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Original Article

Phytochemical Identification and Anti-Oxidant Study of Essential Oil Constituents of *Ocimum basilicum L.* Growing in Iraq

Abed S. Ahmed[1,*], Abo Khthr M. Fanokh[2], Mahood A. Mahdi[3]

ABSTRACT

Introduction: Essential oil of Iraqi growing *Ocimum basilicum* (Thia basil) was studied for their chemical components investigation and antioxidant activity. **Methods:** Essential oils of leaves and stems of *Ocimum basilicum* were collected using hydrodistillation by Clevenger apparatus. Components of the collected essential oils were examined by the techniques such as Gas chromatography and Gas chromatography–mass spectrometry. **Results:** The analysis showed that the major components of essential oils were linalool (48.69%), 1,8-cineole (14.00%), trans-α-bergamotene (8.23%) and eugenol (6.64%). The antioxidant investigation was achieved using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a radical scavenger and bleaching of β-carotene/linoleic assay. The essential oil displayed strong inhibition with 110.8% against autoxidation of linoleic acid, while the scavenging of DPPH radical gave a value of IC_{50} 145.35 µg/mL. **Conclusion:** The results signify that essential oils of *O. Basilicum* could be used for pharmaceutical studies and preservative in the food industry. This is the first study of the essential components of new cultivate of Thai basil in Iraq.

Key words: *O. basilicum*, Thai basil, β-carotene, Linalool, DPPH.

Abed S. Ahmed[1,*], Abo Khthr M. Fanokh[2], Mahood A. Mahdi[3]

[1]Natural Products of Pharmacognosy Department, Pharmacy College, University of Kerbala, Kerbala, IRAQ.
[2]Master in Pharmaceutical Chemistry Department, Pharmacy College, University of Kerbala, Kerbala, IRAQ.
[3]Full Professor Pharmaceutical Chemistry Department, Pharmacy College, University of Kerbala, Kerbala, IRAQ.

Correspondence

Salam Ahmed Abed

Pharmacognosy Department, Pharmacy College, University of Kerbala, Kerbala, IRAQ.

Phone no: 009647713458879

E-mail: salam.a@uokerbala.edu.iq

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INTRODUCTION

Ocimum basilicum L (English name: thai basil) is a member of Lamiaceae family. It is an important species of genus *Ocimum*, which is a group of 150 kinds of aromatic plants that can distributed in a variety parts of the tropic and warm regions.[1] It is present in India, Malaysia, Australia and some Arab countries.[1,2] *Ocimum basilicum* (Ob) is used traditionally for longevity and healthy life purposes due to its revitalizing and tonic effects. flower and leaves are used as aromatic, carminative, antispasmodic and galactogogue. Also they used in folk medicine for the management of fever, nausea, migraine, poor digestion, abdominal cramps, gastroenteritis, insomnia, chronic diarrhea exhaustion and dysentery.[3] *O. basilicum*, sweet basil, was used in traditional medicine of Yemen for the treatment of different ailments: abdominal pain, gastro-enteritis, diarrhea and dysentery. In Sultanate of Oman and Saudi Arabia, leaves juice or crushed leaves juice were used in the management of injuries, acne and vitiligo. It is also used as a antiperspirant, which is considering as an aphrodisiac for men.[4] The plant has important vitamins such as A and C and many metals like Ca^{2+}, Zn^{2+}, Na^{1+}, Mg^{2+} and Fe^{2+}.[5] The essential oil of *Ocimum* species. gained wide importance, because of their many bioactivities. It is used as antiseptic, antihistaminic, antimicrobial, anti-inflammatory, antihelimenthic, antioxidant, immunomodulatory effect, anti-depressant, anti-diabetic, anti-hyperlipidemia, hepatoprotective, neuroprotective effect and cardio-protective and anti-cancer activity.[6,7]

In terms of chemical composition of essential oils, *O. basilicum* characterized by two or three major components (20-70%) of volatile oil and other components present as minor components. It is composed of major constituents such as linalool, eugenol, methyl eugenol and methyl chavicol. However, these major constituents have been reported to occur in different percentages according to geographical locations (Table 1).[6]

Ocimum basilicum is cultivated in Iraq (Figure 1) and it is called "Reyhan". Fresh and dried leaves and stems are widely used in kitchen as an example in vegetable salads, meat and soups. In folk medicine, it is used antispasmodic and carminative. However, information about the essential oil components of *Ocimum basilicum* that was growing in Iraq is not

Figure 1: Ocimum *basilicum* (Iraqi growing).

