INTRODUCTION

Plant kingdom have been and still being considered as available source of medicinal drugs. The Ancient Egyptians, Chinese and Indians, reported the use of plants as remedies to cure various diseases e.g. hypertension, constipation, fever, arteriosclerosis, epilepsy and scabies ***(Svendsen and Scheffer, 1982; Balandrin et al., 1985).***

All over the world, interest has increased in studying the biological effects of traditional medicinal plants as a prerequisite for using them as such or isolating their active components for treatment of illness ***(Meyer et al., 1982; Sofowora, 1982 and Kottb ,1983)*** .

Interest has increased in the possible exploration of the beneficial effects of herbs in the treatment of various diseases. The general public are of the opinion that since herbs are natural, all of them are safe and effective. This opinion has been generated by thousands of years of experience with medicinal plants through which man learned that these natural remedies never bring harm if they do not bring good ***Hanafy (1991).***

Unlike natural medicines, the use of modern chemical drugs have

been associated with many serious problems. For example, the widespread and indiscriminate use of antibiotics, as food additives and for treatment of trivial infections in man and animals has lead to the emergence of resistant strains of bacterial pathogens and has been associated with high incidence of many serious diseases of poorly understood aetiology ***(Booth, 1977; Linkewish, 1980 and Tyler, 1986).***

Man fascinated at the invent of modern drugs by their extreme potency. Lastly he come to realize that his weapons are progressively loosing potency while their devastating effects on health remain. Research in the area of herbal medicine has been hampered by pharmaceutical companies which consider research in the area not financially profitable. In Egypt traditional folklore medicine provides a very rich source of reasonable information that would persuade investigators to start research in that area unlike the situation in the orient. Investigators in West Europe would be unable to find adequate data of an acceptable nature to induce them to indicate major research programmes in herbal medicine ***(Tyler, 1986).***

Five computer assissted literature search in Egypt and Saudi Arabia have been shown that there is little information on the antibacterial effects

of *Nigella sativa* L. and *Peganum harmala* L. Literature on the antibacterial activity of *Alpinia officinarum* Hance., *Ambrosia maritima* L. and *Carthamus tinctorius* L. are neglected. Whereas the attention of previous investigators was primarily focused on the antiparasitic activity of *Ambrosia maritma* L. on other medicinal purposes and chemical purposes of *Carthamus tinctorius* L. and *Alpinia officinarum* Hance., while their antibacterial activity was marginally considered.

Before any plants could be used as the starting material for remedies capable of treating specific infections, several questions had to be answered :

(1) How can suspected antibacterial substances be obtained in a concented form from this plants ?

(2) What are the bacterial pathogens that would be susceptible to the effects of these plants ? and to which range of concentration ?

(3) What is the effect of heat on the antibacterial activity of plant extract ?

AIM OF THE WORK

The aim of this work is to evaluate the antibacterial activity of different organic solvent extracts of *Nigella sativa* L., *Peganum harmala* L. *Carthamus tinctorius* L., *Ambrosia maritima*L. and *Alpinia officinarum* Hance. on some bacterial pathogens and the effect of interaction of these plant solvents with authentic antibiotics.

LITERATURE REVIEW

I- Nigella sativa L.

Plant morphology, taxonomy and geographical distribution

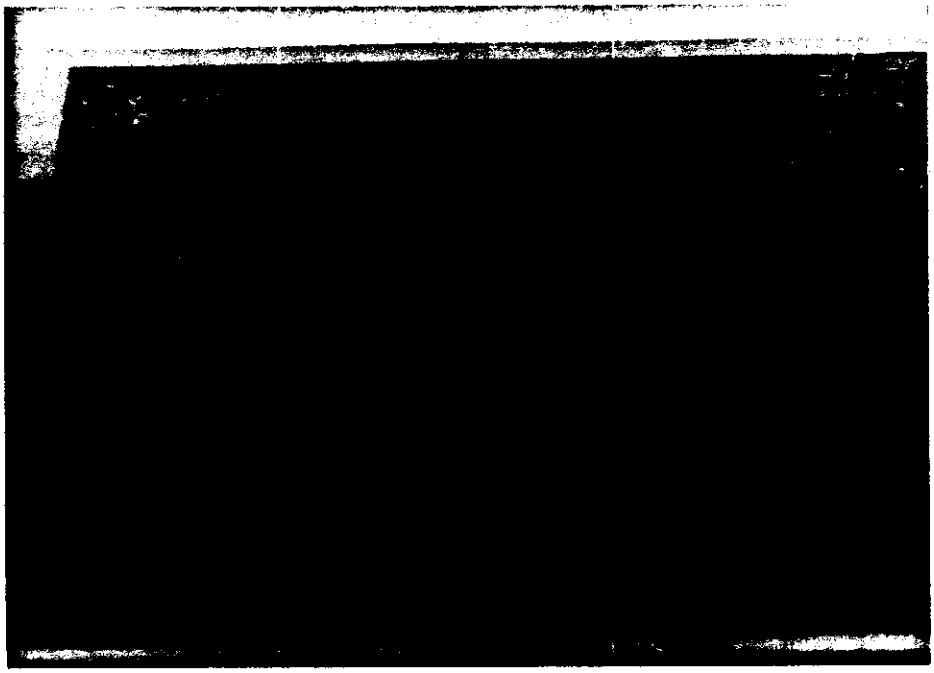
The seeds of *Nigella sativa* L. are known in arabic names as *Habbah sawda, Habbet el baraka, Kamun aswad* and *Shunez*. The genus Nigella include the following species *Nigella sativa* L., *Nigella arvensis* L., *Nigella assynace* Boiss, *Nigella deserti* Boiss, *Nigella hispanica* L., *Nigella damascena* L., Amongst which only Nigella sativa L. was known to be indigenous and grows well in different localities of Egypt. *Nigella sativa* L. is an annual herbaceous plant. It is one of the native plants of Egypt ***(Nagib,1978).***

The genus Nigella family (Ranunculaceae) includes several species and is indigenous to Middle East *Nigella sativa* L. grows in different localities of West and South Asia, East and South Europe and is also cultivated in some countries leaves of Nigella sativa L. finely dissected sepals 5 petaloid, petals 8 transformed into nectaries, small, no distinctly spurred. Carpels more or less united Fig. (A) ***(Perry and Metzger,1980).***

Chemical Composition :

The seeds of *Nigella sativa* L. contain 30-40% fixed oil, 0.1-0.5% essential oil, alkaloids, saponins etc. ***(Akgul,1989).***

Fig. (A) : *Nigella sativa* L. ***(Nagib,1978).***



Photograph (A) : *Nigella sativa* L. ***(Nagib,1978).***

Uses of Nigella sativa L.:

Dried whole seed is used as flavouring agent to some foods *(Perry and Metzger,1980).*

The seeds are very popular spice in Turkish "Core Kotu" for some special backed products and cheesein addition, to imparting flavour, certain spices and essential oils prolong the storage like of foods by an antimicrobial activity *(Akgul, 1989).*

Uses of Nigella sativa L. in traditional medicine :

Mahfouz and El-Dakhakhny (1960) were able to isolate crystalline compound from the volatile oil which was called "Nigellone". This compound is injected I/M to guinea pig to protect it against histamine induced bronchospasm. This drug is now in use as an antiasthmatic drug.

Toppozada et al. (1965) observed that one case of resistant external otitis was markedly improved within a short period of time through the accidental use of the crude oil locally in the ear. Furthermore, it was investigated also clinically to demonstrate its antibacterial action against microorganism affecting the skin and mucosa.

In Egypt the seeds are used in folk medicine as a diuretic and

carminative,this expressed oil is used in treatment of bronchial asthma, and cough ***(Nagib,1978).***

The seeds are valued as carminative and galactagogue; they are warm and stimulating, hense used to mix with unpalatable drugs. In Malay Peninsula : the seeds are a component of poultices for abscesses rheumatism, orchitis, ulcerated nose, headache, also of a lotion to wash fever patients or to gargle, they are taken orally in combination with other drugs as an antiemetic and a laxative. They are found in prescriptions in the Medical book of Malayan medicine for blood poisoning, enlarged liver, nausea, colic, constipation, for women after child birth, and various other troubles. In Indonesia, they are added to astringent medicines for abdominal diseases ***(Perry and Metzger,1980).***

Siddiqui (1988) reported that ethnomedical study of *Nigella sativa* L. terminating pregnancy reporting a survey based on interviews with herbalists and villagers in the districts Sitapur and Aligarh.

