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ANTIMICROBIAL ACTIVITY OF *NIGELLA SATIVA* AND *ZINGBER OFFICINALE*

BY

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SUMMARY

Aqueous and ethanolic extracts of *Zingber officinale* had antimicrobial activitiy against large number of pathogenic bacteria and fungi.

Aqueous extract of *Nigella sativa* possessed antibacterial activity in higher concentration. In addition all fractions of both *Zingber officinale* and *Nigella sativa* exhibited antimicrobial activities against various microorganisms.

Saponin and oil fractions of the test plants increased the anitbacterial acitivity of ampicillin against pyogenic microorganisms while resin fraction decreased that activity.

INTRODUCTION

Nigella sativa and *Zingber officinale* exhibit antimicrobial activities against large number of pathogenic microorganisms (Sinha et al 1977. saxena and Vyas 1986. Hassan et al., 1989 and Mascolo et al., 1989). Alkaloids, saponins and volatile oil (Essential oil) of the tested plants had antimicrobial activity (El-Fatatry et al. 1975. Rathee et al., 1982, Sauza et al., 1987 and Akgul 1989).

The present work was designed to investigate the antimicrobial activity of aqueous and ethanolic extracts of *Nigella sativa* and *Zingber officinale* as well as saponin, alkaloid and oil of *Nigella sativa* and saponin, resin and volatile oil of *Zingber officinal.* Moreover to study their therapeutic uses.

MATERIAL AND METHODS:

Preparation of the tested materials:

Aqueous and ehtanolic extracts were prepared by complete exhausion of *Nigella sativa* seeds and *Zingber officinale* rhizomes with distilled water or ethanol. The solvents were evaporated under reduced pressure till complete dryness. The crude extracts were disolved in tween 80 and then diluted with distilled water to 10-200 mg/ml.

Saponin of the tested plants was isolated and purified according to Basu and Rastorgi (1967) and Sandermann (1962). Extraction of resin (Baily and Pridhan 1962), alkaloid (Seiber, 1970 and Mangold 1969). Essential oil (Nursten 1970) was also carried. out The latter was identified according to (Kaldwey, 1969). Each isolate was dissolved in tween 80 and diluted with distilled water to concentrations of 10-1000 ug/ml.

The prepared concentrations were poured (0.2 ml in each pore) in plates and incubated at optimal temperature for suitable time of each tested organism.

Bacterial strains:

1- Gram positive bacteria.

Staphylococcus aureus, Streptococcus pyogens and *Corynebacterium pyogens.*

2- Gram negative bacteria:

Salmonella typhimurium, Escherichia coli, Psendomonas aeruginosa and *Klebsiella pneumoniae.*

Fungi:

A- Moulds:

Aspergillus niger, aspergillus flavus and *Penicillum spp.*

B- Dermatophytes:

Microsporum canis, Microsporum gypseum.

c- Yeast:

Candida albicans all the tested microorganisms were obtained by personal contact from the Department of Microbiology faculty of et, Med. Cairo University. Antibacterial activity of the graded concentrations of both aqueous and ethanolic extracts (10-200 mg/ml) and isolates principale (Alkaloid, saponin, resin and oils 10-1000 ug/ml) of *Nigella sativa* and *Zingber officinale* was studied by the pore method as described by Cooper and Woodman (1964) using nutrient agar No I and borer No 8 with dianeter (8 mm± 0.1 mm). The plates were incubated at 37°C for 18 hours. Antifungal activity of the studied extracts and isolates were studied *in vitro* as described by Robell and Lamb (1953) using sabaroud agar medium and the plates were incubated at 25°C for 3 days for mould species and 21 days for dermatophytes.

Nutrient agar No 1		Sabaroud agar
Peptone	6.0	glucose 40
Pancreatic digest of casine	4.0	peptone 10
Yeast extract	3.0	Agar 20
Beef extract	1.5	water to 1000
Dextrose	1.0	
Agar	15.0	
Distilled water to	1000.0	
PH 6.55±0.05		

Concomitant combiations of the studied fractions (1 mg/ml) with ampicillin (1 ug/ml) were tested against certain pathogenic bacteria (*Streptococcus pyogens, Salmonella typhimurium* and *Corynebacterium pyogens)* using the sa method.

