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Effect of Plant Alkaloids on Some Pathogens

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ABSTRACT

This study aimed to evaluate the antimicrobial activity of *Rosmarinus officinalis, Origanum vulgare* L. and *Cressa cretica* L. alkaloids on *Staphylococcus aureus*, *Eschrichia coli* and *Candida albicans*. The results showed a prominent inhibitory effect of all alkaloid extracts on all the microbial isolates, noted by the large bacterial growth inhibition zones (0.2-6.8mm), four concentration were used in this study (10, 20, 30 and 40)%, all these concentrations appeared inhibition effect. *C. cretica* had more inhibitory effect on the growth of all microbes than other tested plants.

Keywords: *Effect, alkaloids, pathogens*

Introduction

Alkaloids are the largest group of secondary metabolites, building from ammonia molecules comprising of nitrogen bases synthesized from amino acid building blocks with various radicals and replacing one or more of the hydrogen atoms in the peptide rings and most of them containing oxygen, alkaloids differ in the basicity according to the structure of molecule groups [1]. Alkaloids had so numerous and involve such a variety of molecular structure that lead to difficult in theme classification. So, the best solve to this problem is to group them into families, depending on the type of heterocyclic ring system present in the molecule [2]. Alkaloids are significant for the protecting and survival of plant because of their activity against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrence) and also against other plants by means of allelopathically active chemicals [3]. Many of alkaloids containing plants as dyes, spices, drugs or poisons can be traced back to the beginning of civilization. There are many medical activities of alkaloids including antihypertensive effects like indole alkaloids, antiarrhythmic like quinidine, antimalarial activity like quinine, and anticancer actions like dimeric indoles, vincristine, vinblastine. These are just a few example of the great economic importance of this group of plant constituents [5]. Rosemary (*Rosmarinus officinalis* L.) originally grows in southern Europe. Its herb and oil are commonly used as spice and flavoring agents in food processing for its desirable flavor, high antioxidant activity and lately as antimicrobial agent [(6-8)]. Furthermore, rosemary extracts have been widely used as a preservative in the food industry due to their inherent high antioxidant activity. In addition, it has been used as a medicinal herb for centuries, due to significant activities against many illnesses. In this sense, many major biological properties have been attributed to this plant, mainly hepatoprotective [9], antimicrobial [(10-12)], antithrombotic [13], diuretic [14], antidiabetic (Bakirel *et al*., 2008), anti-inflammatory [15], antioxidant [16], and anticancer [17]. Origanum (*Origanum vulgare* L.) grow abundantly on the stony slopes and rocky mountain areas at a wide range of altitudes (0-400m) [18]. Because of the variability in the chemical and aroma characteristics, different species of origanum ecotypes (biotypes) are widely used in the agricultural, pharmaceutical and cosmetic industries [19]. In addition, they have been used in the folk medicine to treat several illnesses as spasmodic, antimicrobial, digestive, expectorant and aromatic for the whooping and convulsive coughs. Origanum known for its antifungal and antimicrobial properties, its Leaves are traditionally used as antiseptics and disinfectants. This is the very aromatic plant [20]. *Cressa. cretica* L. is a small, erect dwarf shrub [21] the height of it is 38cm. Roots appear horizontal, with lateral branches. It is a perennial sub shrub or herb, usually much-branched. Stems are at first erect and then

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become decumbent, apparently short-lived. Leaves sessile, ovate to lanceolate. *C.cretica* used in all parts as a paste and decoction to treated fungus infection, asthma, blood purifier and eczema treatment [22]. Aqueous and alcoholic extracts of leaves of this plant have a very good activity against some microbial pathogens such as gram – positive, gram negative bacteria and some fungi species, such as *Candida albicans, Aspergillus niger,* and *Penicillium chrysogenum* [23]. The plant can be used as anti-tubercular, and expectorant. Ethanolic extract of *C. cretica* significantly reduced blood glucose, serum cholesterol in rats [24]. Whole plant methanolic extract had antimicrobial activity, Ethylacetate and methanolic extract use as Broncho dilatory activity and mast cell stabilising activity [25]. Aim of this study was evaluate the antimicrobial activity of some alkaloid compounds extracted from *Rosmarinus officinalis*, *Origanum vulgare* and *Cressa cretica*.