In case of Cisplastin induced toxicity in mice (2 mg/kg for 5 day) an extract of *N.sativa* L. seed only tended to protect from cisplastin induced falls in haemoglobin levels and leucocyte counts ***Nair et al. (1991).***

Antimicrobial activity of Nigella sativa L.:

Toppozada et al. (1965) reported that the antibacterial activity of the crude oil of *Nigella sativa* L. was tested by different methods and it was found that the oil inhibited the growth of many strains of gram positive and gram negative bacteria except some strains of pseudo-monas pyocyanea. They showed that the activity was confined solely in the phenolic fraction of the essential oil. Clinically the phenolic fraction was used in the treatment of chronic external otitis and chronic purulent maxillary sinusitis. The pharmacological data of this active fraction showed that it is non toxic and has no action on blood pressure, heart or respiration.

El-Fatatry, et al. (1975) isolated thymohydroquinone from phenolic compound of volatile oil of *Nigella sativa* L. this compound was found to have high antimicrobial effect against gram positive micro-organisms and a low sporocidal but high sporostatic activity.

Rathee et al. (1982) noted that a compound known as carvone in the essential oil of *Nigella sativa* L. have an antibacterial and antifungal activity.

El-Shayeb and Mabrouk (1984) reported that using different concentration of extract of *Nigella sativa* L. in rice corn steep medium inhibit aflatoxin production of a toxinogenic structure of *Aspergillus flavus.*

Namba et al. (1985) reported that methanolic extract of *Nigella sativa* L. inhibits adhesion of viable cells of streptococcus mutans to smooth surface (Plastic tube surface). He also suggested that *Nigella sativa* L.can be of value in inhibition of plaque formation and dental caries.

Saxena and Vyas (1986) studied the petroleum ether and ethanol extracts of *Nigella sativa* L. seeds in vitro. The two extracts were found to have antibacterial activity against (*Escherichia coli, Bacillus subtilis* and *Streptococcus faecalis*) and antifungal activity against (*Aspergillus fumigatus, Trichophyton mentagrophytes* and *Candida albicans*).

Akgul (1989) reported that carbonyl compound, thymoquinone had exhibited antimicrobial activity to some gram positive bacteria. This compound was found to represent the main components of Black cumin seed (*Nigella sativa* L.). The inhibitory effect of *Nigella sativa* L. essential oil on the growth of certain bacteria is an important finding. He showed that the most sensitive bacteria were *E. aerogenes, B. cereus* and *B. subtilis;* and the most resistant bacteria were *E. coli, Ps. aeruginosa* and *Sal. typhimurium.* However the oil exhibited a weak effect on fungi.

Hanafy and Hatem (1991) reported that diethyl ether extract of

Nigella sativa L. seeds caused inhibition of gram positive bacteria represented by *Staph. aureus* and gram negative bacteria represented by *Pseudomonas aeruginosa* and *Escherichia coli* but not *Salmonella typhimurium* and a pathogenic yeast *Candida albicans*. The extract showed antibacterial synergism with streptomycin and gentamicin and showed additive antibacterial action with spectinomycin erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin lincomycin and sulphamethoxyzole - trimethoprim combination.

Anticestodal effect of *Nigella sativa* L. seeds was studied in children infected naturally with the respective worms by usining single oral administration of 40 mg/kg of *Nigella sativa* L. It is conceivable that *Nigella sativa* L. contain active principles effective against cestodes. The crude drug did not produce any adverse side effects in the doses tested ***(Akhtar and Riffat, 1991).***

Antifeedant activity of Nigella sativa L. :

Singh (1983) reported that *Nigella sativa* L. possess antifeedant activity of desert locusts. The acetone extract of *Nigella sativa* L. was sprayed on leaf pieces of maize. When the treated leaf pieces were offered to starved locusts reared on maize, feeding was seen to be inhibited.The *Nigella sativa* L. may change the physical properties of the leaf pieces.

Antidiabetic action of Nigella sativa L. :

Akhtar and Ali (1985) reported that powdered *Nigella sativa* L. methanolic and water extracts produced hypoglycemic effect to normo glycemic animals. Also powdered *Nigella sativa* L. methanolic and water extract caused a well marked decrease in blood glucose level of alloxan hyperglycemic rabbit.

When normal and diabetic rabbits were treated with the same dose of *Nigella sativa* L. seeds, a 16.3% decrease in blood glucose level of normal rabbit was noted, while in the diabetic rabbit this decrease was about 7% after drug administration. It may, therefore be supposed that in the normal rabbit this subtance exerts not only a direct insulin like effect but also acts indirectly by stimulating the release of insulin from pancreatic beta cells that is why it has been found to be more potent in the normal rabbits ***Braunsteiner et al. (1965)*** . Hyperlipaemia was reported to accompany some hyperglycaemic states e.g. patients suffering from diabetes mellitus. They have estimated, the blood total lipid contents in normal and alloxan diabetic rabbits after oral administration of various doses of *Nigella sativa* L. seed as well as their extracts in water and methanol. The data obtained showed that *Nigella sativa* L. decreases the blood sugar level in normal rabbit but were ineffective in lowering the normal blood total lipid level. In alloxan induced diabetic rabbit, the total blood lipids were found to be markedly increased. The aqueous and methanolic extract of the drug also failed to decrease the raised lipid

contents of diabetic rabbit. Acut toxicity studies revealed no visible signs and symptoms of toxicity and none of the rabbits had died after 7 days even at a high dosage like 8 g/kg body weight (orally).

Al-Awadi et al. (1991) studied the antidiabetic action of the plant mixture extracts (*Nigella sativa* L. *Myrrh, Gum olibanum, Gum Asafoetida* and *Aloe*) on liver gluconeogenesis in streptozotacin induced diabetic rats.The extracts were proved to be useful therapeutic agents in the treatment of non-insulin dependent diabetes mellitus (NIDDM).

Anti-tumour activity of Nigella sativa L.:

Salomi et al. (1991) reported that intraperitoneal administration of *Nigella sativa* L. extract (100 mg/kg body wt.) per mouse restricted tumour incidence. Topical application of *Nigella sativa* L. extract inhibited two stage initiation and promotion of skin carcinogenesis in mice.This dose delayed the onset of papilloma formation and reduced the mean number of papillomas per mouse.

The active principle of *Nigella sativa* L. seeds containing certain fatty acids was studied for anti-tumour activities against Ehrlich Ascites Carcinoma (EAC). In vivo (EAC) tumour development was completely inhibited by the active principle at the dose of 2 mg/mouse per day ***(Salomi et al.,1992).***

Antihistaminic action of Nigella sativa L.:

Charkravarty (1993) reported that isolation of "Nigellone" carbonyl polymer of thymoquinone from the *Nigella sativa* L. seeds inhibite histamine release from mast cells. The experiment was carried on rat peritoneal mast cells. In vitro, it was shown that Nigellone in relatively low concentrations is very effective. The Nigellone inhibited histamine release by : 1- decreasing intracellular calcium, 2- inhibition on protein kinase C and 3- mild inhibition of oxidative energy metabolism.

Effect of Nigella sativa L. on non target organism :

When the aqueous extract of the seeds of *Nigella sativa* L. was administrated orally (10 ml/kg) under light ether anaesthesia to male Sprague-Dawley rats for 14 days, it produced an increase in gamma-glutamyl transferase and alanine aminotransferase concentrations in the absence of hepatocyte degeneration. This suggests that these enzymes may have been released due to hepatocellular damage that occured at the molecular level. However an enzyme-inducing effect of *N. sativa* L. extract cannot be ruled out. ***(Tennekoon et al., 1991)***

II- Peganum harmala L.

