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SOME PHARMACODYNAMIC EFFECTS AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF CERTAIN PLANTS USED IN EGYPTIAN FOLK MEDICINE

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SUMMARY

In this study ten essential oils were prepared from their respective natural sources namely: *Cinnamomum cassia* barks (cassia). *Curcuma* sp. rhizomes (curcuma), *Elettaria cardamomum* fruits (cardamom), *Eugenia caryophyllus* flower buds (clove), *Origanum syriacum* herb (za'tar), Origanum *majoranum* herb (sweet majoram). *Piper nigrum* fruits (black pepper), *Rosmarinus officinalis* leaves (rosemary), *Salvia triloba* L. (maryamiyah) and Zingiber *officinalis* rhizomes (ginger). Their percentage yields, specific gravities and refractive indices were determined.

The essential oils ginger and black pepper markedly stimulated the motility of rabbit's jejunum at concentrations more than 47.2 and 70.0µg/ml respectively. While the other essential oils possessed intestinal antispasmodic effects on isolated rabbit's jejunum. All tested oils produced inhibitory effect on the uterus of the pregnant rat. Concerning the antimicrobial study, the sensitivity of nineteen microbes (six Grampositive and six Gram-negative bacteria, and seven fungi), to tested essential oils was investigated at different concentrations (10, 25,50, 100 and 200 mg/ml). Cassia oil showed a pronounced antibacterial activity against all tested bacteria *in vitro*. Essential oils of cardamom, curcuma, za'tar, sweet majoram and maryamiyah showed a moderate antibacterial activity. Results of the antifungal study showed that cassia and clove essential oils caused a pronounced antifungal activity *in vitro* and *in vivo*. Curcuma, za'tar and sweet majoram showed a marked activity against *Trichophyton mentagrophytes*. za'tar showed also a moderate inhibitory activity against the other tested fungi.

INTRODUCTION

Plants which contain essential oils are commonly used as spices, aromatic, carminative, stimulant, tonic, antispasmodic and stomachic (Chopra et. al., 1956). Cardamom, cassia, clove curcuma, ginger and black pepper are generally used for culinary purposes and in confectionery (Purselgove et. al., 1981). Curcuma is externally applied to sprains and wounds. While black pepper is used as rubefacient and as a local application for relaxed sore-throat and skin diseases. The friuts are used as aphrodisiac, diuretic, emmenagogue and galactagogue (Chopra et. al., 1956). They are also used as tonic and stimulant to digestive functions (Purselgove et. al., 1981 and Boulos, 1983). Mixed with oil, it is used by rubbing in treatment of acne and leprosy. Ginger is used as stimulant to the gastrointestinal tract and as a rubefacient, counter irritant and aphrodisiac. Cardamom is used as diuretic and has aphrodisiac properties (Purselgove et.. al., 1981). Clove acts as antispasmodic. It is used in dentistry and as diuretic (Boulos, 1983). The essential oil of rosemary is incorporated in ointments for rheumatism, eczema, ulcers and wounds. It is also used as rubefacient and insecticide (Boulos, 1983).

Although essential oils are generally used as flavouring agents and to impart fragrance in the pharmaceutical and cosmetic industries, they have

been widely reported to possess antibacterial activity (Soliman et al., 1994). The antimicrobial activity of essential oils of *Salvia triloba* L. (Mahmoud et al. 1992) and rosemary (Soliman et al., 1994) was previously reported.

The purpose of this study was to evaluate the activity of the essential oils of the mentioned plants on rabbit's jejunum and pregnant uterus preparations. Moreover, their antimicrobial activities against representatives of bacteria, yeasts, moulds and dermatophytes commonly isolated from man and animals *in vitro* as well as in experimentally infected guinea pigs were also investigated.