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Table 1: The effect of geographical location of the chemical component of *O. basilicum* essential oil.

Location	Percentage of major constituents (%)[8]
Turkey	Linalool (45.7), Eugenol (13.4), methyl eugenol (9.57) and Funchyl alcohol (3.64)
Sudan (Um-Ruaba)	Methyl chavicol (70.0), Linalool (25), Eugenol (5.0)
Pakistan	Methyl chavicol (87.3), Linalool (5.4), methyl eugenol (1.5), β-Caryophyllene (2.4) α-Pinene (1.00)
Western of Cameroon	Linalool (50.8), Limonene (10.4)
Guinea	Linalool (69.0), Eugenol (10.0), (E)-α-Bergamotene (3.0)
Egypt	Linalool (44.18), 1,8-Cineole (13.65), Eugenol (8.59)
India (Attarakhand)	Methyl chavicol (70.04), Linalyl acetate (22.54), camphene (7.32).
European Basil	Linalool (53.0), Methyl chavicol (29.0)
Togo (Lome)	Estragole (85.5), Linalool (1.71)[8]

available. Therefore, it was worthy to undertake such investigation by isolations of the essential oils of the leaves and stem of *Ocimum basilicum L* which cultivated in Iraq. The oil, extensively identifies using GC and GC-MS. Then, the anti-oxidant action of the essential oil was evaluated using bleaching of β-carotene and DPPH scavenging assay.

EXPERIMENTAL

Plant material

Leaves and stems were collected during October / 2010 from the garden of the medicinal plants of the Kerbala University in Iraq. The sample was identified by the Iraqi National Herbarium in Abu-Graib. The drying was under shade at room temperature. Then the plants' leaves and stems were ground by blender and the total weight of the powder was 300 gm.

Hydrodistillation

The plant material of *O. basilicum* was submitted to hydrodistillation for 8 h, to Clevenger system. Then, the oils were extracted from the water layer with diethyl ether and dried over sodium sulfate anhydrous. These oil samples are kept in the refrigerator (2- 4)°C before the submitting for the chemical analysis.

Gas chromatography (GC)

Oil constituents were analyzed by Gas Chromatography (Hewlett-Packard HP-6890) with FID and setted with Ultra-1 column (100% polymethylsiloxanes) (25 m × 0.33 µm thickness of film × 0.20 mm i.d.). The temperature of the column was programmed from 50°C for 5 min to 300°C for 5 min at rate 4°C/min. The carrier gas was Helium with a flow rate 1 ml/min. Diethyl ether was used as a solvent for sampling. The sample (0.1 µL) was prepared by diluting 1/100 (v/v). On the other hand, standard hydrocarbons (C_9 – C_{20}) were used as reference in the Kovats Index of *O. basilicum* oil.

Gas chromatography– mass spectroscopy (GC-MS)

The analyses in GC-MS carried out using GC (Hewlett Packard model 5890 A) and MS (Hewlett Packard model 5989 A). The GC was prepared with an Ultr-1 column (25 m × 0.33 µm thickness of the film × 0.20 mm i.d.). The oven temperature was 50°C for (5 min) at the beginning and it was increased to 280°C at a rate (8°C / min) for (8 min). The solvent was dimethyl ether for dissolving and preparing the sample. Helium was a carrier gas with flow rate 1 ml/min.

Measurement of antioxidant activity using DPPH

The essential oil capacity of *O. basilicum* to scavenge free radicals was evaluated by the ability to capture the radical of DPPH.[8] First, a stock solution (1000 µg/mL) was prepared in methanol and then a series of a diluted sample in methanol were prepared to yield a sample (200 µL, 15.63-1000 µg/mL) in disposable cuvettes. Then DPPH (3800 µL, 1000 µg/mL) in methanol was added to each cuvette. The reaction was kept over 30 min in the dark at room temperature. The absorbance was measured for each sample at (λmax = 517 nm). The absorbance measurement of the negative control (DPPH and methanol) was at (0 min). In this test, the increment in the absorbance of the sample is associated with the lowest scavenging activity. The inhibition percentage (I%) of DPPH radical was measured using the subsequent formula:

$$I\% = [(Abs_{blank} - Abs_{sample})/ Abs_{blank}] \times 100$$

Abs_{blank} = Control absorbance of the: (DPPH and methanol).