Plant morphology, taxonomy and geographical distribution

Peganum harmala L. Commonly known as : *L. Peganum harmala* L., *E. Harmal wildrue, F. Pegane, Harmale* and *A. Harmal, Harmalan, Khardal Abiad, Khineza, Khayas* and *Cays.* ***(El-Khalifa and Sharkas,1984 and Gabre, 1987)***

Peganum harmala L. (Family zygophylaceae) is a perennial small bushy plant up to 90 cm high with much branching stems and oval shaped leaves, many leaves 3-divided; flowers, white and at the end of stem spetals, fruits are 3 valved round triangular shaped seeds. It flowers from february to June. The plant is common in deep sandy places and sand dunes and is found over the north coast of Egypt. Fig. (B) ***(Helal and Heberyounigesky, 1984).***

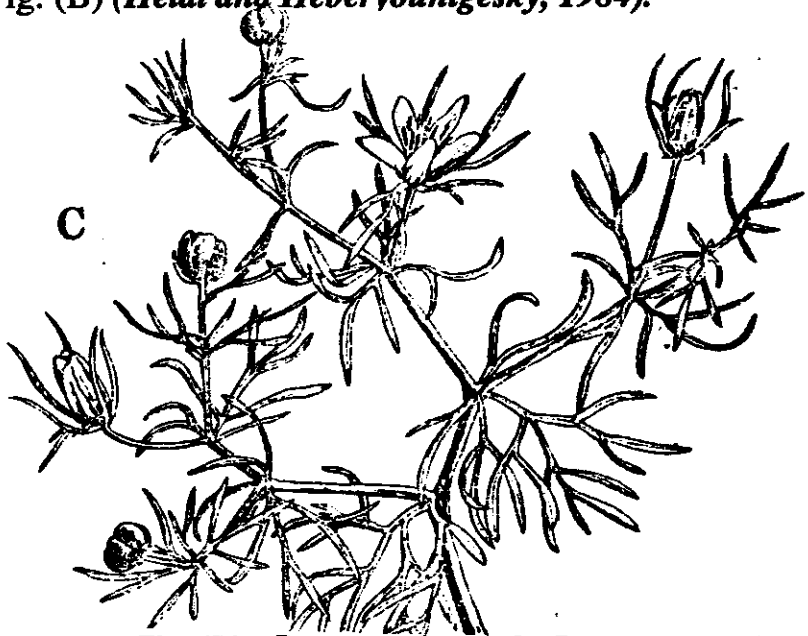
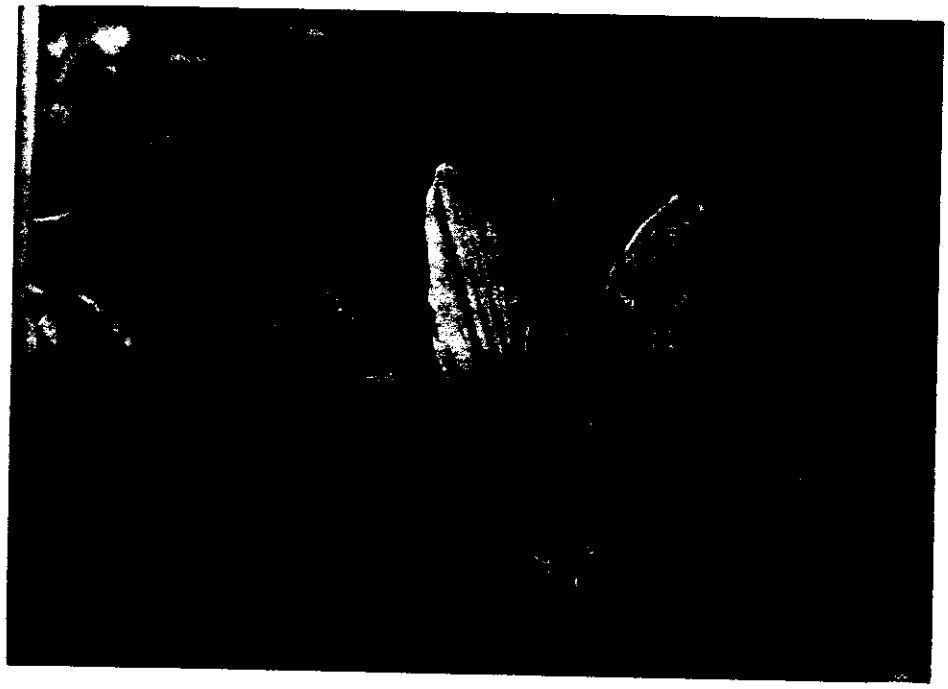


Fig. (B) : *Peganum harmala* L. ***(Tackholm, (1974)***



Photograph (B) : *Peganum harmala* L.

Uses of peganum harmala L. in traditional medicine :

Peganum harmala L. used to induce vomiting followed by strong tea or tannin containing drink, Physicians use stimulants and/or demulcents. Also used as anthelmintics ***(Helal and Heberyounigesky, 1984).***

Peganum harmala L. is an ancient plant widely used in many areas in folklore medicine as an antiseptic and for the treatment of skin diseases. The smoke of this plant was used as a disinfectant ***Adaay et al. (1989).***

Twaij, et al. (1989) reported that extracts of *Peganum harmala* L. and other plants exhibited significant analgesic activities in either the hot-plate test or in benzoquinone-and/or acetic acid induced writhing in mice.

The effect of methanol and acetone extracts of the epigeal parts of *Peganum harmala* L. was studied on several parameters of reproduction in female rats. The methanol extracts at a dose of 2.5 gm/kg/day, offered in food or in drinking suspension for 30 days, significantly prolonged diestrus by 1.0 day. The methanol extracts at doses of 2.0, 2.5 and 3.5 gm/kg/day appeared to produce a dose-dependent significant decrease in litter size. No change in the physical and nutritional status of the animals and no adverse toxicological effects were observed ***(Shapira et al., 1989).***

In Indo-Pakistan, Iran, Central Asia countries and North Africa. various parts of the plant are highly used in the traditional systems of medicine for the treatment of a variety of human ailments as lumbago, asthma, colic and jaundice; also as a stimulant emmenagogue. The possible therapeutic application of the *Peganum harmala* L. alkaloids as protozoacidol agents,coronary dilators, ecbolics as well as in nervous diseases has been described by various authors ***(Siddiqui et al., 1990).***

Antispasmotic action of Peganum harmala L.:

The effects of an aqueous extract of *Peganum harmala* L. seed were tested in vitro using isolated segments of intestine, trachea, and aorta of rabbit and guinea pig. The extract exhibited, the spontaneous movement of rabbit jejunum and guinea pig ileum. It is suggest that this seed extract has antispasmodic, anticholinergic, antihistaminic and antiadrenergic effects ***(Aqel and Hadidi, 1991).***

Hypoglycemic action of Peganum harmala L.:

The hypoglycemic effect of orally administrated seed suspensions of *Peganum harmala* L., was investigated in both normal and streptozotocin-induced diabetic rats. *Peganum harmala* L. resulted in high mortality and a moribund state and its medicinal use should be discouraged ***(Al-Zaid, et al., 1991)*** .

Antimicrobial activity of Peganum harmala L.:

Jit et al. (1986) reported that shoots or fruits of plants have a medicinal value in ayurvedic literature, including *Peganum harmala* L. and other plants. An ethylether and 50% ethanolic extract of the test samples were active against *Staph. aureus. E. coli* and *Candida albicans*. Maximum activity was noted in *Peganum harmala* L. against *E. coli.*

Adaay et al. (1989) studied the effects of the different *Peganum*

harmala L. seed extracts on different species of bacteria. Five different fractions from *Peganum harmala* L. seeds (aqueous fraction, alkaloid fraction, non-alkaloid fraction, ethanolic fraction and methanolic fraction) were tested for their in vitro antimicrobial activity against seven economically significant microbes. (*E. coli, Staph. aureus, Proteus vulgaris, Ps. aeruginosa Salmonella, typhi, Salmonella typhimurium* and *Shigella flexeneri*). It was found that all fractions possess a good antimicrobial activity against all tested microbes,excèpt non-alkaloid fraction which was not active.Comparison of the activity of the fractions showed that the aqueous fraction was somewhat less active than the other fractions, it still got a good activity against all the microbes used in their study.

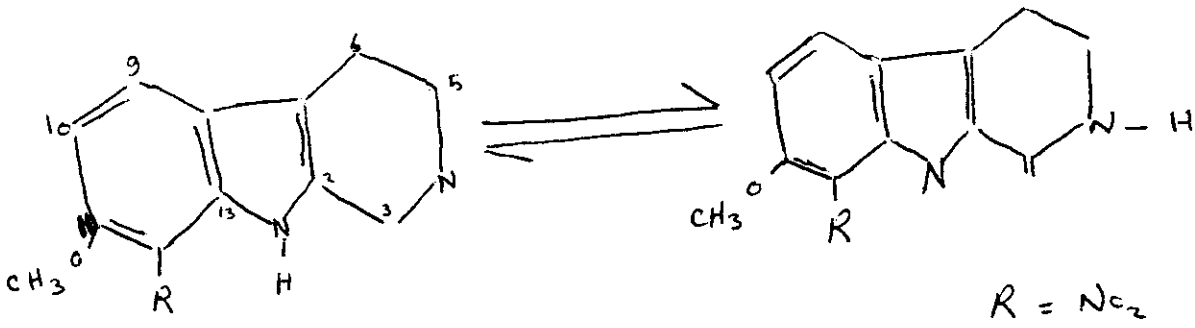
They also reported that the aqueous extract contains 2.08% total alkaloids (harmaline, harmine and harmalol) in comparison with 4-5% of the same alkaloids in the ethanolic extract. The same study also showed the presence of quercetin and kaempferol. In conclusion they said that, whatever the active compound(s), the results obtained in this study give a special importance for the use of water in extraction procedures. In addition to that, it gives some support to the ancient claims about the utility of this plant in the treatment of some diseases such as the infections of the respiratory tract.