EXPERIMENTAL

Plant Material

Cinamomum cassia Blume bark, (lauraceae), *Curcuma* sp. rhizomes, (Zingiberaceae) *Elettaria cardamomum* Maton fruits (Zingiberaceae). *Eugenia caryophyllus* (Spreng.) Sprague flower buds (Myrtaceae), *Piper nigrum* L. fruits (Piperaceae) and Zingiber *officinale* Roscoe rhizomes (Zingiberaceae) were purchased from the local market at Cairo. They were kindly verified by Prof. F. M. Soliman, Department Pharmacognosy, Faculty of Pharmacy, Cairo University. *Origanum majorana* L.herb and *Rosmarinus officinalis* L. leaves (Labiatae) were obtained from the Experimental Station of Medicinal Plants (ESMP), Department of Pharmacognosy, Faculty of Pharmacy, Cairo University at Giza. *Origanum syriacum.* L. herb and *Salvia* leaves (Labiatae) were obtained from El-Arish, Sinai. The identity of *Origanum majorana* L., *O. syriacum* L., *Salvia triloba* L. and *Rosmarinus officinalis* L. was kindly confirmed by Dr. Nahed El-Husseini, Department of Botany, Faculty of Science, Cairo University. Herbarium specimens are kept at the Department of Pharmacognosy. Faculty of Pharmacy, Cairo University.

Preparation of essential oils:

The essential oil of each sample was prepared (Egyptian Pharmacopoeia, 1984) by hydrodistillation of the dried plants, except rosemary which was used fresh. The volatile distillate in each case was separated, and submitted for biological screening. Their percentage yields, specific gravities and refractive indices were determined (Table 1).

Preparation of solutions for pharmacological investigation:

Each essential oil was dissolved in a known volume of diluted (10%) aqueous Tween 80. These emulsions were used for all pharmacodynamic assays.

I. Pharmacological Effects on Isolated Preparations:

a- Intestinal Motility:

Experiments were carried out using a glass jar bath apparatus of 50 ml capacity organ bath. A piece of rabbit's jejunum was suspended into the inner vessel of the apparatus containing Tyrode's solution at 37°C as described by the method of Perry (1968). The normal motility of isolated rabbit's jejunum was recorded. Graded concentrations of each essential oil was added to the organ bath and their responses were then recorded and EC_{50} values (concentration inhibiting or stimulating muscle contraction by 50%) were also determined. Trials were also made to determine the site of action for each oil on the isolated rabbit's jejunum.

b- Uterine Motility:

The method described by Robella et al. (1925) was used . Uteri of Wistar rats at early pregnancy were isolated and mounted in a 50ml capacity organ bath containing oxygenated Dale's solution at 38°C. Normal rhythmic contractions were recorded using Harvard apparatus. Graded concentrations of each tested oil were added to the bath and their effects were demonstrated. The EC_{50} values for each oil were also calculated.

II. Antimicrobial Activity:

Microorganisms used for the antimicrobial screening:

a. Bacteria

1- Gram-positive: *Staphylococcus aureus, Staphylococcus* methicillin resistant, *Streptococcus* types B and D, *Streptococcus agalactiae*, and *Bacillus subtilis.*

2- Gram-negative: *Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae, Haemophilus influenzae* and *Proteus mirabilis.*

b. Fungi:

Candida albicans, Cryptococcus neoformans, (yeasts), *Penicillium* sp., *Aspergillus niger* and *A. fumigatus* (molds), *Microsporum gypseum* and *Trichophyton mentagrophytes* (dermatophytes).

1- Antibacterial Activity:

The sensitivity of the previously mentioned bacteria to each essential oil was carried out *in vitro* by the agar diffusion sensitivity test using the bore method as described by Cooper and Woodman (1946). Different concentrations (1,10,25,50,100 and 200mg/ml) from each tested essential oil were prepared in Twen 80 (10%). Eight bores were made by a sterile metallic borer (8mm innerdiameter) at adequate distances. Five plats were made for each tested concentration of essential oil. The inhibitory zones were measured (mm) and calculated as means ± S.E. M. (Stantared Error Mean).

2- Antifungal activity:

All essential oils tested were sterilized through Seitz filter and used directly after dilution with agar for testing their antifungal activities *in vitro.* For studying the antimycotic effects *in vivo,* the essential oils were directly prepared in 20% concentration in lanolin, sterilized and used as dressing for the infected skin areas.

A. *In vitro* study:

The antifungal activity of the tested oils was studied *in vitro* as described by Robell and Lamb (1953) and Abdel-Naby (1985). The Sabouraud agar with and without oils (at concentrations of 2.5, 5, 10 and 20%) were inoculated in a zigzag form (4 arms) with the test fungi. All plates were incubated at 30°C for 7 days with yeast and molds and 21 days with dermatophytes and the growth of fungi was evaluated in comparison with the control by one + for each arm.

