In vitro antimicrobial activity of total sesquiterpene lactones and phenols isolated from some Iraqi plants

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Abstract

The antimicrobial potency of the crude ethanolic extracts from different Iraqi plants were evaluated . Further more, total sesquiterpene lactones and phenolic compounds were isolated and their antimicrobial activity attempted. The results indicated that crude extracts have no activity except that of Callistemon lanceolatus. Also, the sesquiterpene lactones and phenolic compounds isolated from Callistemon lanceolatus were the most significant antimicrobial active constituents of the studied plants.

Introduction

A different variety of important plants have been reported to be used in folk medicine. Iraq is among the developing countries which commonly use plants in treatment of many diseases (1,2).These plants contained a various chemical constituents and sesquiterpene lactones were among these which showed considerable antimicrobial activity against different types of pathogenic microorganism (3,4).The sesquiterpene lactones have been identified from many species of higher and lower plants (5,6)and their constituents were found to be high in the leaves and flowering heads specially in Compositae family (7).

Phenolic compound are among the plants constituents which widely reported to be a disinfectant for many pathogenic microorganisms (8).Many of these compounds have been isolated and characterized and their antimicrobial potency were reported (9). In the present study some of never been studied Iraqi plants were selected to evaluate their antimicrobial potency especially their isolated sesquiterpene lactones and phenolic compounds.

Materials and methods

Sampling:

Callistemon lanceolatus (Sm.)DC.; Vince rosea L.; Bouhinia variegata L.; Descurainia sophia L.; Lactuca serriola L. and Bougainvillea glabra choisy plants were collected from environs of west Baghdad during April and identified by the Iraqi National herbarium(Baghdad Abu-Ghraib).The aerial parts of these plants were air dried at room temperature and ground to powder form.

Plant extraction:

The powdered plants material (100g) was extracted (Soxhelet) with 80% ethanol until exhaustion. The extracts were evaporated to dryness under reduced pressure at 50°C.

Sesquiterpene lactones and phenolic compounds isolation:

Half of the ethanolic extract was dissolved in 80 ml. warm mixture of ethanol and water(50% v / v)which contained lead acetate(3 - 4 g.) .After Standing overnight, the resulted precipitate

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Was filtered and the filtrate was concentrated in a rotary evaporated until the ethanol had been removed at a temperature not exceeding 40°C. The yellow – orange syrup which separated from aqueous phase was extracted with chloroform. The chloroformic layer was separated and dried over anhydrous sodium sulphate.The solvent was evaporated and the resulted thick syrup was analysed for sesquiterpene lactones presence using infrared spectroscopy. Infrared analysis were carried out using Beckman acculab 8 instrument. The lactonic carbonyl groups were monitored at a frequencies between 1740 to 1770 Cm^{-1} (10).

The second half of the ethanolic extract was subjected to a column chromatography using polyamide. Fractions were eluted first with distilled water and then with methanol. The eluted fractions were analysed using T.L.C. and FeCl3 reagent.The phenolic contained fractions were collected and evaporated to dryness (11).

Test micro-organisms and culture media:

Bacillus subtilis ATCC 6633; Staphylococcus aureus NCTC8532; Escherichia coli k12 10538; Pseudomonas aeruginosa NCTC 6750; Salmonella typhimurium ATCC 14628; Candida albicans(local isolate)and Sacharomyces cerevisiae (local isolate)were used as a representative micro-organisms. Nutrient broth (oxoid)and yeast extract glucose were used for the cultivation of bacteria and fungi respectively. The medium was solidified by the addition of 2% Oxoid agar No.3 if necessary.

A particular amount of overnight culture was added to agar medium to get final concentration of $1x10^6$ cells/ml. Glass plates(35x25cm)with a 2.5cm altitude edge sealed with agar were prepared for each microbe. wells(10mm. in diameter)were made using the cork borer and vacuum to pull out the pilletes.0.2ml.of each sample was added in each hole at a concentration of 20mg/ml , then incubated at 37±1° for 18 hours. Absolute ethanol was used as experimental control when necessary; 0.5 mg/ml streptomycin sulphate and 2% phenol were used as a standard bacterial and fungal growth inhibitors, respectively .