Rashan et al. (1989) reported that from the ancient time,

Peganum harmala L. seeds have been claimed to possess analgesic, diuretic, anthelmintic, antimicrobial and other biological activities. They also reported that an aqueous seed extract has good antiviral activity against DNA-containing herpes virus horminis type I (HSV-1) but not against influenza viruses A and B.

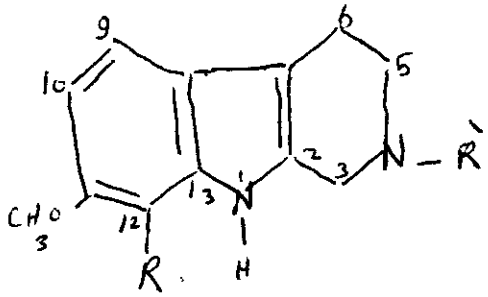
Siddiqui et al. (1990) reported that a number of B. carboline and tryptamine derivatives have been prepared from harmaline and studied for their antibacterial activity against various gram positive and gram-negative organisms. The electron withdrawing substituents in these bases resulted in a decrease of activity. The new derivatives are :

1. Harmaline 2. Nitroharmaline 3. B. carboline
4. Benzoyl- II- methoxy-3-methylene- 12-nitro-3,4,5,6 - tetrahydro B. carboline.
5. Cinnamoyl - II -methoxy 3-methylene-12-nitro 3,4,5,6-tetrahydro B. carboline
6. Benzenesulfonyl-II-methoxy-3-methylene-12-nitro 3,4,5,6- tetrahydro B. carboline.
9. 3- (2-Acetamidoethyl)-2-acetyl-7-methoxy-8-nitroindole 10-2-Acetyl -3-(2-benzamidoethyl)-7methoxy-8-nitroindole.
10. 2-Acetyl-3-(2-benzamidoethyl)-7-methoxy-8-nitroindole.
11. 2-Acetyl-3-(2-cinnamidoethyl)-7-methoxy-8-nitroindole.
12. 2-Acetyl-3-(2-Benzenesul fonamidoethyl)-7-methoxy-8- nitroindole



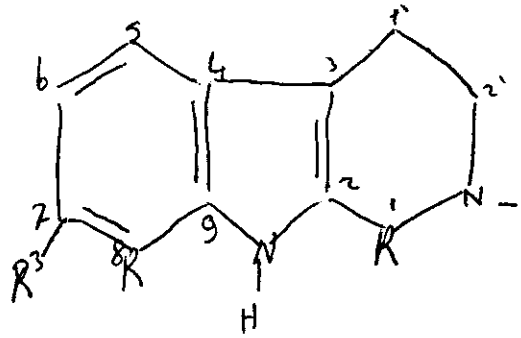
1 harmaline

2 nitroharmaline



B. carboline

3 $R = No_2$, $R^1 = -COCH_3$
4 $R = No_2$, $R^1 = -COC_6H_5$
5 $R = No_2$, $R^1 = -COCH : CHC_6H_5$
6 $R = No_2$, $R^1 = -SO_2C_6H_5$



tryptamine

7 $R = R^1 = R^2 = R^3 = H$

8 $R = H, R^1 = R^2 = COCH_3, R^3 = OCH_3$

9 $R = No_2, R^1 = R^2 = COCH_3, R^3 = OCH_3$

10 $R = No_2, R^1 = COCH_3, R^2 = CHC_6H_5\ R^3 = OCH_3$

11 $R = No_2, R^1 = COCH_3, R^2 = COCH : CHC_6H_5 R^3 = OCH_3$

12 $R = No_2, R^1 = COCH_3, R^2 = SO_2C_6H_5\ R^3 = OCH_3$

Table (A) : Antimicrobial activity of *Peganum harmala* L. derivaties against (25) species of bacteria

Compound No.												Micro-organism	Gram type
1	2	3	4	5	6	7	8	9	10	11	12		
Inhibition zone in mm													
12	-	-	-	-	-	-	-	-	-	-	-	*Escherichia coli*	-
13	-	-	-	-	-	-	-	-	-	-	-	*Enterobacter aerogenes*	-
24	-	-	-	-	-	-	-	-	-	-	-	*Enterbacter cloacae*	-
15	17	-	-	-	-	10	-	-	-	-	-	*Shigella flexneri*	-
12	-	-	-	-	-	-	-	-	-	-	-	*Shigella sonnei*	-
14	15	16	-	-	-	-	-	-	-	-	-	*Citrobacter fraundii*	-
11	-	-	-	-	-	-	-	-	-	-	-	*Salmonella typhi*	-
14	16	10	-	-	-	-	-	-	-	-	-	*Salmonella paratyphi A*	-
10	-	-	-	-	-	-	-	-	-	-	-	*Salmonella schotmuelleri*	-
14	12	-	-	-	-	-	-	-	-	-	-	*Klebsiella pneumoniae*	-
14	12	-	-	-	-	-	-	-	-	-	-	*Klebsiella ozaenae*	-
20	13	-	-	-	-	-	-	-	-	-	-	*Serratia marciscens*	-
14	16	-	-	-	-	-	-	-	-	-	-	*Proteus vulgaris*	-
-	-	-	-	-	-	13	-	-	-	-	-	*Pseudomonas aeruginosa*	-
10	12	10	-	-	-	10	-	-	-	-	-	*Pasteurella moltocida*	-
16	13	-	-	-	-	18	10	-	10	10	-	*Corynbacterium d'phtheria*	+
26	21	12	-	-	-	20	-	-	-	-	-	*Corynbacterium hofmannii*	+
22	21	-	-	-	-	15	-	-	11	-	-	*Corynbacterium xerosis*	+
-	-	-	-	-	-	-	-	-	-	-	-	*Staphylococcus aureus*	+
13	-	-	-	-	-	10	-	-	15	-	-	*Staphylococcus epidermidis*	+
22	-	-	-	-	-	-	22	-	10	-	-	*Staphylococcus citreus*	+
-	-	-	-	-	-	-	-	-	-	-	-	*Streptococcus pyogenes*	+
13	11	-	-	-	-	-	-	-	-	-	-	*Streptococcus faecolis*	+
24	12	10	-	-	-	11	25	-	-	-	-	*Streptococcus lactis*	+
18	-	-	-	-	-	-	20	-	-	-	-	*Sarcina lutea*	+

Nitroharmaline and harmaline is more effective against *Serratia marcescens, Corynbacterium hofmannii* and *Streptococeuslactis* indicating an activity reducing effect of nitro group in the benzene ring of the B. carboline skeleton. However 1, and 2 were found almost equally effective against *Shigella flexeneri, Citrobacter freundii, Sal. paratyphi A, Klebsiella pneumonia, Klebsiela ozaenal, Proteus vulgaris, Pasteurella maltocida* and *Streptococcus faecalis, while Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* were found resistant. The electron withdrawing substituents of pyrido ring nitrogen in the B-carboline further decreased the antibacterial activity thus 3 was active only against *C. freundii Sal. paratyphi A, P. maltocida, C. hofmanni* and *S. lactis* while derivatives 4-6 were found inactive against all the organisms tested. On the other hand tryptamine 7 showed activity against *S. flexeneri, Ps. aeruginosa, P. maltocida, C. diphtheria, C. hofmannii, C. xerosis, S. epidermidis* and *S. lactis*, whereas 3-with an electron withdrawing substituent. Lost of the inhibitory effect against all gram negative bacteric but showed significant inhibitory effect against *S. citreus S. lactis* and *S. lutea.* Compounds 9-12 having the nitro group in benzene ring, were found to be almost ineffective against these organisms it may be summarily stated that the electron withdrawing substituents of B. carbolines or tryptamines generally decrease the antimicrobial activity.

Alkofahi et al. (1990) reported that ethanolic extracts of *Peganum harmala* L. was examined for cytotoxicity, mutagenecity and antimicrobial activity.

1- Cytotoxicity testing: *Peganum harmala* L. extract was tested against 3 types of tumours. This extract was not active against tumour.
2- Mutagenecity testing : *Peganum harmala* L. extract was mutagenic against *Salmonella typhmurium* strains (TA 98 and TA 100).
3- Antimicrobial activity : Peganum harmala L. extract showed antibacterial activity on *E. coli* and *Bacillus subtilis* : It might be possible that the antimicrobial activity is due to mutagenic effect of the extract.

Effect of Peganum harmala L. on non target organism :

The whole plant especially the seeds cause nausea, and vomiting. If not removed from the stomach soon after ingestion, poisoning progresses to severe inflammation and swelling of tissues. Poisonous constituents include the alkaloids Peganine, harmine and harmaline ***(Helal and Heberyounigesky ,1984).***

III- Ambrosia maritima L.