B. *In vivo* study:

Fifty guinea pigs were divided into two equal groups of 25 animals each. Animals in each group were experimentally infected with either *Microsporum gypseum* or Trichophyton mentagrophytes culture suspenison using the scarification method (Amer, et. al., 1985). Five infected animals from each group were left non treated as control, whereas the others were treatedwith za'tar, sweet majoram, cassia and clove oils. Each oil was used in 20%concentration in lanolin for treatment of five animals from each group. Treatment of infected animals was done by local application of the prepared onitment of each oil twice daily, for 20 days and the percent of cure for each sample was determined. The curative effects were confirmed by microscopic examination and culturing of samples obtained from the treated and non-treated skin lesions.

RESULTS AND DISCUSSION

The percentage yields of the essential oils of black pepper, cardamom, cassia, clove, curcuma, ginger, rosemary, maryamiyah, sweet majoram and za'tar were 2.40, 4.01, 0.52, 4.90, 2.20, 1.28, 0.40, 1.45, 1.25 and 4.50 respectively. The specific gravities and refractive indices were also determined (Table 1). These data are significant criteria for the identity and purity of these oils.

Effect of isolated organs:

This study revealed that the essential oils of ginger and black pepper at concentrations more than 47.2 and 70.0µg/ml bath markedly stimulated the motility of isolated rabbit's jejunum

respectively, as shown in Fig. (1). The essential oils of cardamom, curcuma, za'tar, sweet majoram, cassia, maryamiyah, clove and rosemary at concentrations more than 24.4, 22.1, 30.0, 55.0, 87.6, 26.2, 67.0 and 41.7µg/ml bath, markedly inhibited the motility of isolated jejunum, respectively as shown in Fig (1). The degre of stimulation or inhibition was found to be in harmony with the concentration added, since larger concentrations completely stimulated or relaxed the intestinal strip. Moreover, addition of acetylcholine (1.1×10^{-7} m mol/L) produced stimulation of the intestinal motility in the presence of cardamom, curcuma, za'tar, sweet majoram, cassia, maryamiyah, clove and rosemary oils. Each of the essential induced its effect directly on the muscle except cassia oil (beside its effect on muscle) and rosemary. Nicotine sulphate (10 µg/ml) produced no effect of the intestinal motility in the presence of cassia and rosemary oils (Fig. 2 and table II). These trials proved that the site of action of these essential oils is neither cholinergic nor ganglionic (except rosemary and cassia oils) in nature and may be directly on the muscle of rabbit's jejunum.

The calculated EC_{50} values and their ranges were shown in Table II.

The stimulating effect of black pepper and ginger on the intestine, which is in agreement with that previously reported (Purselgove et al., 1981 and Boulos, 1983) is possibly due to their essential oil contents.

All the essential oils tested produced an inhibitory effect on the rat uterus at pregnant stage (Table III and Fig. 3) This effect was found to be in harmony with the concentrations used, since larger concentration caused complete relaxation of the uterus. The calculated EC_{50}., values and their ranges were shown in Table III.

Hence all the tested essential oils exhibited an inhibitory effect on the uterus at pregnant stage they can be used safely for treatment of dysmenorhea and uterine spasms during pregnancy if it is proved to produce uterine relaxation in intact animals. This needs further *in vivo* experiment.

Table (I): Percentage yields, specific gravities and refractive indices of essential oils

Essential Oil	Percentage yield *	Specific gravity *	Refractive index *
Black pepper	2.40	0.8872	1.48046
Cardamom	4.01	0.9329	1.46245
Cassia	0.52	1.0355	1.59832
Clove	4.90	1.0290	1.52323
Curcuma	2.20	0.9756	1.50943
Ginger	1.28	0.9417	1.49132
Rosemary	0.40	0.9205	1.47141
Maryamiyah	1.45	0.9394	1.46439
Sweet majoram	1.25	0.9891	1.46748
Za'tar	4.50	0.9249	1.48833

* Average of three determinations.

Table (IV): Antibacterial activity of different concentrations of tested essential oils.