Materials and Method

Alkaloids extract preparation: 100 g from dried plant powder was putted in a flask (500 ml), added 350 ml methanol: distilled water (1:4), putted in a horizontal shaker for 24 h, filtered throw filter paper (whatman No1), concentrated in a rotary evaporator, added drops from sulfuric acid (2%) until PH become 1-2, placed in a separatory funnel and extracted with chloroform (three times), mixed and shaken for about five times and allowed to separate into two layers. The lower layer of chloroform contained the alkaloids and the upper layer the aqueous portion, then added ammonium hydroxide (NH_4OH) until PH become 9, transferred to a separatory funnel and another extracted with chloroform: methanol (3:1) for two times, the lower layer was taken, concentrated and dried at 40-45^{o}c, then kept in refrigerator.

Test for alkaloids:

A. **Dragendorff's reagent:** 20 g from Bismuth Nitrate in 40 ml distilled water and 16 g from sodium Iodide in 40 ml distilled water, mixed together and added 1-2 ml from this reagent to 5 ml from the extract, a prominent orange color was indicated the test as positive.

B. **Tannic acid reagent:** 2 ml from 10% Tannic acid solution was added to 5 ml from extract, Alkaloids give orange color precipitate.

C. **Hager's test:** Hager's reagent is saturated solution of Picric acid ($C_6H_3N_3O_7$), after added a few drops from this this reagent appear yellow color precipitate, that's positive indicator to the presence of alkaloids.

Microorganisms tested: Microbial cultures of three different species of microorganisms were used for determination of the antimicrobial activity, they were *Staphylococcus aureus*, *Eschrichia coli* and *Candida albicans*. All the test cultures were maintained on nutrient agar media with regular sub-culturing.

Test antifungal activity of the plant extracts

Preparation of Inoculum: Suspended isolated colonies from nutrients agar was added to 5 ml from 0.85% sterile normal saline to achieve 0.5 McFarland turbidity to form a yeast stock suspension of 1 × 106 to 5 × 106 cells/mL, which should produce semi confluent growth with most microorganisms isolates.

Preparation of the 0.5 McFarland standards: 0.5 ml of 0.048 M from $BaCl_2$ (1.17% w/v $BaCl_2.2H_2O$) was added to 99.5 ml of 0.18 M H_2SO_4 (1% v/v) with constant stirring. Distribute the standard into screw cap tubes of the same size and with the same volume as those used in growing the broth cultures. Seal the tubes tightly to prevent loss by evaporation. Store protected from light at room temperature. Vigorously agitate the turbidity standard on a vortex mixer before use.

Agar well diffusion method: Determine the antimicrobial activity of plant extracts was followed by agar well diffusion method. Nutrient agar plates were swabbed (sterile cotton swabs), wells in about 10 mm in diameter were made in each plate using sterile cork borer. Stock solution of each extract was prepared at a concentration 40 % in different plant extract (aqueous and alcoholic), about 100 µl of different concentration of extract were added using micropipette into the well and allowed to diffuse at room temperature for 2 hrs. The plates incubated at 28^{o}c for 24 -48 hrs. After that measured the diameter of inhibition zone (mm), The experiment were maintained triplicates, for each replicate the reading were taken in three different fixed directions and recorded the average values.

Results and Discussion

Table (1) represent the alkaloids constituents that present in the plants under the study. Hager and Tannic acid reagents appear a positive result, while Dragndrof reagent appear a negative result. There was a significant differentiation between the extracts and the microbes according to Pv. Test by using Anova. figure (1) appear the diameter of inhibition zone of *S. aureous* at concentrations 10, 20, 30 and 40 % for all plant alkaloid extracts, it was appear that the inhibitory effect increase with the concentration increasing. figure (2,3) appear the diameter of inhibition zone of *E. coli* and *C. albicans* at concentrations 10, 20, 30 and 40 % for all plant alkaloid extracts, it was appear that the inhibitory effect increase with the concentration increasing. *C. cretica* alkaloid appear most inhibitory effect on microorganisms growth, which the diameter of inhibition zone reach to 6.8mm on *C. albicans*, followed by *R. officinalis* (4.6 mm) then *O. vulgare* (3.2 mm), on *E. coli* as appear in table (2). *S. aureous* was the most affected by alkaloids, followed by *E. coli*, then *C. albicans* as shown in table (2). Plants have ability to synthesize chemical products. These products are secondary metabolites and serve as plant defense mechanisms against microorganisms, like alkaloids. This study agreed with the study of Ivanovska *et al*. (1999) which showed that the alkaloid berberine expresses a protective effect on *C. albicans* infection induced in arthritic mice, Alkaloids targeting cell wall and ergosterol biosynthesis leading to cell death. [25-30], also alkaloids lead to inhibition of extracellular enzyme activity of *Candida* and that might be related to decreased pathogenicity [26]. Issabeagloo1 *et al*. (2012) found that extract of rosemary inhibit the growth of *S.aureus* and this inhibitory effect increase with the increasing concentration. Oliveira *et al*. (2009) evaluate the antimicrobial activity of *O. majorana* essential oils on the growth of *S. aureous* and they found that this inhibitory effect increase with increasing concentration.