Plant morphology, taxonomy and geographical distribution :

Ambrosia maritima L. is one of species of the genus, Ambrosia which belongs to the family compositae ***Mahran (1967).***

Ambrosia maritima L. is a herbaceous weed, widely distributed over the Mediteranean region and Africa (Algeria, Tunisia, Libya, Egypt, Senegal, Guinea, Mali, Burkina faso, Ivory coast, Ghana, Niger, Nigeria, Cameroon, Chad, Central African Republic, Sudan, Ethiopia, Uganda, Kenya, Tanzania, Burundi, Zaire, Angola Malawi, Zambia, Zimbabwe, Mozambique, Republic of South Africa, Madagascar). ***(Geerts et al., 1991).***

The plant especially grows on slightly humid soils in the coastal areas and in the great river basins it is often reported as a weed in cultivated fields. The plant is much branched and attains 30-90 cm height. It is known to be prostrate or erect, the latter at least in several regions of Egypt and Senegal. Fig. (C) ***(Geerts et al., 1991).***

Ambrosia maritima L. seems to be annual herbaceous plant ***(Fahmy and Darwish, 1949).*** *Ambrosia maritima* L. is growing in Egypt, it is known in Arabic by the common name Damaseisa ***(Bailey, 1968 and Tackholm, 1974).***

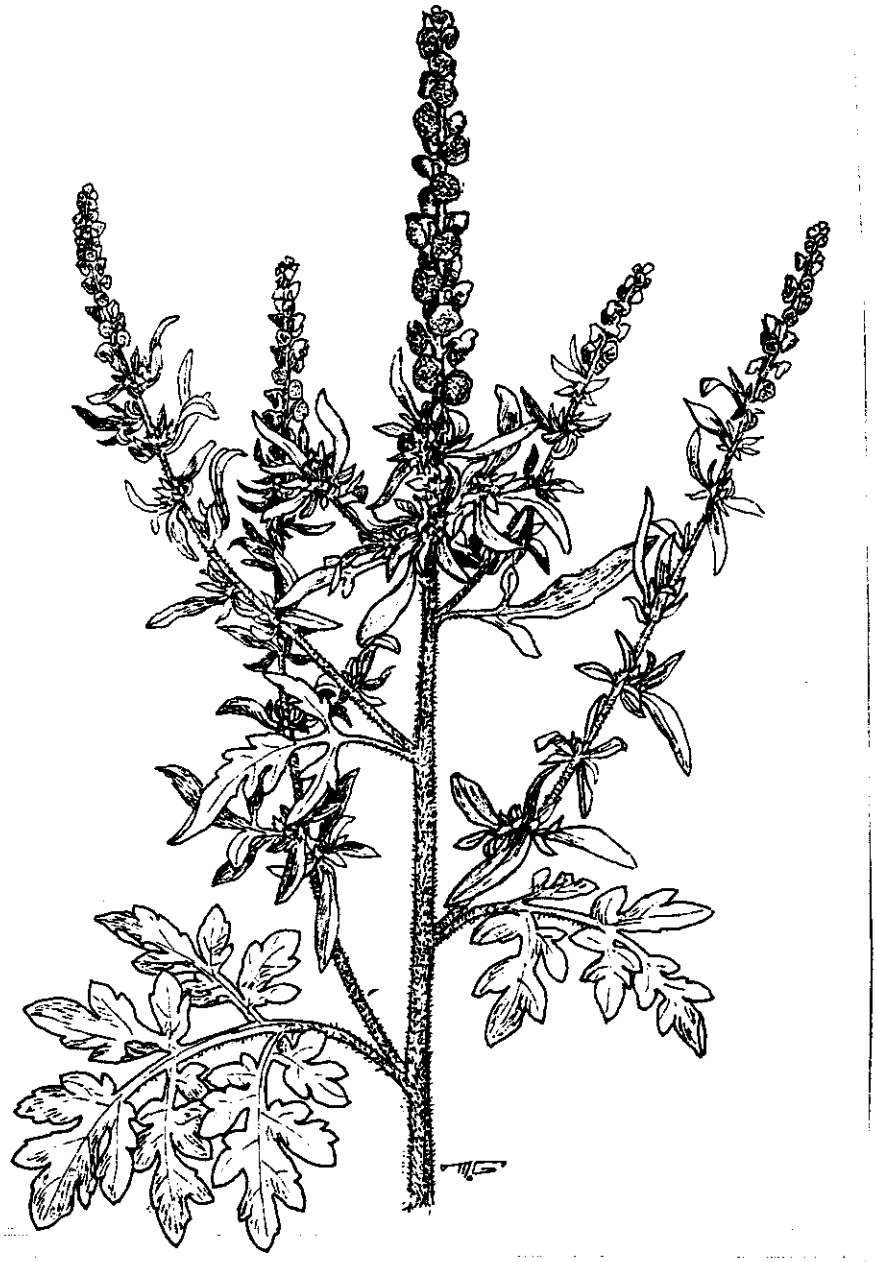


Fig. (C) : Ambrosia maritima L.
(Tackholm, 1974)

Uses of Ambrosia maritima L. in traditional medicine :

In upper Egypt, it was shown that at least 40% of the people interviewed had used *Ambrosia maritima* L. as a drug against schistosomiasis, the leaves and flowering parts of the plant are generally used to prepare an infusion or a decoction. Sometimes the leaves are smoked or the steam of a boiled solution is inhaled to treat cold and rheumatism respectively ***(Kloos et al., 1982).***

The most cited uses of *Ambrosia maritima* L. in traditional medicine are as an antispasmodic, diuretic or in cases of kidney stones ***(Fahmy and Darwish, 1949; Helal and Hilmy, 1951; Sherif and El-Saw y, 1962).***

The plant seems to be used less frequently as a stomachic, cardial or carminative drug ***(Fournier, 1948; Abu-Shady and Soine, 1953; Helal and Hilmy, 1951).*** Its astringent activity used against nose bleeding or blood in sputum ***(Fournier, 1948; Abu-Shady and Soine, 1953)*** and its anthelmintic activity ***(Helal and Hilmy, 1951).***

In Senegal *Ambrosia maritima*L. is known as "ngadol nak" and is used to treat inflammations ***(Vassiliades and Diaw, 1980).***

Helal and Hilmy (1951) also mentioned the use of the powdered leaves (with olive oil) as a dressing for wounds and burns.

Furthermore, the plant is used in combination with other plants as a stimulant or against syphilis, as mentioned by ***Kerbaro and Adam (1974).***

Only two studies have been carried out to evaluate the pharmacological activity of *Ambrosia maritima* L. The preliminary results of ***Helal and Hilmy (1951)*** showed that there might be some antispasmodic activity on smooth muscle.

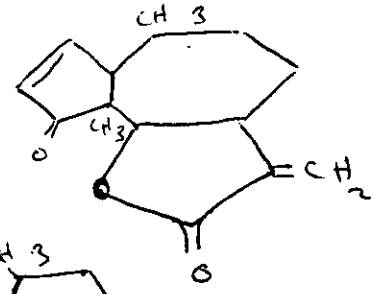
Abu-Shady and Soine (1953), however, could detect only pharmacological activity of the two major sesquiterpene lactones present in *Ambrosia maritima* L., ambrosin and damsin.

Sesquiterpene lactones : the molluscicidal principles of Ambrosia maritma L.:

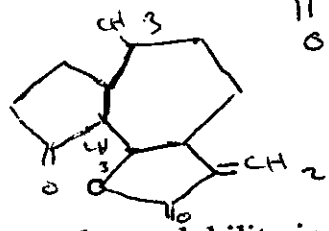
Sesquiterpene lactones, which are secondary metabolites of the compositae, contain basically three isoprene (C_5H_8) units and are formed by the oxidation of a terminal methyl group of the sesquiterpene followed by the incorporation of an oxygen atom in the cyclic skeleton. They are colorless, bitter, relatively stable, lypophilic constituents which are biogenetically derived from trans, trans farnesyl-pyrophosphate, and are essentially present in the leaves and flowering heads, rarely in the stems and the roots ***(Rodriguez et al., 1976)..***

Up till now 21 sesquiterpener lactones, mainly pseudo-guaianolides and norsesquiterpenes, have been identified in the leaves, flowers and seeds of *Ambrosia maritima*L. ***(Abu-Shady and Soine, 1953; Salama et al., 1984; Pickman et al., 1986; Jakupovic et al., 1987; Ali et al., 1990).***