Tested oils	Concentrations (mg/ml)	Diameter of inhibition zones in mm (Mean ± S. E.)											
		Gram-positive strains						Gram-negative strains					
		Staph. aureus	Staph. Methicillin Resistant	Strept. type (B)	Strept. type (D)	Strept. galactiae	Bacillus subtilis	Escherichia coli	Salm. typhimurium	Pseudomonas aeruginosa	Klebsiella pneumoniae	Haemophilus influenzae	Proteus mirabilis
Cardamon	1	--	--	--	--	--	--	--	--	--	--	--	--
	10	--	--	--	--	--	--	--	--	--	--	--	--
	25	--	--	--	--	--	--	--	--	14.0±0.45	--	--	--
	50	--	--	--	--	--	--	--	--	16.0±0.32	--	--	--
	100	--	--	--	--	--	--	--	--	18.0±0.32	--	--	13.4±0.25
	200	--	--	--	--	--	--	--	--	20.2±0.37	--	--	15.0±0.32
Curcuma	1	--	--	--	--	--	--	--	--	11.0 ±0.32	--	--	--
	10	--	--	--	--	--	--	--	--	12.6±0.25	--	--	--
	25	--	--	--	--	--	--	--	--	14.6±0.25	--	--	--
	50	--	--	--	--	--	--	--	--	16.4±0.25	--	--	--
	100	--	--	--	--	--	--	--	--	17.8±0.37	--	--	--
	200	--	--	--	--	--	--	--	--	20.02±0.20	--	--	--
Za'tar	1	--	--	--	--	--	--	--	--	--	--	--	--
	10	--	--	--	--	--	--	--	--	--	--	--	--
	25	--	13.4±0.51	--	--	--	--	--	--	--	--	--	--
	50	--	20.2±0.20	--	--	--	--	--	--	16.8±0.37	--	--	--
	100	--	27.0±0.71	--	--	--	--	11.4±0.25	--	20.6±0.40	--	--	--
	200	--	28.8±0.37	--	--	--	--	13.4±0.25	--	27.6±0.51	--	--	--
Cassia	1	--	--	--	--	--	--	--	--	--	--	--	--
	10	--	--	--	--	--	--	--	14.6±0.25	16.6±0.25	16.4±0.51	--	--
	25	--	17.0±0.32	20.2±0.37	12.6±0.40	--	--	--	24.4±0.51	18.6±0.40	20.8±0.25	16.2±0.37	12.6±0.25
	50	--	20.2±0.20	30.6±0.40	24.0±0.32	12.2±0.37	--	17.0±0.32	28.8±0.37	25.4±0.25	30.0±0.32	27.0±0.32	16.0±0.32
	100	12.0±0.45	28.6±0.25	36.4±0.51	30.0±0.32	15.0±0.32	--	19.4±0.40	31.2±0.37	30.6±0.40	39.2±0.37	36.0±0.32	18.0±0.32
	200	23.8±0.37	35.0±0.32	40.8±0.37	40.0±0.63	19.4±0.40	15.0±0.3	29.0±0.32	39.6±0.40	38.6±0.40	45.4±0.51	39.8±0.49	20.0±0.32
Maryamiyah	1	--	--	--	--	--	--	--	--	--	--	--	--
	10	--	--	--	--	--	--	--	--	--	--	--	--
	25	--	--	--	--	--	--	--	--	10.2±0.20	--	--	--
	50	--	--	--	--	--	--	--	--	12.2±0.58	--	--	--
	100	--	--	--	--	--	--	--	--	17.2±0.37	--	--	--
	200	--	--	--	--	14.0±0.45	--	--	--	21.0±0.45	--	--	--

Ginger, black pepper, sweet majoram and clove oils have no antibacterial activity.

Table (V): Effect of tested essential oils on the growth of some fungi.