Abs_{sample} = Extract or Standard absorbance: (BHT).

Bleaching assay by Beta-carotene/linoleic acid

The bleaching assay using β-carotene/ linoleic acid used to evaluate the inhibition ability of chemical components by β-carotene oxidation in the presence of (O_2). The procedure was achieved as detailed in the literature, with slight modification.[9] β -Carotene was mixed with linoleic acid by dissolving β- carotene (0.5 mg) in chloroform (1 mL) and linoleic acid (25 µL) and Tween (200 mg). Then, the chloroform was vaporized under vacuum. Oxygenated D. W (100 ml) was consequently added and diverse gently to obtain a clear yellow emulsion. *O. b* oil and BHT were dissolved individually in methanol (2 mg/ml) and (350 µL) of each were added to the yellowish emulsion (2.5 mL) in test tube and mixed carefully. The mixture and the blank were incubated in a water bath for (2 hrs) at 50°C. The absorbance was measured at 470 nm. Percent of Inhibitions (I%) of the samples were measured according to the subsequent equation:

$$I\% = [A_{\beta\text{-carotene after 2 h}} / A_{\text{initial }\beta\text{-carotene}}] \times 100$$

RESULTS AND DISCUSSION

The essential oil of *O. basilicum* was collected by hydrodistillation as pale yellowish (0.40 g, 0.40%). The chemical components were investigated by GC and GC-MS. The identification of essential oil components was achieved by comparing

their Kovat's indices with the reported values of authentic compounds in the literature.[10] The results of GC and GC-Ms analysis displayed that the major components of essential oil of *O. basilicum* from Iraq were linalool (48.69%), 1,8-cineole (14.00%), trans-α-bergamotene (8.23%) and eugenol (6.64%) (Figure 2). Moreover, it has shown that there are other minor components of *O. basilicum* which were represented in (Table 2).

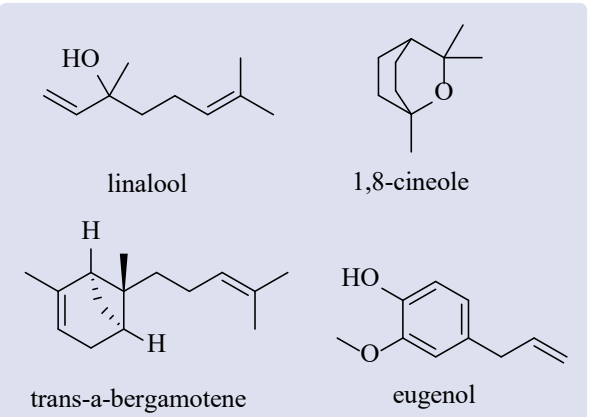

Figure 2: The structure of major components of *O.basilicum* essential oil.

Many studies have reported the effect of cultivation site on the components of the essential oil of O. *basilicum*. For example, methyl chavicol presents as the highest main content in the Nigerian *O. basilicum* (60.30%).[11] where it was considered as a main component for Um-Ruaba and Pakistan in a percent about 70% and 87.30%, respectively.[6] Moreover, the site of cultivation has an effect not only on the percent of the composition but also on the type of the main constituents. Linalool, for an instant, is the major component in *O. basilicum* essential oils from North-eastern Brazil (42.5%),[7] Greek (43.1%),[12] Egypt (44.18%) and Turkey (45.70%).[6] More interestingly, the essential oil of Iraqi grown *O. basilicum* was showed, that linalool was the main component (48.69%, Table 2). The major differences in the contents of essential oil were attributed to the change in the location and climate differences.[13]

Linalool is an aliphatic compound that has three allylic hydrogens. These threes can participate potentially in antioxidant effect. The extract of essential oil has also phenol and terpene components which are also having antioxidant effect. The antioxidant action was examined by β-carotene-linoleic acid bleaching assay and DPPH scavenging assay. The results of the scavenging activity are reported in Table 3.