RESULTS

The obtained data showed that aqueous a ethanolic extracts of *Zingber officinale* concentrations ranged from 10 to 200 mg exhibited a powerful antibacterial and antifu activity against, the tested pathogenic a nonpathogenic bacteria and fungi (Table 1-2). addtion, aqueous extract of *Nigella sa* possessed antibacterial activity in concentrati over 50 mg/ml especially against *Staphyloco aureus, Streptococcus pyogens, E. coli* a *Pseudomonas. aeruginosa*, while the ethan one had no effect. Both extracts of *Nigella sa* at the tested concentrations had no antifu activity *in vitro.*

Both antibacterial and antifungal activities of studied fractions of *Zingber officinale* and *Nig sativa* are recorded in tables (3-6). It is clear volatile oil of *Zingber officinale* is the most ac fraction against the tested bacterial and fu strains while saponin and resin fractions are l active than volatile oil (Table 3).

Saponin, alkaloid and fixed oil fraction of *Nig sativa* in concentrations over 50 ug/ml exhibi potent antibacterial activity especially aga *Streptococcus pyogens. E. coli* and *Salmon typhimurium* (Table 4) Furthermore sapo alkaloid and oil fractions of nigella sativa concentrations over lmg/ml had the abilit inhibit the growth of *Candida albic Aspergillus niger. penicillium* spec *Microsporum gypseum* and *Microsporum can* recorded in table (6).

Our findings showed that saponin and oil fra of *Nigella sativa* and *Zingber officinale* incre the antibacterial activity of ampicillin in v against *Streptococcus pyogens, Salmo typhimurium* and *Corynebacterium pyogens* the other hand resin fraction of the tested p decreased the antibacterial activity of ampicil

DISCUSSION

Table 1: *In vitro* antibacterial activity of ethanolic and aqueous extracts of *Zingber officinale*.

Microorganism	Concentration mg/ml	Diameter of the ihibition Zone in (mm) ±S.E.	
		Ethanolic	Aqueous
Staphylococcus aureus	10	--	--
	25	12.0 ± 0.50	--
	50	13.5 ± 0.67	--
	100	15.0 ± 0.50	13.30 ± 0.67
	200	17.67 ± 0.33	17.67 ± 0.88
Streptococcus progens	10	--	--
	25	12.5 ± 0.29	--
	50	14.0 ± 0.29	--
	100	17.0 ± 0.58	--
	200	20.67 ± 0.67	15.67 ± 0.67
E.Coli	10	--	--
	25	--	--
	50	--	--
	100	--	12.33 ± 0.33
	200	--	15.63 ± 0.57
Salmonella Typhimurium	10	--	--
	25	--	--
	50	11.33 ± 0.33	--
	100	13.0 ± 0.00	10.00 ± 0.00
	200	14.67 ± 0.67	14.67 ± 0.33
Klebsiella pneumoniae	10	--	--
	25	12.67 ± 0.17	--
	50	14.83 ± 0.17	--
	100	16.67 ± 0.58	12.33 ± 0.33
	200	21.20 ± 0.49	18.32 ± 0.88
Pseudomonus aeruginosa	10	--	--
	25	--	--
	50	--	--
	100	--	12.33 ± 0.33
	200	--	15.67 ± 0.67
Corynebacterium progens	10	--	--
	25	13.83 ± 0.44	--
	50	15.50 ± 0.50	--
	100	17.0 ± 0.58	--
	200	20.67 ± 0.67	--

Table (2): *In vitro* anitfungal activity ethanolic and aqueous extracts of *Zingber officinale*.