Types of oil	Concentrations of oil (%)	Candida albicans	Cryptococcus neoformans	Penicilium spp.	Aspergillus niger	Aspergillus fumigatus	Microsporum gypseum	Trichophyton mentagrophytes
Control plates		++++	++++	++++	++++	++++	++++	++++
Curcuma	2.5	++++	++++	++++	++++	++++	++++	+++-
	5	++++	++++	++++	++++	++++	++++	++--
	10	++++	++++	++++	++++	++++	++++	+---
	20	++++	++++	++++	++++	++++	++++	----
Za'tar	2.5	++++	++++	++++	++++	++++	++++	+++-
	5	+++-	+++-	++++	++++	++++	++++	++--
	10	++--	++--	+++-	+++-	+++-	+---	+---
	20	+---	+---	+---	+---	+---	+---	----
Sweet majoram	2.5	++++	++++	++++	++++	++++	++++	++++
	5	++++	++++	++++	++++	++++	++++	+++-
	10	++++	++++	++++	++++	++++	++++	++--
	20	++++	++++	++++	++++	++++	++++	+---
Cassia	2.5	+++-	++--	+---	+---	----	----	----
	5	++--	+---	----	+---	----	----	----
	10	+---	+---	+---	+---	+---	+---	+---
	20	----	----	----	----	----	----	----
Clove	2.5	++++	++++	+++-	+---	++--	++--	+++-
	5	+++-	+++-	++--	+---	+---	+---	++--
	10	++--	++--	+---	----	----	+---	----
	20	+---	+---	+---	----	----	+---	----

++++ = nomal growth, +++- = slight inhibition, ++-- moderate inhibition, +--- = high degree of inhibition, ---- = complete inhibition

Essential oils of ginger, black pepper, maryamiyah and cardamom have no antifungal activity.

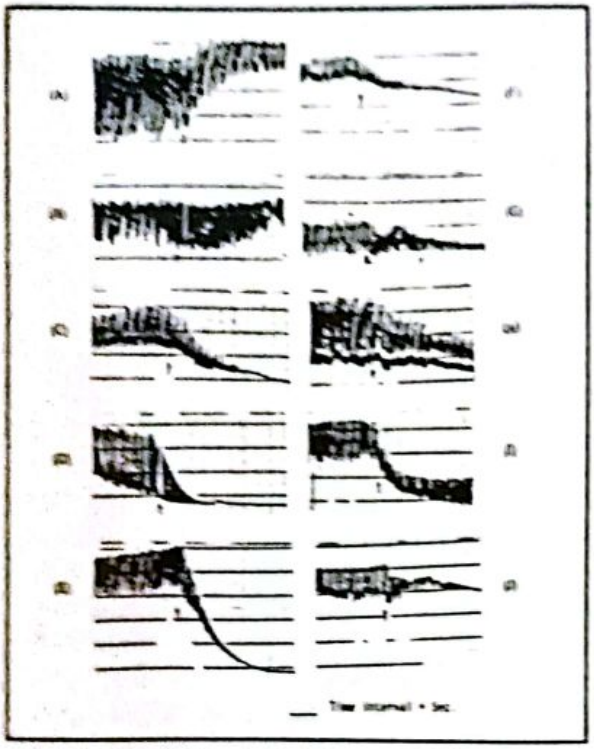

Fig. 1: Effect of 100 µg/ml of Ginger (A), Black pepper (B), Cardamom (C), Curcuma (D), Za'tar (E), Sweet majoram (F), Cassia (G), Maryamiyah (H), Clove (I) and Rosemary oil (J) on isolated rabbit's jejunum.