In terms of DPPH scavenging assay, it bases on the capturing free radical DPPH by an antioxidant (HA). The ability of HA as an antioxidant was determined by measuring the decreasing in the absorbance of the purple color of the DPPH.[9] DPPH radical received hydrogen radical from an antioxidant HA and as illustrated in Figure 3.

It has been reported that essential oil of *O. basilicum* showed antioxidant activity, which was relatively more than a quarter the antioxidant activity of ascorbic acid. In terms of numbers, the essential oil exhibits IC_{50} 145.35 µg/mL, while the standard ascorbic acid shows a high scavenging potency for DPPH radical with IC_{50} 35.1 µg/mL. This finding indicates that the activity of antioxidant from major component linalool; as well as, the other minor components of the volatile oil.

Moreover, β-carotene-linoleic acid bleaching assay exhibited that the volatile oil has a potential scavenging efficacy. The potency of *O. basilicum* was very strong (110.8%) and it is equivalent to the standard BTH (110.7%). This activity was attributed to the presences of antioxidant components of the essential oil such as linalool.

According to the GC-MS analysis of Iraqi *O. basilicum,* linalool is the major component of essential oil. The result of β-carotene-linoleic acid bleaching assay showed that essential oils of *O. basilicum* exhibited a high antioxidant potency (110.8%). Linalool (HA) possesses high potency as antioxidant[13] due the presence of allylic hydrogens (Figure 4). In fact, the free peroxy linoleic radical (LOO•) has a good opportunity to abstract allylic H of linalool, which then converts to (LOOH). This could explain the strong inhibitory activity of lipid peroxidation of essential oil as a result of the high content of linalool and other major components such as trans-α-bergamotene (8.23%) and eugenol (6.64%), which are all having allylic hydrogen in their structures (Figure 4). While, in the absence of the anti-oxidant (HA), linoleic acid undergoes rapid peroxidation and produce peroxy linoleic

Table 2: The components of essential oil of *O. basilicum* growing in Iraq.

No.	Compound	Rt_x	Area%	KI	KI_L
1	β-Pinene	10.41	0.86	949	949
2	1,8-cineole	11.748	14.00	1014	1016
3	p-cymene	11.836	0.49	1016	1015
4	(E)-B-ocimene	13.036	0.32	1047	1048
5	γ-terpinene	13.64	0.55	1061.90	1060
6	***Linalool***	***14.806***	***48.69***	***1087.94***	***1086***
7	terpinen-4-ol	17.523	3.79	1157.03	1155
8	α-terpineol	18.023	1.71	1169.32	1173
9	exo-fenchyl acetate	19.276	0.56	1198.69	1214
10	Linalool propanoate	24.395	1.2	1340.84	1337
11	exo-2-hydroxy cineole acetae	24.597	0.25	1346.71	1354
12	eugenol	25.264	6.46	1365.75	1368
13	Geratnyl acetate	25.468	0.75	1371.48	1365
14	α-copaene	25.476	0.23	1371.70	1376
15	β-elemene	25.748	1.13	1379.26	1375
16	Z-caryophyllene	26.601	2.37	1402.99	1404
17	Trans-caryophyllene	27.367	0.58	1427.46	1419
18	α-trans-bergamotene	27.507	8.23	1431.85	1434
19	β-humulene	27.712	0.24	1438.25	1438
20	epi-bicyclosesquiphellandrene	27.97	0.33	1446.24	1438
21	Germacrene-D	29.4	1.83	1489.22	1485
22	Germacrene-A	30.011	0.23	1508.00	1508
23	Germacrene-B	31.38	0.94	1552.27	1559

KI is Kovats index from our experimental.

KI_L is Kovats index obtained from literature by Adams.[10]

Table 3: β-Carotene-linoleic acid bleaching and the scavenging activity on free radical DPPH of *O. basilicum* volatile oil.