Microorganism	Concentration mg/ml	Diameter of the in ihibition Zone in (mm) ± S.E.	
		Ethanolic	Aqueous
Candida albicans	10	--	--
	25	--	--
	50	11.66 ± 0.33	--
	100	14.00 ± 0.58	11.00 ± 0.58
	200	15.67 ± 0.67	17.33 ± 0.67
Asperigillus niger	10	--	--
	25	--	--
	50	--	--
	100	11.67 ± 0.88	--
	200	16.00 ±0.58	--
Asperigillus falvous	10	--	--
	25	--	--
	50	11.33 ± 0.67	--
	100	14.00 ± 0.58	--
	200	17.00 ± 0.58	--
Pencillium Spp	10	--	--
	25	--	--
	50	--	13.00 ± 0.33
	100	11.33 ±0.33	18.00 ± 0.58
	200	14.00 ± 0.58	23.33 ± 0.88
Microsporum gypseum	10	--	--
	25	--	--
	50	--	--
	100	--	15.33 ± 0.33
	200	--	18.67 ± 0.67
Microsporum canis	10	--	--
	25	--	--
	50	--	12.67± 0.33
	100	--	18.67 ± 0.67
	200	--	22.33 ± 1.20

Table(3): *In vitro* antibacterial activity of *Zingber officinale* isolated fractions.

Microorganism	Concentration mg/ml	Diameter of the ihibition Zone in (mm) ±S.E.		
		Saponin	Resin	Volatile oil
Staphylococcus aureus	10	--	--	--
	50	--	--	--
	100	--	--	12.0 ≠ 0.57
	1000	--	--	14.5 ± 0.47
	5000	--	--	15.0 ± 0.00
Streptococcus pyogens	10	--	--	--
	50	--	--	11.7 ± 0.16
	100	12.2 ± 0.33	10.5 ± 0.16	13.0 ± 0.00
	1000	15.5 ± 0.57	13.2 ± 0.33	15.5 ± 0.28
	5000	17.0 ± 0.33	15.0 ± 0.00	19.0 ± 0.50
E.Coli	10	--	--	11.0 ± 0.00
	50	--	--	13.0 ± 0.16
	100	11.5 ± 0.47	11.0 ± 0.00	14.5 ± 0.28
	1000	13.5 ± 0.57	13.5 ± 0.47	17.2 ± 0.33
	5000	15.2 ± 0.88	17.0 ± 0.00	20.0 ± 0.57
Salmonella Typhimurium	10	--	--	--
	50	--	--	11.2 ± 0.13
	100	--	10.5 ± 0.16	13.0 ± 0.57
	1000	10.5 ± 0.16	13.3 ± 0.33	16.3 ± 0.33
	5000	13.0 ± 0.57	15.0 ± 0.57	18.0 ± 0.00
Kiebsiella pneumoniae	10	--	--	--
	50	--	--	--
	100	11.0 ± 0.10	--	--
	1000	14.5 ± 0.47	--	12.0 ± 0.47
	5000	16.2 ± 0.33	--	14.2 ± 0.50
Pseudomonus aeruginosa	10	--	--	--
	50	--	--	--
	100	--	--	11.5 ± 0.15
	1000	--	10.5 ± 0.15	13.0 ± 0.28
	5000	--	12.0 ± 0.00	14.2 ± 0.33
Corynebacterium pyogens	10	--	--	10.3 ± 0.15
	50	--	--	12.2 ± 0.33
	100	--	--	13.2 ± 0.33
	1000	11.5 ± 0.28	--	15.7 ± 0.47
	5000	13.0 ± 0.15	--	17.0 ± 0.00

Table(4): *In vitro* antibacterial activity of *Nigella sativa* isolated fractions