Table 1: Qualitative tests of alkaloids according to plant type

Test	R. officinalis	O. vulgare	C.cretica
Hager	+	+	+
Dragndrof	_	_	+
Tannic acid	+	+	+

+ positive - negative

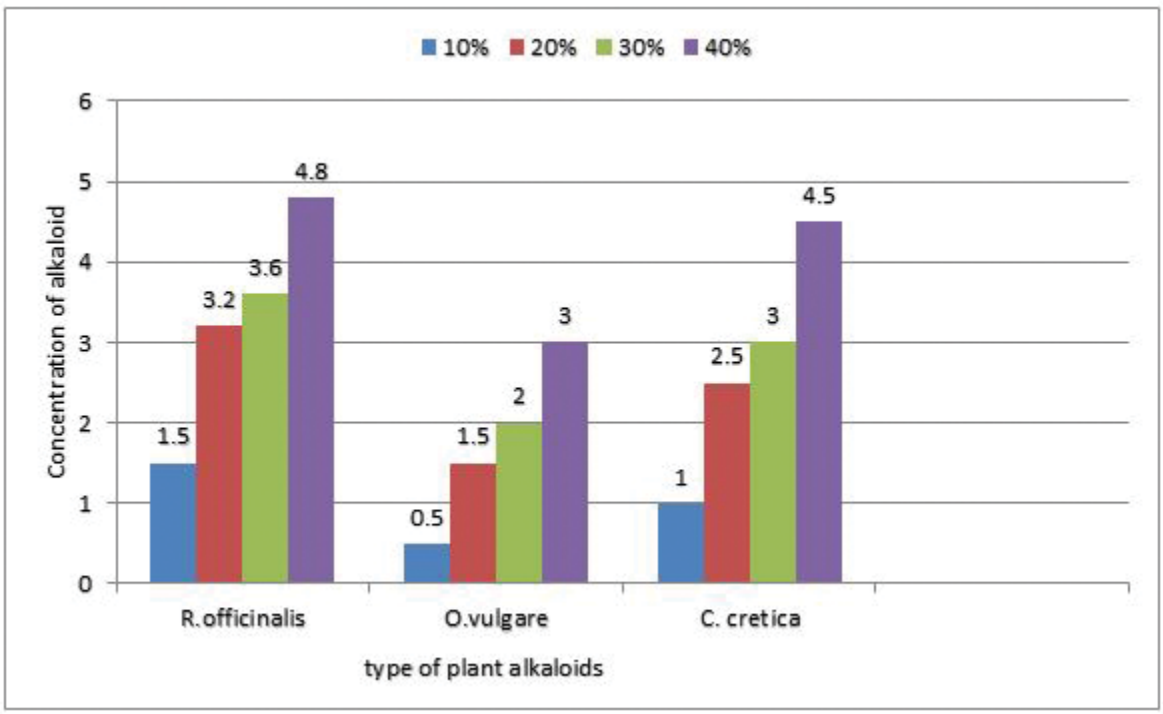

Figure 1: Effect of alkaloid concentration on *S.aureous* growth

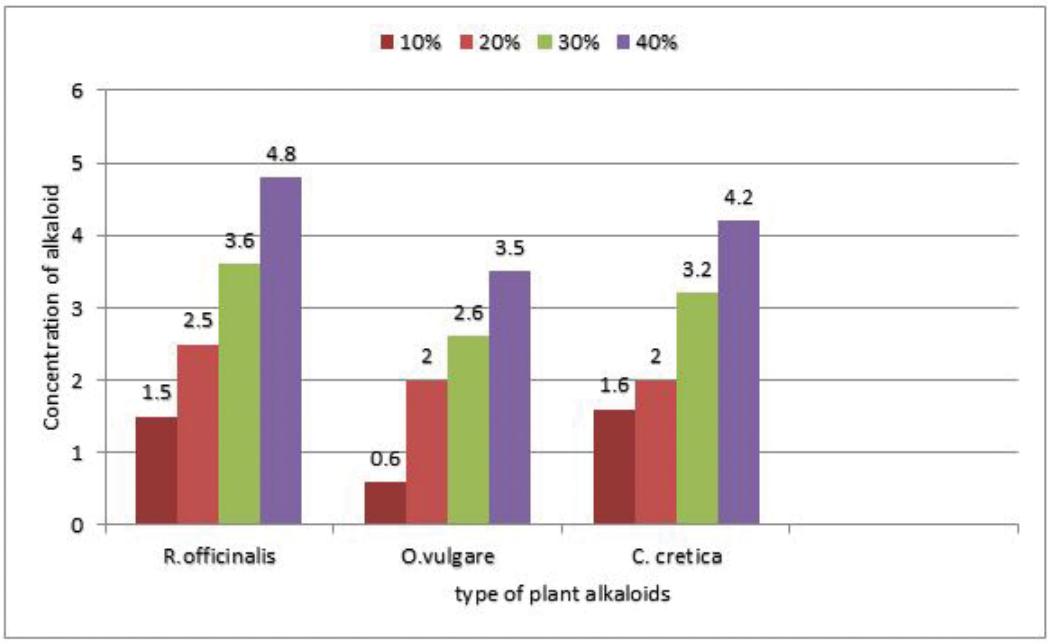

Figure 2: Effect of alkaloid concentration on *E.coli* growth

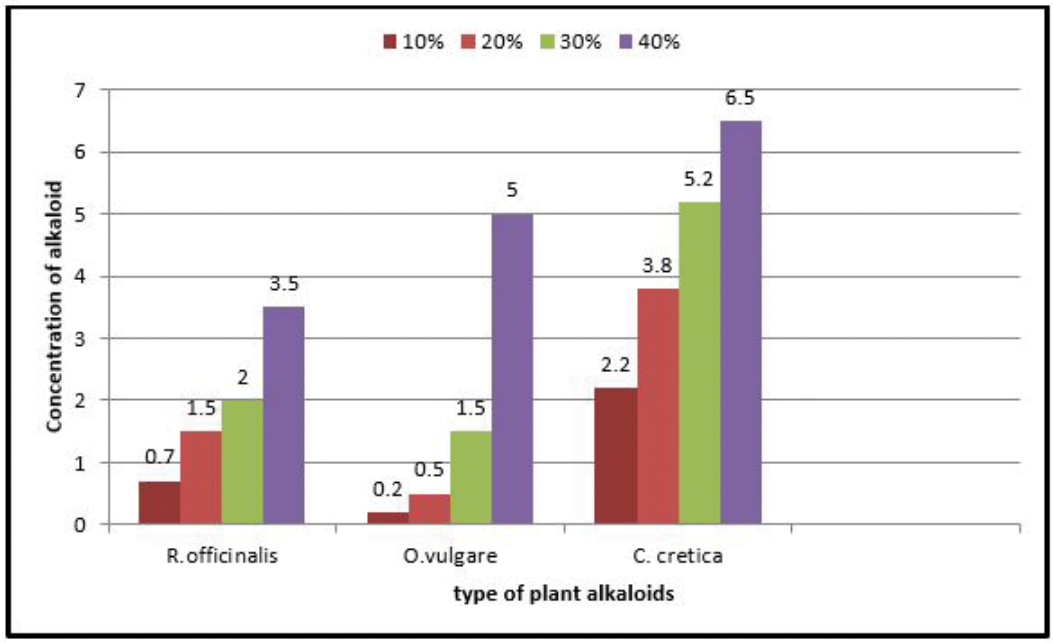

Figure 3: Effect of alkaloid concentration on *C.albicans* growth

Table 2: Effect of alkaloid extracts of different plants on microorganisms growth

Plant extract / Microorganisms	*R. officinalis*				*O. vulgare*				*C. cretica*			
	Concentration (%)											
	10	20	30	40	10	20	30	40	10	20	30	40
S. aureous	2	3.5	4	5	0.5	1.5	2	3	1	2.5	3	4.5
E.coli	1.4	2	3.5	4.6	0.5	2	2.5	3.2	1.5	2	3.5	4
C. albicans	0.6	1.5	2	4	0.2	0.5	2	2.5	2	4.5	5.5	6.8

Pv. 0.000

Conclusion

Results revealed a strong activity of plant alkaloid extracts on the growth of the microorganisms, *C. cretica* was most inhibitory effect on microorganisms than *R. officinalis* and *O. vulgare,* and this effect increase with concentration increasing. *S. aureus* was most affected than *E. coli* and *C. albicans*.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Pharmacy, University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines

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