Samples	β-carotene-linoleic acid	Scavenging DPPH IC_{50} (µg/mL)
O.basilicum volatile oil	110.8 ± 0.02	145.35
BHT	110.7 ± 0.01	-
AA	-	35.135

AA = Ascorbic Acid; BHT = Butylatedhydroxytoluene

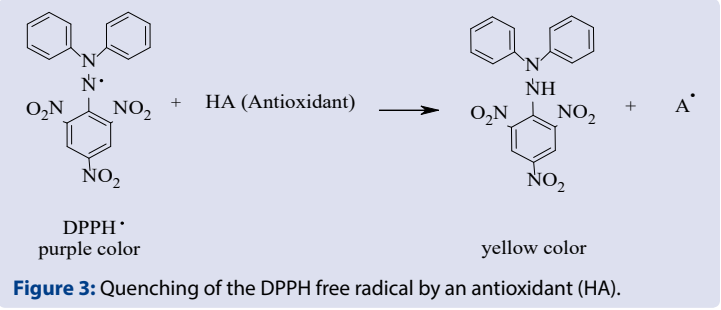

Figure 3: Quenching of the DPPH free radical by an antioxidant (HA).

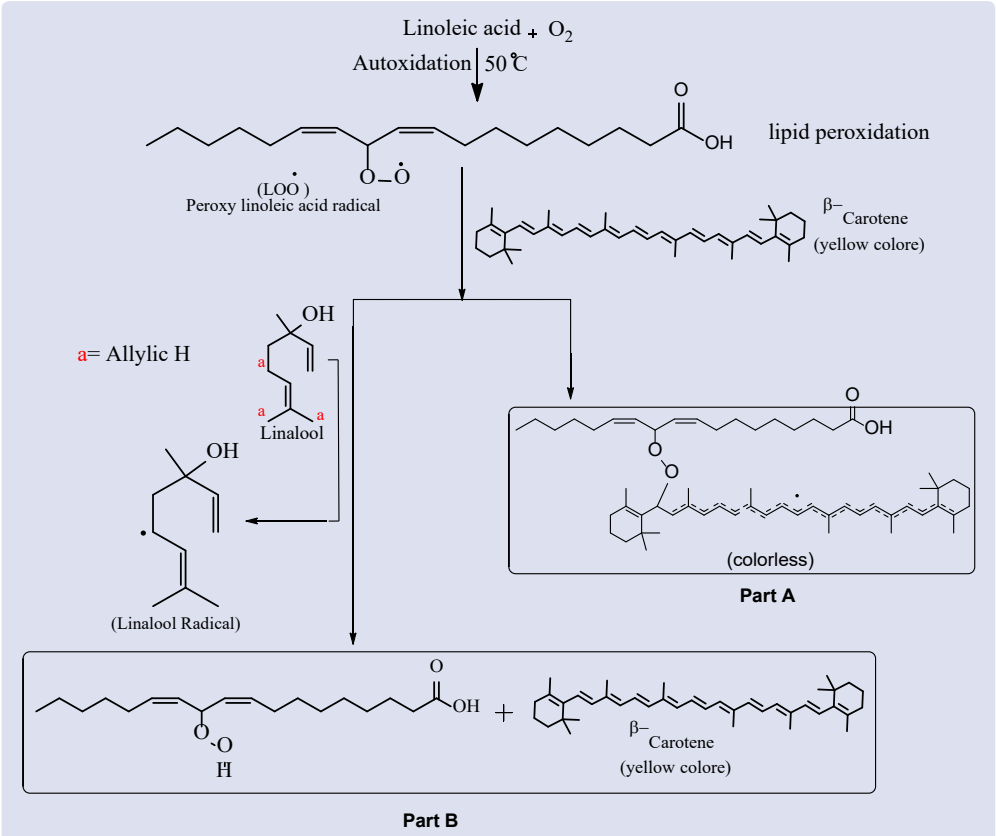

Figure 4: The suggested hyperoxidation of β-carotene-linoleic acid by atmospheric oxygen in the absence/ presence of an anti-oxidant linalool.

acid radical (LOO•) which then attacks β-carotene molecules and led to discoloration of β-carotene. The discoloration is due to the lost of the double bond from the conjugate system as explained in (Figure 4).

