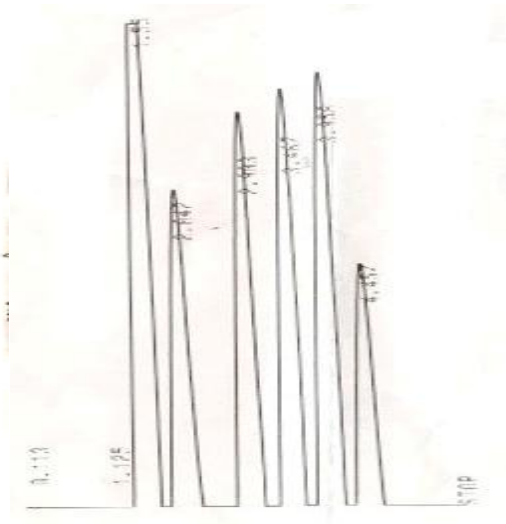
Table(2) Sudan compounds in Red Beet Root plant

No.	subject	Retention time minute	Area	Con. µg/ml.	%
1	Sudan B	2.042	180327	22.96	20.9868
2	Sudan G	2.903	127424	14.54	14.8298
3	Sudan 7B	3.958	116281	9.74	13.0577



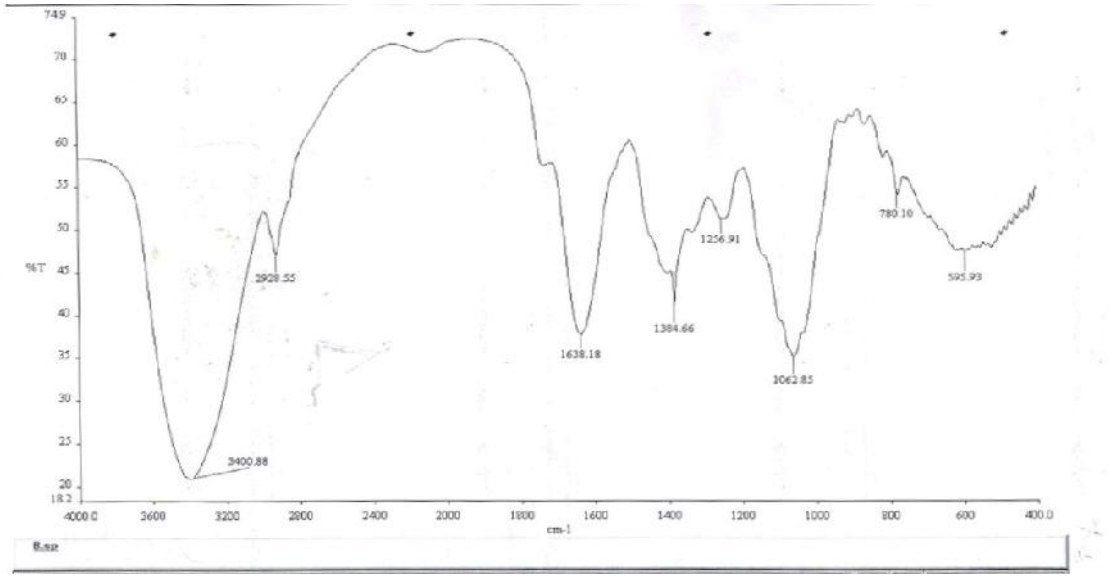
Fig(2) SudanB,7G and 7B compounds in natural Red Beet Root

Fig(3) HPLC chromatogram of standered Sudan dyes



Fig(4) HPLC chromatogram of Sudan dyes in natural Red Beet Root

The IR. Spectra fig (5) shows absorption bands of C–OH, C=C, phenyl and C– O–C in the wave range number of 3397cm-1,1632cm-1,2850-3000cm-1and1110-1200cm-1respectively, which demonstrate that graphene has been successfully prepared and the graphene platelets contain abundant C–O–C and C–OH functional groups of sudan dye.



Fig(5) IR Spectroscopy of Beet Root

Interpretation

The IR spectroscopy of Beet Root shows the range of
Phenol compound - 3397nm (3200-3550nm)
Alkanes - 2928nm (2850-3000nm)
Alkenes - 1632nm (1630-1680nm).