(I) Ambrosin ($C_{15}H_{18}O_3$) m.p. 150°C



(II) Damsin ($C_{15}H_{20}O_3$) m.p. 102°C



Sesquiterpene lactones are known to have a low solubility in cold water, this characteristic could be responsible for a kind of slow release effect which causes the death of the snails when exposed to low concentrations for prolonged periods, the solubility of the sesquiterpene lactones in water seems to improve, in the presence of impurities***Duncan, (1985)*** or by dissolving them in ethanol and further diluting in 0.9% NaCl ***(Abu-Shady and Soine, 1953).***

The mechanism of action of the sesquiterpene lactones on the snails remains unknown until now ***(El-Sawey et al., 1978; El-Sawey et al., 1981 and Duncan, 1985).***

The molluscicidal and ovicidal activity of Ambrosia maritma L.:

*Ambrosia maritima*L. is able to control the snail vectors of both schistosomosis and distomatosis. The activity of the plant has been proven against biomphalaria alexandrina ***(Sherif and El-Sawey, 1977 and El-Sawey et al., 1978, 1981, 1984).***

Ambrosia maritima L. produces a powerful molluscicidal effect. Leaves and flowers of *Ambrosia maritima*L. infusion of 1: 1000 parts of the weed, killed snail host of the parasite The plant potency lasted 2 days and extented to the eggs and larvae of the schistosome ***(El-Sawey, 1979).***

El-Magdoub et al. (1980) showed that the *Ambrosia maritima*L. was highly effective against the intermediate host of liver fluke, the snail lymnaea caillaudi, and it was without toxicity for fish and ruminants. The molluscicidal properties of damsin (I) Ambrosin (II) and Tribromodamsin (III) were investigated against the intermediate hosts of shistosomiasis, biomphalaria alexandrina and bulinus truncatus. Although damsin was to some extent more toxic against. B. alexandrina than the other two compounds, it was less toxic against B. trancatus after 24 hour exposure period.

Concerning the ovicidal activity of *Ambrosia maritima*L., laboratory experiments have clearly shown that the plant is able to kill the snail eggs ***(Sherif and El-Sawey, 1962; Sidhom and Geerts, 1984).***

On the field study the Egyptian experiments seems to confirm this ovicidal effect, given the long duration of the molluscicidal activity after one application of *Ambrosia maritma* L. ***(El-Sawey et al., 1981 & 1984).***

Dried whole *Ambrosia maritima* L. was applied (35,70 or 140 mg/litre) to irrigation canals and drains in Egypt, in June 1981. Differences in the numbers of live snails that remained after treatment at the concentrations were small. The reduction in number of live biomphalaria in June, July and August were 21, 90 and 100%, respectively in canals and 3, 76 and 97% in drains, the snails population remained at a low level until September and October ***(El-Sawey et al., 1984).***

Ambrosia maritima L. from both Egypt and Senegal showed significant molluscicidal and ovicidal activity in relation to snail vectors of bilharzia. The Egyptian material was the more active, the aerial parts of material from both sources yielded in addition to known pseudoguaianolides,nine new compounds including 2 nor-sesquiter-pene lactones and 2 dimeric lactones. There was a far greater concentration of the more highly oxygenated pseudoguaianolides in the Egyptian material ***(Jakupovic, et al., 1987).***

El-Sawey et al. (1987) showed that the effect of *Ambrosia*

*maritima*L. on the snails biomphalaria alexandrina, the irrigation water courses at dosage levels of 560 mg/litre (fresh) and 70-140 mg/litre (dry) in May and 280 mg/litre (fresh) and 70 mg/litre (dry) in June has resulted in the reduction of snails numbers to the lowest level after 2 weeks by all treatments. The snails number reduction was maintained to the end of the year in all treatments. This confirms an earlier finding that a correctly-timed, single application of the plant is capable of controlling snails throughout the entire schistosomiasis transmission season in lower Egypt.

Belot et al. (1989) studied that molluscicidal properties of several generations of *Ambrosia maritima* L. originating in Egypt and cultivated in Senegal four generations are tested. The plants had same molluscicidal activity as the strain in Egypt.

Effect on non-target organisms :

Sesquiterpene lactones are known to have abroad range of biological activities, cytotoxic and antitumoral (ambrosin and damsin). antimicrobial, anthelmintic, phytotoxic (parthenin) and allergic effects (parthenin and ambrosin) have been described ***(Rodriguez et al., 1976; Ivie and Witzel, 1983)***

Ambrosia maritima L. has a very low toxicity to aquatic non-target organisms, it is not toxic when used at the molluscicidal

concentration of 35 to 70 mg/litre but alcoholic extracts of *Ambrosia maritima*L.were somewhat more toxic to all aquatic organisms tested due to the better solubility of the sesquiterpene lactones. Algae (selenastrum capricornutum) however, were not affected by these extracts even at doses corresponding to 1000 mg/litre of *Ambrosia maritima*L. powder ***(Alard, et al., 1991).***

Ambrosia maritima L., has been tested on rats. No toxic signs could be detected neither after oral administration of 5 g/kg. of dried leaves of the plant as a powder or as a methanolic extract nor after the incorporation of powdered leaves in the feed for 4 weeks. Using an aqueous extract of the plant material of *Ambrosia maritima*L. or using ambrosin, one of the active molluscicidal components of the plants, no mutagenic activity could be detected in the *Sal. typhimurium* strains TA 97, TA 98, TA 1538, TA 100 and TA 1535 ***(Alard, et al., 1991).***

It is generally accepted that sesquiterpene lactones are more toxic, when administrated parenterally as compared to oral treatment ***(Ivie and Witzel, 1983).***

During field experiments using a concentration of 70 mg/litre of the Egyptian *Ambrosia maritima*L. no irritation or other toxic effects in fishes have been noticed. Tadpoles and Frogs were affected by *Ambrosia maritima*L. at dose of about 375-400 mg/litre ***(Vassiliades and Diaw, 1982).***

Up till now only one case of irritant contact dermatitis in a technician, grinding the plant, was noticed by ***Abu-Shady and Soine (1953).*** This was never reported again, although many workers in different countries have manipulated the plant intensively. *Ambrosia maritima* L. belongs to the ragweeds, which are also known as "sneezeweeds", possible allergic effects should be carefully looked for. Although quite a lot of information is available on allergic responses to all kinds of plants containing sesquiterpene lactones ***(Rodriguez et al., 1976; Duncan, 1985).*** not much is known about *Ambrosia maritima* L. it

Allergic contact dermatitis is also known to be caused by sesquiterpene lactones ***(Rodriguez et al., 1976). Mitchell (1975)*** mentions Ambrosia spp. among the plants, which have been reported to cause this phenomenon. Ambrosin and Damsin, however have not been proven to be the direct cause of allergic contact dermatitis. Since they contain an α - methylene-gamma-lactone moiety and since this group was found to be responsible for allergic effects ***(Mitchell, 1975).***

The insecticidal and larvicidal properties of *Ambrosia maritima* L. have been examined by ***Sherif and El-Sawey (1962)*** on Culex and Anopheles. No toxic effects at all were noted at 1000 mg/litre whereas later more detailed experiments by ***El-Sawey et al. (1986)*** showed toxicity at 2000 mg/litre. The susceptibility of the larvae increased when the stage of development progressed.

Effect of storage on Ambrosia maritima L.:

Storage of dried *Ambrosia maritima* L. for 2 years did not affect the efficacy of the plant according to ***Sherif and El-Sawey (1962).*** This finding was not confirmed by ***Sidhom and Geerts (1984),*** however who reported a significant decrease in activity during the second year of storage of the plants. Therefore, it should be advised to use the dried plant during the first year after harvesting ***(Geerts et al., 1991).***

The physicochemical stability of Ambrosia maritima L. :

The physicochemical stability of *Ambrosia maritima* L. has been studied only partially. The effects of temperature, sunlight, silt, mud and. pH were thoroughly examined for damsin ***(Shoeb and El-Emam, 1976)*** , but not ambrosin and other sesquiterpene lactones. ***Sherif and El-Sawey (1962),*** on the other hand did some preliminary studies on the physicochemical stability of the whole dried plants. More detailed and precise experiments are needed ***(Geerts et al., 1991).***

IV- Alpinia officinarum Hance

Alpinia officinarum Hance commonly known as *L* : *Alpinia officinarum* Hance *E* : *Galangal*, *F* : *Galanga* and *A* : *Kholingan* ***(Gabre, 1987).***

Plant morphology, taxonomy and geographical distribution :

Alpinia officinarum Hance family "Zingiberaceae" a reed like plant, attaining about metre in height a native of and cultivated on the island of Hainan and the neighbouring South-East Coast of China ***(Wallis ,1967).***