Results and discussions

All ethanolic crude extracts of the investigated plants showed no significant antimicrobial activity except that from Callistemon lanceolatus which exhibited antimicrobial potency against Staphylococcus aureus ; Bacillus subtilis and Saccharomyces cerevisiae (Table1).Chemical analysis exhibited that all the investigated plants contain different quantities of sesquiterpene lactones and phenolic compounds as presented in table1.The sesquiterpene lactones of the studied plants have been subjected to antimicrobial assay(Table 1). The growth of Staphylococcus aureus and Bacillus subtilis were particularly inhibited in addition to some other test microorganisms but not from all the sesquiterpene lactones of the investigated plants. Salmonella typhimurium and Pseudomonas aeruginosa were found resistance to attack by all the sesquiterpene lactones obtained. However, the results indicated that these compounds are mostly active against Gram positive bacteria and more potent than other Iraqi plants evaluated previously(7,12) .

The phenolic compounds did not show a significant activity against the test microorganisms except for Callistemon lanceolatus which exhibited an exceptional antibacterial activity specially against Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa. A moderate antifungal activity was observed as shown in table 1.

These results indicated that Callistemon lanceolatus is the most interesting plant due to its higher activity

compared with other plants under investigation.

Various chemical constituents were isolated from Callistemon lanceolatus such as triterpenoids, flavonoids, saponins and polyphenolic compounds (13 - 16).However, only the volatile oil of this plant reported to have fungistatic activity (17).Therefore, the total sesquiterpene lactones and phenolic compounds of this plant which proved to have higher antimicrobial potency in the present study need to be purified and identified in order to evaluate their therapeutic applications.

Table (1) Antimicrobial potency of crude ethanolic extracted and islated sesquiterpene lactones and phenols from different Iraqi plants.

Plant species and families	Type of extract	% Yield	Test microorganisms						
			S.a.	B.s.	P.a.	E.c.	S.t.	S.c.	C.a.
Callistemon lanceolatus (Myrtaceae)	EtOH		20	20	-	-	-	15	-
	Ses.	0.2	35	35	-	15	-	25	19
	Phen.	20.6	27	27	24	-	-	15	15
Bouhinia variegata (Legmuminosae)	EtOH		-	-	-	-	-	-	-
	Ses.	0.16	15	15	-	12	-	-	12
	Phen	2.1	-	-	-	-	-	-	-
Descurainia Sophia (Cruciferae)	EtOH		-	-	-	-	-	-	-
	Ses.	0.11	12	13	-	-	-	-	-
	Phen	1.65	13	12	-	-	-	-	-
Vinca rosea (Apocynaceae)	EtOH		-	-	-	-	-	-	-
	Ses.	0,25	16	16	-	-	-	-	14
	Phen	19.9	-	-	-	-	-	14	-
Bougainvillea glabra (Nyctaginaceae)	EtOH		-	-	-	-	-	-	-
	Ses.	0.11	17	15	-	-	-	20	13
	Phen	7.9	-	-	-	-	-	15	-
Lactuca serriola (Compositae)	EtOH		-	-	-	-	-	-	-
	Ses.	0,19	16	14	-	-	-	-	-
	Phen	16.4	15	12	-	-	-	-	-

Inhibition zones were determined in mm. - =No inhibition zone detected. Inhibition zone of 0.5%mg/ml streptomycin sulphat on S . a.(Staphylococcus aureus)=18mm;B.s(Bacillus subtilis)=20mm,P.a.(Pseudomonas aeruginosa) =20mm, E.c.(Escherichia coli)=18mm, S.t.(Salmonella typhimurium)=18mm. Inhibition zone of 2% phenol on S.c.(Saccharomyces cervisea)=14mm, C.a.(Candida albicans)=14mm. Ethanol inhibition was between 11 – 12mm.
Ses.=Total sesquiterpene lactones, phen.=Total phenolic compounds.

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الفعالية ألمضادة للأحياء ألمجهرية خارج ألجسم ألحي لمركبات ألسيسكويتربين لاكتون وألفينولات ألمعزولة من بعض ألنباتات ألعراقية

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الخلاصة:

لقد تم في هذه ألدراسة تقييم ألفعالية ألمضادة للأحياء ألمجهرية للمستخلصات ألكحولية لبعض ألنباتات ألعراقية.اضافة الى ذلك فقد تم عزل ألفينولات ومركبات السيسكويتربين لاكتون من هذه ألنباتات وتقييم فعاليتها ألمضادة أيضاً.

أظهرت ألنتائج بأن ألمستخلصات ألكحولية للنباتات ألمدروسة ليس لها أي فعالية تذكر ماعدا تلك ألمستخلصة من نبات فرشاة ألبطل Callistemon lanceolatus حيث أظهرت فعالية مضادة لبعض البكتريا المستخدمة في ألدراسة...وكذلك لوحظ أن مركبات ألسيسكويتربين لاكتون والفينولات ألمعزولة من هذا ألنبات ذات فعالية عالية جداً قياساً الى بقية ألنباتات ألاخرى ألتي تضمنتها ألدراسة.