Microorganism	Concentration mg/ml	Diameter of the ihibition Zone in (mm) ±S.E.		
		Saponin	Alkaloid	Volatile oil
Staphylococcus aureus	10	--	--	--
	50	--	--	--
	100	12.0 ± 0.00	--	11.5 ± 0.28
	1000	15.0 ± 0.28	10.5 ± 0.16	14.5 ± 0.33
	5000	17.3 ± 0.17	12.0 ± 0.16	17.0 ± 0.00
Streptococcus pyogens	10	--	--	--
	50	11.3 ± 0.16	--	10.3 ± 1.60
	100	13.0 ± 0.16	--	12.5 ±0.00
	1000	16.3 ± 0.33	12.7 ± 0.78	14.0 ± 0.16
	5000	19.5 ± 0.57	14.5 ± 0.16	19.0 ± 0.28
E.Coli	10	--	--	--
	50	11.3 ± 0.33	11.5 ± 0.00	--
	100	13.0 ± 0.10	12.0 ± 0.16	12.0 ± 0.00
	1000	16.6 ± 0.28	14.5 ± 0.28	15.5 ± 0.57
	5000	18.0 ± 0.16	17.2 ± 0.16	18.7 ± 0.68
Salmonella Typhimurium	10	--	--	--
	50	--	--	--
	100	--	10.5 ± 0.00	11.0 ± 0.00
	1000	12.3 ± 0.33	13.3 ± 0.16	13.2 ± 0.16
	5000	14.0 ± 0.16	16.0 ± 0.28	15.5 ± 0.47
Klebsiella pneumoniae	10	--	--	--
	50	--	--	--
	100	--	--	12.2 ± 0.16
	1000	--	10.5 ± 0.16	14.5 ± 0.47
	5000	12.0 ± 0.00	12.7 ± 0.60	17.2 ± 0.33
Pseudomonus aeruginosa	10	--	--	--
	50	--	--	11.5 ± 0.28
	100	10.0 ± 0.47	--	13.0 ± 0.16
	1000	13.0 ± 0.00	12.3 ± 0.16	16.5 ± 0.97
	5000	14.5 ± 0.28	13.0 ± 0.00	20.0 ± 0.57
Corynebacterium pyogens	10	.	--	--
	50	.	--	--
	100	11.0 ± 0.00	12.0 ± 0.16	12.0 ± 0.28
	1000	13.0 ± 0.16	14.0 ± 0.57	14.5 ± 0.16
	5000	14.3 ± 0.33	15.3 ± 0.33	17.0 ± 0.33

Table(5): *In vitro* antibacterial activity of *Zingber officinale* isolated fractions.

Microorganism	Concentration mg/ml	Diameter of the in ihibition Zone in (mm) ± S.E. ±S.E.		
		Saponin	Resin	Volatile oil
Candida albicans	1	11.0 ± 0.00	--	12.2 ± 0.33
	5	14.7 ± 0.57	11.9 ± 0.73	17.4 ± 0.41
	10	18.2 ± 0.33	14.2 ± 0.33	21.0 ± 1.50
	25	20.0 ± 0.28	17.2 ± 0.33	25.9 ±0.57
	50	23.7 ± 0.57	20.0 ± 0.00	29.2 ± 0.33
Asperigillus niger	1	--	--	--
	5	--	--	--
	10	--	--	10.5 ± 0.10
	25	--	12.2 ± 0.33	13.2 ± 0.33
	50	--	15.0 ± 0.00	17.5 ± 0.20
Asperigillus fulvous	1	--	--	--
	5	--	--	--
	10	--	--	11.9 ± 0.57
	25	10.0 ± 0.00	11.2 ± 0.30	14.3 ± 0.70
	50	12.5 ± 0.10	13.5 ± 0.27	17.0 ± 0.00
Pencillium Spp	1	--	--	--
	5	10.0 ± 0.00	--	11.2 ± 0.27
	10	12.5 ± 0.57	--	13.2 ± 0.33
	25	14.2 ± 0.33	11.5 ± 0.50	17.7 ± 0.57
	50	17.3 ± 0.39	13.1 ± 0.43	22.0 ± 1.50
Microsporum gypseum	1	--	--	--
	5	10.5 ± 0.30	--	12.1 ± 0.30
	10	12.0 ± 0.00	10.5 ± 0.01	13.0 ± 0.50
	25	14.2 ± 0.33	12.0 ± 0.30	16.8 ± 0.44
	50	16.8 ± 0.73	14.5 ± 0.00	18.7 ± 0.61
Microsporum canis	1	--	--	11.0 ± 0.57
	5	11.0 ± 0.00	--	14.0 ± 0.00
	10	13.2 ± 0.33	11.5 ± 0.57	18.2 ± 0.33
	25	17.9 ± 0.50	14.3 ± 0.44	25.7 ± 0.33
	50	23.2 ± 0.33	17.0 ± 0.00	29.6 ± 0.88