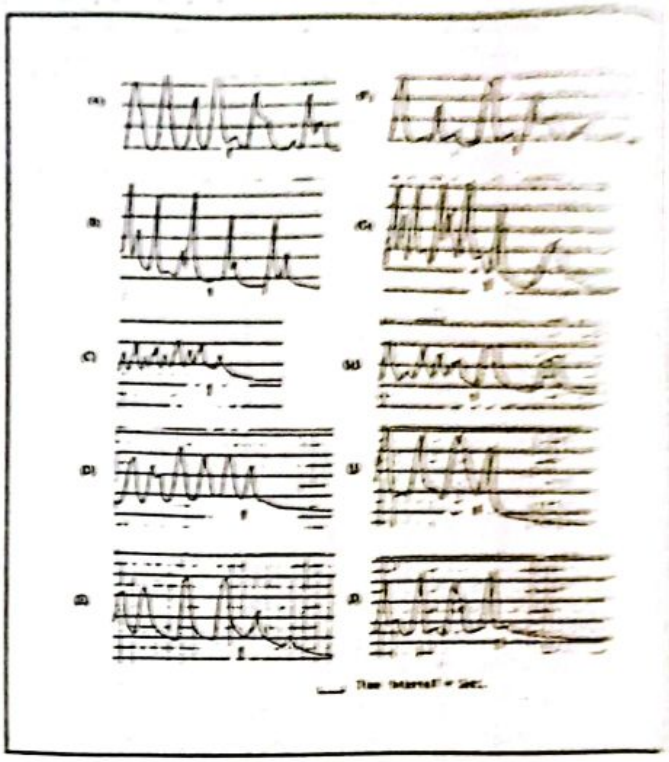

Fig. 3: Effect of 100 µg/ml of Ginger (A), Black pepper (B), Cardamom (C), Curcuma (D), Za'tar (E), Sweet majoram (F), Cassia (G), Maryamiyah (H), Clove (I) and rosemary oil (J) on isolated uterus of pregnant rats.

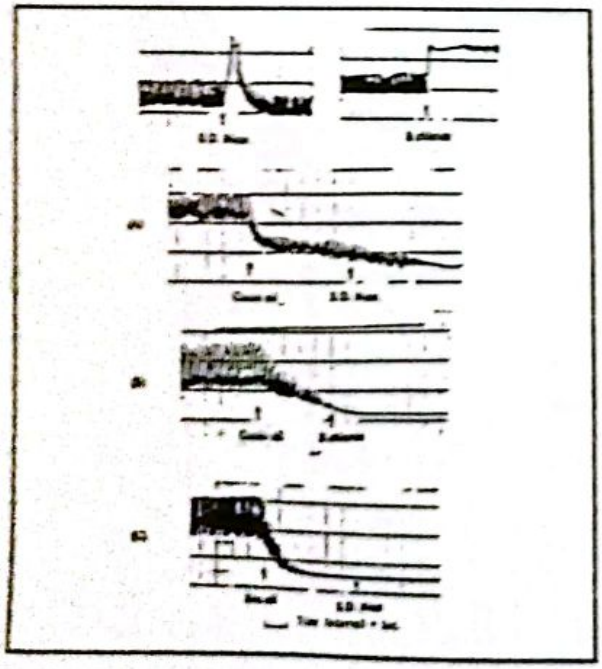

Fig. 2: Site of action of cassia oil (100 µg/ ml) and rosemary oil (Ros. oil, 100 µg/ml) on isolated rabbit's jejunum.

A- Cassia oil followed by small dose of nicotine (S. D. Nicot.).

B- Cassia oil followed by barium chloride (B. chloride)

C- Ros. oil followed by barium chloride (B. chloride).

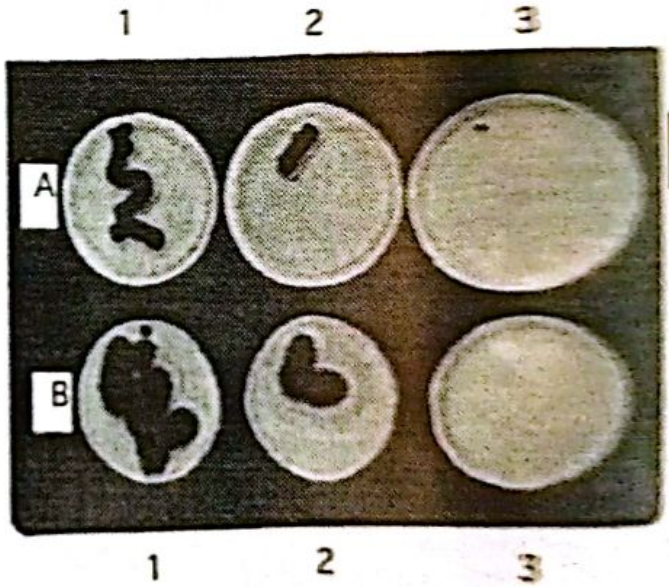

Fig. 4: (A) Normal growth of *Penicillium spp* (1) and high degre of inhibition (2) and complete inhibition (3) of its growth caused 2.5 and 5% of cassia oil, respectively.

(B) Normal growth of *A. niger* (1) and moderate inhibition (2) and complete inhibition (3) of its growth caused by 2.5 and 10% of cassia oil, respectively.

Table (II): *In vitro* EC_{50} values of essential oils on rabbit's jejunum preparation.