CONCLUSION

The chemical composition of *O. basilicum* is changing according to the geographical location. Iraqi growing *O.basilicum* has linalool as the highest constituent of the essential oil. The antioxidant activity was evaluated through β-carotene-linoleic acid and DPPH assays. In β-carotene-linoleic acid assay, the essential oil showed antioxidant activity equivalent to what was reported for BHT. Whereas it is about one quarter of ascorbic acid in DPPH assay. Linalool and other main components contain an allylic hydrogen, which can easily abstract by ($LOO^{\cdot}$) in β-carotene-linoleic acid and exhibition the antioxidant activity.

DEDICATION

S. A. A. dedicates this study to Prof. Dr. Hasnah Mohd Sirat of Universiti Teknologi, Malaysia.

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CONFLICTS OF INTEREST

Authors declare that there is no conflicts of interest.

ABBREVIATIONS

DPPH: 1,1-diphenyl-2-picrylhydrazyl; KI: Kovats Index; AA: Ascorbic Acid; BHT: Butylatedhydroxytoluene; IC_{50}: Inhibitory Concentration; µg/ml: Microgram per Mililiter; °C: Degree Celsius; mg/mL: Milligram per Mililiter; µL: Microliter; min: Minutes; µL: Mililiter; HA: Linalool; LOO•: Peroxy Linoleic Radical.

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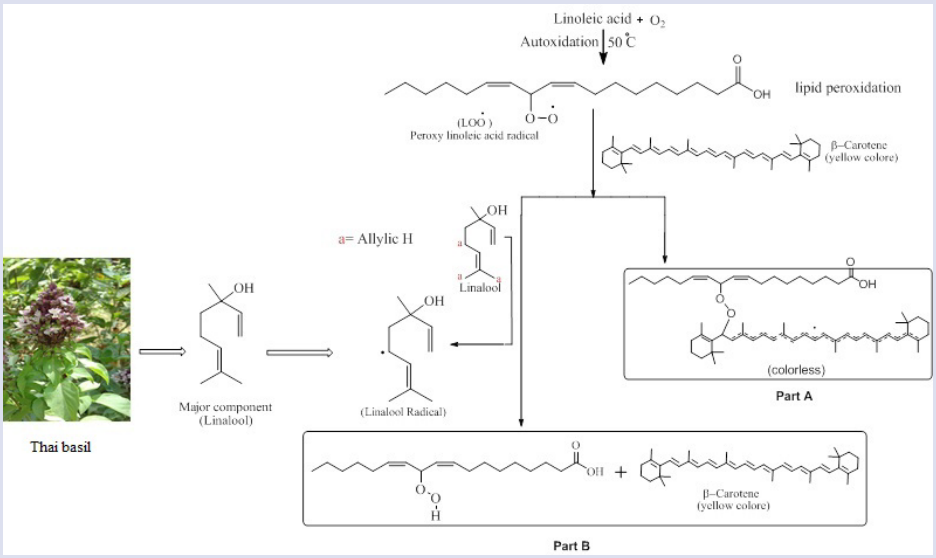
GRAPHICAL ABSTRACT



SUMMARY

Essential oil of Iraqi growing *Ocimum basilicum* was studied for chemical investigation and antioxidant activity. The analysis showed that the major components of essential oils were Linalool (48.69%), 1,8-cineole (14.00%), trans-α-bergamotene (8.23%) and eugenol (6.64%). The essential oil displayed strong inhibition with 110.8% against autoxidation of linoleic acid, while the scavenging of DPPH radical gave a value of IC_{50} 145.35 µg/mL. This is the first study of the essential components of new cultivate of Thai basil in Iraq.

ABOUT AUTHORS

Salam Ahmed Abed is specialized in Natural Product and Organic Chemistry. Ph.D. degree in Natural Product Chemistry, from Universiti Teknologi Malaysia (UTM). Currently, works at Pharmacognosy Department/ Pharmacy College, University of Kerbala.

Abo Khthr M. Fanokh is a Lecturer in Pharmaceutical Chemistry Department, MSc Drug Chemistry/ Newcastle University/ UK and his research areas of interest are studying the antioxidant effect of natural product, molecular docking, and organic synthesis of bioactive molecules.

Prof. Dr. Abdulbari Mahdi Mahood is an head department of Pharmaceutical Chemistry, works in Analytical chemistry-Chromatography-HPLC and Gas Chromatography, TLC and flow-injection spectrometer-Uv-Visible, FT-IR and Atomic absorption.

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