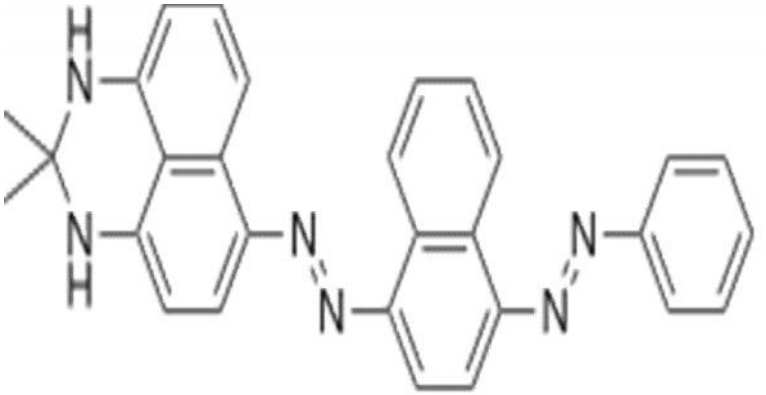


Fig (6) Sudan Black B
(2,2-dimethyl-1,3-dihydroperimidin-6-yl)-(4-phenylazo-1-naphthyl)diazene

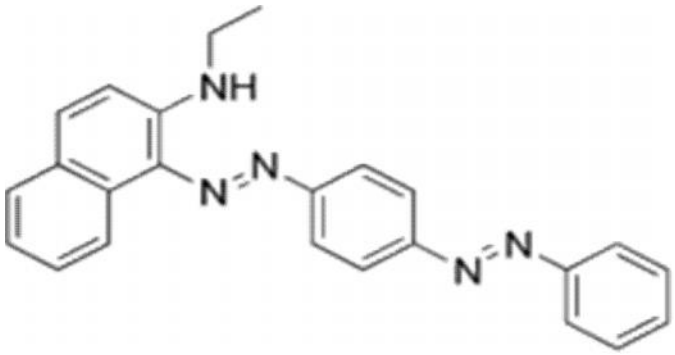
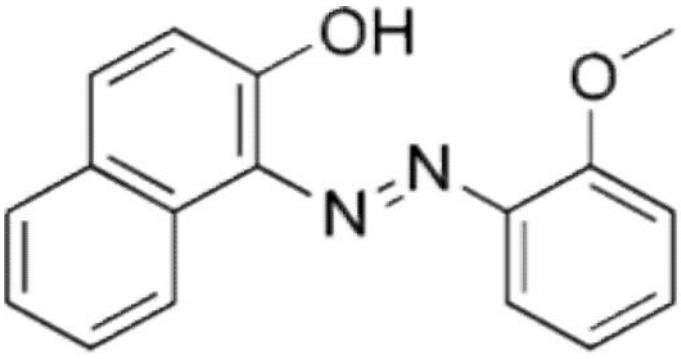


Fig (7) Sudan Red 7B
N-Ethyl-1-((4-phenyldiazenyl)phenyl)diazenyl)naphthalen-2-amine



Fig(8) Sudan G amethoxybenzenazo-β-naphthol

The result of antimicrobial activity of methanol, water, ethylacetate, chloroform and BHT extracts of plant was presented in table-3-showed that methanol extracts higher antimicrobial activity against all bacteria and fungi and chloroform extracts show no inhibition against most of microorganism

Table (3) Antimicrobial activity of Beet root extracts

Extracts	Microorganism										
	B.PU	***E.coli***	***S.au***	***B.Ce***	***KLb***	***E.NT***	***B.sub***	***St.Ep***	***PS***	***Can***	***Sac***
Methanol	14[a] (15)[b]	12 (15)	12 (16)	12 (16)	18 (8)	14 (15)	12 (15)	12 (15)	5 (32)	10 (10)	11 (10)
water	14 (15)	11 (15)	12 (15)	11 (15)	10 (10)	12 (15)	11 (10)	12 (15)	-	0	11 (10)
Ethyl acetate	11 (15)	12 (15)	14 (15)	0	11 (15)	10 (10)	11 (10)	12 (15)	-	-	0
chloroform	0	11 (15)	-	-	-	-	10 (10)	10 (10)	-	-	-

a Diameter of inhibition zones (mm) including diameter of sterile disk (6 mm).
b Minimum Inhibitory Concentration, values as mg/ml.
c -, not active; (7–14) moderately active; (>14) highly active

Conclusion

Sudan compounds occur in relatively high concentration in the natural red beet root plant grow in Sulaimani City, Kurdistan Region –Iraq like Sudan B, Sudan G and Sudan 7B occur as organic According to European Food Safety Authority, Sudan Red G is considered genotoxic and/or carcinogenic, Sudan Black B can be used to stain some other materials than the other Sudan dyes also Sudan 7B It is used in biology for staining.

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