Table(6): *In vitro* antifungal activity of *Nigella sativa* isolated frations.

Microorganism	Concentration mg/ml	Diameter of the inhibition Zone in (mm) ± S.E. ±S.E.		
		Saponin	Alkaloid	Fixed oil
Candida albicans	1	12.5 ± 0.17	11.5 ± 0.90	13.0 ± 0.16
	5	15.2 ± 0.33	13.5 ± 0.44	17.2 ± 0.33
	10	18.7 ±0.57	15.2 ± 0.33	21.7 ± 0.57
	25	21.0 ± 0.67	17.9 ± 0.47	30.1 ± 0.88
	50	25.9 ± 0.83	21.2 ± 0.5	39.5 ± 1.87
Asperigillus niger	1	--	--	12.5 ± 0.10
	5	--	--	14.6 ± 0.16
	10	10.7 ± 0.13	11.5 ± 0.88	19.8 ± 0.33
	25	12.5 ± 0.30	13.2 ± 0.43	25.2 ± 0.88
	50	15.0 ± 0.50	11.5 ± 0.27	33.7 ± 0.67
Asperigillus fulvous	1	--	--	11.5 ± 0.33
	5	10.7 ± 0.30	12.0 ± 0.10	13.5 ± 0.44
	10	12.7 ± 0.44	14.2 ± 0.33	16.5 ± 0.51
	25	13.7 ± 0.80	17.9 ± 0.57	19.7 ± 0.56
	50	15.2 ± 0.33	23.7 ± 0.88	25.0 ± 0.67
Penicillium Spp	1	--	--	--
	5	10.5 ± 0.30	12.0 ± 0.10	12.5 ± 0.16
	10	12.4 ± 0.44	13.2 ± 0.33	15.4 ± 0.28
	25	13.5 ± 0.33	15.9 ± 0.88	19.5 ± 0.33
	50	17.0 ± 0.57	19.5 ± 0.70	22.7 ± 0.88
Microsporum gypseum	1	11.5 ± 0.17	12.5 ± 0.70	14.0 ± -.16
	5	13.1 ± 0.37	14.2 ± 0.33	18.5 ± 0.33
	10	16.1 ± 0.27	18.6 ± 0.90	20.0 ± 0.28
	25	21.9 ± 0.44	22.7 ± 0.64	27.2 ± 0.57
	50	27.0 ± 0.78	29.2 ± 0.88	35.3 ± 0.58
Microsporum canis	1	--	--	11.0 ± 0.00
	5	12.1 ± 0.17	13.5 ± 0.37	13.2 ± 0.33
	10	14.0 ± 1.00	15.7 ± 0.80	16.3 ± 0.16
	25	16.0 ± 0.64	18.2 ± 0.33	21.0 ± 0.88
	50	20.6 ± 0.84	22.7 ± 0.88	32.0 ± 0.67

Table(7): *In vitro* study on the activity of ampicillin (1 ug/ml), isolated tested plant fractions (1 mg/ml) and their combination aqainst three bacterial strains.