Galangal rhizome : lesser galangal rhizome galangae the rhizome is dug up in the autumn, washed, trimmed cut into pieces, and dried, during the process the pale colour of the fresh rhizome turns to a reddish-brown the drug is exported in bales made of splite cane, plaited and bound round with cane ***(Wallis,1967).***

Description :The drug consists of a branched rhizome, about 12mm thick, in pieces about 5 or 10 cm long. These are frequently cylindrical but sometimes tapering or enlarged and often branched. They are dull reddish, brown, longitudinally striated or shrivelled. Here and there the broken upper end of a root remains attached to the rhizome, it is hard, tough, and difficult to break. The interior of the drug has a reddish

brown colour, the smoothed transverse surface exhibits a stele, occupying about one-third of the bundles and numerous, deep red, resin cells may be distinguished, the drug has an agreeable, spicy odour and a strongly pungent spicy taste. Fig. (D) ***(Wallis,1967).***

Constituent of Galangal rhizome :

Galangal rhizome contains a little volatile oil (cineol methyl cinnmate) and a pungent oily body, Galangal, it also contain three tasteless yellow crystalline substances, kaempferide; galangin and the monomethyl ether of galangin ***(Wallis,1967).***

Fig. (D) : Alpinia officinarum
(Wallis, 1967)

Uses of Alpinia officinarum Hance in traditional medicine :

The galangal rhizome has stimulant and carminative properties and used for dyspepsia. It is not much used in England, but is still employed in some countries both as a medicine and as a spice ***Wallis (1967).***

The rhizome is pungent, warming and promotes digestion it is prescribed to treat stomach pain and diarrhea. It is also sialogogue, tonic, and antiperiodic their use as remedy for heartburn, cholera, toothache, ague, and diseases arising from damp and chills. Indo-China, the rhizome is aromatic, stimulant and excitant in powdered form or alcoholic extract, it may be chewed to soothe, toothache and headache ***(Perry and Metzger, 1980).***

A large number of herbal drugs are used in the traditional medicine of Saudi Arabia for treatment of rheumatism, arthritis, gout and other forms of inflammation, seven of these crude drugs, namely (*Francoeuria crispa, Hammad a elegans, Malus pumila, Ruta chalepensis, Smilax sarparilla Achilleo fragrantissima* and *Alpinia officinarum* Hance) were tested against canageenan-induced acute inflammation in rats. The plant materials were extracted with 96% ethanol, the dried extract was dissolved in water for pharmacological testing. The rats were administered an oral dose of 500 mg/kg body weight of each extract 1 h. prior to production of inflammation by carrageenan of the right hind foot. Four of the seven plants, namely *Francoeuria crispa* (24%), *Malus pumila* (23%), *Ruta chalepensis* (30%) and *Smilax sarsaparilla* (25%), produced significant inhibition of carrageenan induced inflammation in rats. These plants also inhibited cotton pellet-induced exudation ***(Aqeel, et al. 1989).***

Antifungal activity of Alpinia officinarum Hance:

The pet ether extract of *alpinia officinarum* Hance was tested for antifungal activity against *Trichophyton rubrum, Trichophyton mentagrophytes, Candida albicans* and *Candida tropicalis* responsible for skin diseases in tropical countries, the pet. ether fraction containing the active substance.

The properties of active compound : The compound was highly soluble in dimethyl sulphoxide, acetone, ethyl alcohol, methyl alcohol and ethyl ether, it was moderately soluble in benzene, chloroform and carbon tetrachloride. The compound gave yellow colour in alkaline solution but on acidification it became colorless. The ethanolic solution of the compound reduced potassium permanganate solution and produced purple colour with ferric chloride solution ***(Ray and Majumdar, 1975).***

Antihepatotoxic action of Alpinia officinarum Hance:

Naturally occuring and synthetic compounds were tested at 0.01-1.0 mg/ml against carbon tetrachloride and galactosamine induced cytotoxicity in rat hepatocytes. They included three diarylheptanoids from *Alpinia officinarum* Hance, the antihepatotoxic activity of shogaols was dependent on the length of the side chains ***(Kikino et al., 1985).***

Alpinia officinarum Hance used against kidney stones :

In Saudi folk medicine : Powdered seeds of rhizomes of *Alpinia galanga* were tested for their effects on oxalate urolithiasis in male rats *Alpinia officinarum* Hance showed marked anticalculi activity and also had diuretic effects ***(Ahsan et al., 1990).***

V- Carthamus tinctorius L.

Carthamus tinctorius L. commonly known as *L* : *Carthamus tinctorius* L., *E* : *Safflower, F* : *Carthane officinal* and *A* :*Gawzer-zaapharan-Asphore-Kortom* ***(El-Khalifa and Sharkas , 1984 and Gabre, 1987).***

Plant morphology, taxonomy and geographical distribution:

Carthamus tinctorius L. family (compositae). Safflower is only known in cultivation, with primary centres in Afghanistan and the Nile Valley and Ethiopia. It is supposed to have originated from *C. lunatus* L. which occurs wild over the entire range of the genus, or more probably from *C. oxyacantha* Bieb., which occurs as a weed from Northern India to Turkey. *Carthamus tinctorius* L. was cultivated in Egypt in very early times and spread throughout the Mediterranean region and eastwards to China. It was introduced experimentally as an oil crop into the United States in 1925, particulary in California, Trials have been made in Australia and South Africa ***(Purseglove, 1974)***..

Plant morphology :

A much-branched, glabrous, herbaceous annual, 0.5-1.5 m tall, with varying degrees of spininess. Tap-root long. brnahces stiff and cylindrical, whitish in colour, leaves spirally arranged, dark green glossy, florets all tubular, usually orange-yellow in colour, seeds striated whitish in colour Fig. (E) ***(Purseglove, 1974 and Gabre, 1987).***

Fig. (E) : Carthamus tinctorius L.
(Okyl et al., 1987)

Chemical composition :

The oil content of the seeds varies from 20-38 percent. The drying oil has a high linoleic content. The florets contain 0.3-0.6 percent of scarlet red dye, carthamin, which is insoluble in water, and about 30 percent of yellow pigment, which is soluble in water and which is removed by washing the dried florets ***(Purseglove, 1974).***

Uses of Carthamus tinctorius L. :

The dried florets were the source of the red dye, "Safflower carmin" in Egypt, the Middle East and India, the colour is still used for dyeing cloth. it is also used for colouring cakes and biscuits and for rouge. Safflower is now grown mainly as an oil seed crop. In India the oil is used for cooking and soap manufacture. Else-where it is used mainly in paints and varnish; because of its low linoleic content it has excellent colour-retention properties. The tender shoots used as fodder ***(Purseglove, 1974).***

Use of Carthamus tinctorius L. in traditional medicine :

Carthamus tinctorius L. is native, from China and India into south east Asia the flowers are regarded as having stimulant, sedative, laxative (in large dose), sudorific in a warm infusion. They are used as treatment during pregnancy***(Perry and Metzger, 1980).***

In China, from April to June the flower is in full bloom; as it

opens the petals change from orange to red, the petals are picked with care and dried in the sun or heat. The drug is warming and help in circulation of the blood. It is prescribed especially for women with amenorrhea, or carrying a dead fetus or with post partum discharge. The *carthamus tinctorius* L. also prescribed for patients spitting blood. Indo-China the flowers are used as a tonic, a remedy for paralysis and dysmenorrhea. In Philippine : the dried floculi of the flower heads are used to treat Jaundice, an aqueous maceration is employed as an eyewash ***(Perry and Metzger, 1980).***

The most cited uses of *carthamus tinctorius* L. seed in traditional medicine are as a remedy for apoplexy and dropsy. The seeds contain oil, principally linoleic acid and glyceride another analysis yielded myristinic, arachidic, lignoceric, and linoleic acid ***(Perry and Metzger 1980).***

In Saudi Arabia seeds are purgative diuretic, reduce cholesterol bl. level treatment of arteriosclerosis, and in cases of eczyma in children and the dried flowers are used in treatment of psycholo gical and neurological disturbance, emmenagogue, and usefull in cardiac pain, while the boiling root extract is used as analgesic in toothache but leaf juice used as potent emetic ***(Okyl et al. 1987).***

Suppressive effect of safflower yellow on immune function :

Safflower yellow (SY) extracted from *Carthamus tinctorius* L. contained chalconoid compounds, 75% of which is safflomin A. (SY) 50-450 mg/kg in mice decreased serum lysozyme concentration and phagocytosing functions of both peritoneal macrophages and peripheral leucocytes. It diminished the production of plaque forming cells, specific rosette forming cells, and antibody; inhibited delayed type hypersensitivity reaction. Experiments in vitro showed inhibitory effects incorporation during human peripheral T and B lymphocyte proliferation by (SY), respectively, murine mixed lymphocyte culture response and the production of interleukin. In conclusion (SY) produced declines, in both non specific and specific immune functions ***(Lu et al., 1991).***

The mutagenic potential of Carthamus tinctorius L. :

After extraction with boiling water and frozen vaccum drying, preparation from the drug was tested with the Ames test, and the micronucleus and chromosomal aberration assays in mice in vivo. The extract was found to induce revertants in *Salmonella typhimurium* TA 98 and or TA100 beside that the extract was positive in the chromosomal aberration and micronucleus assays in mice ***(Yin et al., 1991).***

Reduction of cellular damage induced by cerebral ischemia in rats :

Herbal preparation and nifedipine was investigated for reduction of cell damage following cerebral ischemia. The herbal preparation contained ginsengosides and extracts of *Carthamus tinctorius* L., *Panax notoginseng* and *Ligusticum chuanxiong* and *Salvia militorrhiza* Bge. The histological evidence of cell damage and the formation of paroxidation products were both reduced in rats pretreated with the herbal preparation or with nifedipine.