Tested oils	EC_{50} (µg/ml)	Range for EC_{50} (µg/ml)	Activity and site of action
Ginger	66.322	47.19-85.45	Stimulation of muscle
Black pepper	71.842	69.99-73.68	Stimulation of muscle
Cardamom	54.624	24.40-84.85	Inhibition of muscle
Curcuma	51.612	22.14-81.08	Inhibition of muscle
Za'tar	47.018	30.00-64.04	Inhibition of muscle
Sweet majoram	71.418	55.00-87.84	Inhibition of muscle
Cassia	90.133	87.62-92.65	Inhibition of muscle & ganglia
Maryamiyah	54.191	26.19-82.19	Inhibition of muscle
Clove	84.109	67.00-101.22	Inhibition of muscle
Rosemary	51.137	41.67-60.61	Inhibition of ganglia

Table (III): *In vitro* EC_{50} values of essential oils on pregnant uterus of Wistar rats.

Tested oils	EC_{50} (µg/ml)	Range for EC_{50} (µg/ml)	Activity
Ginger	225.035	182.55-267.52	Inhibition
Black pepper	135.185	129.96-140.41	Inhibition
Cardamom	52.445	46.17-58.72	Inhibition
Curcuma	93.080	80.45-105.71	Inhibition
Za'tar	22.795	20.59-25.00	Inhibition
Sweet majoram	47.495	21.75-73.24	Inhibition
Cassia	119.435	87.81-151.06	Inhibition
Maryamiyah	146.815	122.37-171.26	Inhibition
Clove	45.815	42.98-48.65	Inhibition
Rosemary	75.910	62.31-89.51	Inhibition

Antibacterial effect:

Cassia essential oil in concentrations of 100, 25, 25, 50 and 200 mg/ml inhibited the growth of Staph. aureus, Staph. methicillin resistant, Strept. type 8 and D, Strept. agalactia and Bacillus subtilis, respectivley (Table IV). Moreover, al the tested gram-negative strains are inhibited by cassia oil in concentrations more than 25 mg/ml.

The essential oil of Za'tar at concentration of 25, 100 and 50 mg/ml showed a marked inhibitory activity against Staph. methicillin resistant, E. coli and P. aeruginosa, respectivley. Cardamom, curcuma and maryamiyah showed an inhibitory activity against P. aeruginosa in concentrations more than 25 mg/ml. The other tested oils showed no activity against all investigated microbes. The difference in the antimicrobial activity of maryamiyah (Salvia triloba) from that previously reported (Mahmoud et al., 1992) for the samples obtained from Jordon is attributed to envirommental conditions.

Antifungal activity:

The essential oil of cassia at 2.5% concentration inhibited the growth of all tested fungi (Fig. 4). This effect was found to be in harmony with the concentration used (Table V).

At concentrations 2.5 and 5%, essential oil of clove produced inhibition of the growth of *(Penicillium spp., A. niger, A. fumigatus, M. gypseum, T. mentagrophytes)* and (all tested fungi) respectively. Ginger, black pepper, cardamom and marymiyah showed no antifungal activity against all selected strains of fungi (Table V). Za'tar showed complete inhibition of *T. mentagrophytes, C. albicans* and C. neoformans. It showed a moderate inhibition to *Penicillium spp, Aspergillus niger, A. fumigatus and M. gypseum. Sweet majoram* showed high degree of inhibition of *T. mentagrophytes* at a dose of 20 mg/ml. Curcuma essential oil caused complete inhibition to *Trichophyton mentagrophytes* at a dose of 20 mg/ml.

Topical application of cassia oil at 20% concentration completely cured all skin lesions (100%) of experimentally infected guinea pigs

with dermatophytes after 16 days treatment. Non treated skin lesions were still inflamed, hairless, ulcerated and covered with crusts after 20 days post infection. The oils of clove and za'tar caused complete cure of skin lesions in 60 and 40% of animals after 18 and 20 days treatment, respectivley. The oil of sweet majoram caused complete cure of skin lesions in 20% of animals after 20% of animals after 20 days treatment with *T. mentagrophytes* only. The curative effect of all oils were confirmed by microscopic examination and culturing of the samples obtained from treated and non-treated skin lesions on agar medium.

The broad spectrum antimicrobial activity of essential oil of cassia makes it the drug of choice for tratment of infections caused by sensitive strains of bacteria and fungi.

Acute and chronic toxicity of the investigated essential oils are needed to prove their safety for animal and human therapy.

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