Plant	Isolate	Strain	Diameter of inihibition Zone		
			Ampicillin	Fraction	Ampicillin + Fraction
Zingber officinale	*Saponin*	Strept.	24.1 ± 0.3	28.7 ± 0.7	35.9 ± 0.3
		Sal.	12.5 ± 0.0	14.1 ± 0.2	14.1 ± 0.3
		Coryne.	47.7 ± 0.3	40.1 ± 0.9	50.5 ± 0.9
	Volatile oil	Strept.	16.0 ± 0.0	18.0 ±0.1	22.3 ± 0.2
		Sal.	13.3 ± 0.3	28.0±0.0	20.1 ± 0.3
		Coryne.	45.3 ± 0.7	40.0 ±0.3	46.7 ± 0.9
Nigella stiva	*Saponin*	Strept.	25.7 ± 0.3	40.0 ± 0.3	40.0 ± 0.3
		Sal.	12.0 ± 0.0	14.0 = 0.1	16.5 ± 0.9
		Coryne.	45.7 ± 0.6	44.0 ± 0.3	-47.5 ± 0.9
	Alkaloid	Strept.	26.0 ± 0.2	40.7 ± 0.3	45.2 ± 1.5
		Sal.	13.5 ± 0.7	18.2 ± 0.2	16.1 ± 0.1
		Coryne.	44.3 ± 0.9	40.3 ± 0.5	40.3 ± 0.4
	Fixed oil	Strept.	26.7 ± 0.3	25.7 ± 0.3	25.6 ± 0.4
		Sal.	13.1 ± 0.2	14.0 ± 0.0	--
		Coryne.	44.7 ± 0.8	45. 5 ± 0.7	45.6 ± 0.3

Streptococcus pyogens.
Salmonella typhimurium.
Corynebacterium pyogens.

The obtained data proved that aqueous and ethanolic extracts of *Zingber officinale* exhibit a potent antimicrobial activity in low concentrations. This effect may be attributed to the presence of saponin, resin and volatile oil as it has been previously recorded by Kaur and Sinha (1982) and Sauza et al., (1987). In addition, the antimicrobial activity of both extracts of *Zingber officinale* recorded here was similar to that finding recorded by Gugnani and Ezenwanze (1985),and Mascolo et al (1989).

Our findings showed that saponin, resin and volatile oil fractions of *Zingber officinale* exhibited a more potent antibacterial and antifungal activities against several pathogenic and nonpathogenic bacterial and fungal strains *in vitro*. The present antimicrobial activities of equeous, ethanolic extracts and fractions was in consistence with the findings recorded by El-Fatatry et al (1975). Rathee et al., (1982), Saxena and Vays (1986) and Hassan et al (1989).

The ability of aqueous and ethanolic extracts as well as the tested fractions of *Zingber officinale* to inhibit the growth and multiplication of various pathogenic fungi and bacteria, encouraged the authors to use these materials as a chemotherapeutic agents in treatment of different diseases as ring worm, wound infection and diahrriea in veterinary medicine but these trials still need further field application study to demonestrate the ability of these extracts and fractions for treatment of systemic infections and also to determine the proper form and dose for use on larg scale.

The obtained results demonstrated that aqueous extract of *Nigella sative* seeds has a potent intibacterial activity against pathogenic bacteria. In addition, alkaloids, saponin and fixed oil fractions of *Nigella sativa* seeds exhibit very strong antibacterial and antifungal activities so they can be used effectively in therapy in the form

of solution.

The present data showed that saponin and oil fractions of *Nigella sativa* and *Zingber Officinale* increased the antibacterial activity of ampicillin. This effect may be attributed to a tested synergestic action with amipicillin. On the other hand, resin fraction of the tested plants decreased the antibacterial effect of ampicillin against pathogenic bacteria. This may be referred to the formation of less active compound during their combination resulted in antagonestic effect, therfore this fraction must be used alone as antibacterial substance.

Nearly the same synerge-stic activity between *Nigella sativa* seeds extracts and streptomycin and gentamycin antibiotics was recorded by Hanafy and Hatem (1991).

Conclusively the extracts and isolates of *Zingber Officinale* and *Nigella sativa* are effective against pathogenic bacteria and fungi and they are safer than other antibacterials of synthetic origin.

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