The results show that in this model of in complete cerebral ischemia, the degree of lipid peroxidation can be lowered by the pretreatment with Chinese herbs containing ginsengosides or with nifedipine. These drugs may be beneficial in the treatment of cerebral ischemia in humans ***(Leung, et al., 1991).***

Hypotensive effects of Carthamus tinctorius L.:

Safflower yellow (SY) is a mixture of chalconoid compounds extracted from *Carthamus tinctorius* L. (SY) lowered the blood pressure of spontaneously hypertensive rats (SHR) for about five weeks after administration of (SY). The plasma renin activity and angiotension II level diminished in (SHR) experimental groups. These suggest that the decrease of blood pressure is mediated by the renin angiotensin system ***(Liw et al., 1992).***

The plant reported to possess antibacterial and antiviral activity :

In this part scientific literature that deal with the chemical studies on plants with antimicrobial activity have been surveyed up to 1992, the plant species are categorized table (B) Plants reported to possess antibacterial activity and table (C) Plants reported to possess antiviral activity.

Table (B) : Plants reported to possess antibacterial activity

Plant species	Active compound	Active against	References
Achillea conferta	Flavonoids	Gram+ and Gram - bacteria	Nadir et al. (1991)
A. fragrantissima	Volatile oil	Gram+ and Gram - bacteria	Barely et al. (1991)
Adiantum capillus-veneris	Flavonoids	Gram+ and Gram - bacteria	Mahmoud et al. (1989)
Aframomum melegueta	Volatile oil	Gram+ and Gram - bacteria	Igwilo et al. (1991)
Allium ampeloprasum L. (elephant gralic)	Diallyl thiosulphinate (Allicin)	E. coli, staph aureus and antifungal	Hughes and Lawson (1991)
A. sativum (gralic)	Methyl allyl thiosulphinate, and allyl methyl thiosulphinate	E. coli, staph aureus and antifungal	Hughes and Lawson (1991)
A. cepa (onion)	" " " " " " " " " " " "	E. coli, staph aureus and antifungal	Hughes and Lawson (1991)
Albizia amara	Novel macrocytic alkaloids (budmunchiaminus)	Sal. typhimurium (TM 677)	Mar et al. (1991)
Anacardum occidentale	Cashew gum exudate	Bacteriocidal and fungicidal	Marques et al. (1992)
Annona salzmanii D.C.	Benzylis oquinoline alkaloids	Gram+ and Gram - bacteria	Paulo et al. (1992)
Andrographis paniculata (Kal-megh)	4 diterpenes (Andrographolibe,neoandrographolide deoxyandrographolide and andrographiside	E. coli enterotoxin (LT and LT/st)	Gupta et al. (1990)
Artemisia caerulescens subsp.	Volatile oil	Bacillus subtilis and E. coli	Moran et al. (1989)
A. afra jacq.	Volatile oil	Posses broad-spectrum activity	Graven et al. (1992)
A. barrelieri	Sesquiterpene lactones	Antibacterial activity	Marco et al. (1991)
A. granatensis	Volatile oils, tannins, sterols, triterpenes, saponins, flavonoids, leucoanthycanins, heterosides and anthraquinones	Bacillus subtilis, E. coli and micrococcus luteus	Diaz et al. (1989)
Brosimopsis oblongifolia	Three new flavone derivatives (brosimone G,H and I)	Staph. aureus and Candida glabrata	Ferrari et al. (1989)
Borreria ocymoides	Alkaloids, cardiacglycosides, Aqueous and Alcoholic extract	Gram+ and Gram - bacteria	Ebana et al. (1991)
Brachylaena hutchinsii	Sesquiterpenoids (ylangenol)	Strept. mutans and Brevibacterium	Vieira et al. (1991)
Bixa orellana	Pentacyclic triterpene maslinic acid	Bacillus subtilis	Simpol et al. (1989)
Buddleia asiaticalour	Volatile oil	Sal. spp., Shigella shiga and vibriocholera	Garg et al. (1992)
Bystropogon plamosus	Volatile oil	Staph aureus,Ps..aeruginosa and E.coli	Economow et al. (1991)
B. origanifolium var. palmenises	Volatile oil	Staph aureus,Ps..aeruginosa and E.coli	Economow et al. (1991)
B. widpretii	Volatile oil	Staph aureus,Ps..aeruginosa and E.coli	Economow et al. (1991)
B. moderensis	Volatile oil	Staph aureus,Ps..aeruginosa and E.coli	Economow et al. (1991)
B. canariensis var. smithianus	Volatile oil	Staph aureus,Ps..aeruginosa and E.coli	Economow et al. (1991)
Cassia alata	Rhein	Antibacterial and antifungal	Ogunti et al. (1991)

Table (B) : Continued

Plant species	Active compound	Active against	References
Calamintha sylvatica subsp. Ascendens	Volatile oil	Bacteria and fungus	Ortiz de Urbina et al. (1988)
Capsella	Alkaloid compound (yohimbine and erojgeristine) flavonoid compound (flavone diosmin)	Actinomyces P. and yeast	El-Abyad et al. (1990)
Carex kobomugi ohwi	Kobophenol A., a unique tetrastilbene	Staph. aureus	Kawabata et al. (1989)
Chaenactis douglasii	Thiarubine A, adithiacyclohexane dinepolyine	Bacteria and fungus	Constabel and Towers (1989)
Chamaemelum nobile	VI hydroperoxides compound	Antibacterial activity	Mericli (1990)
Cinnamomum/zeylanicum	Cinnamon	Antibacterial activity	Islam et al. (1990)
Cola nitida	Alkaloids, cardiac glycosides aqueous and alcoholic extract	Gram+ and Gram - bacteria	Ebana et al. (1991)
Chenopodium botrys	Volatile oil	Gram+ and Gram - bacteria	El-Sayed et al. (1989)
Cupressus funebris	Flavonoid	Gram+ and Gram - bacteria	Parveen et al. (1989)
Eucalyptus perriniana	Grandinol and phloiroglucinol-terpene	Staph. aureus and bacillus subtilis	Nakayama et al. (1990)
Eucalyptus	Volatile oil	Gram+ and Gram - bacteria	Dellacassa et al. (1989)
Eugenia uniflora	Volatile oil	Antibacterial and antifungal	Adebajo et al. (1989)
Elettaria cardamomum	Cardamom	Antibacterial activity	Mericli (1990)
Epimeredi indica	Volatile oil	Gram+ and Gram - bacteria	Yadava and Saini (1991)
Eriodictyonangusti folium	Benzyl-trans-4-coumarate and 16-hydroxy carnosic acid	Gram+ and Gram - bacteria	Dentali and Hoffman (1992)
Garcinia kola	Alkaloid is an cardiac glucosides, aqueous and alcoholic extract	Gram+ and Gram - bacteria	Ebana et al. (1991)
Guardiola platylla	O-catecholderivaties	Gram+ and Gram - bacteria	Wahyouno et al. (1991)
Gerbera anandia	3,8-dihydroxyle-methoxy-coumarine 3,8-dihydroxyle methoxy 2-oxozhi benzopyrans curboxjlic 5,8-dihydroxyle 7-14-hydroxy-5 methyl (coumiriae 3)-coumarine	Antibacterial activity	Gu et al. (1989)
Glycyrrhiza (glabra)	Licoflavonone	Bacillus subtilis,staph.aureus, candida albicans	Fukui et al. (1988)
Ghaphalium robustum	Acylated flavonoid agylcones	Bacillus anthracis	Urzua and Cuadra (1990)
Helichrysum species	Flavonoids	Gram+ and Gram - bacteria	Cosar and Cubukcu (1990)
H. odoratissimum	Flavonoids and chalcone	Possess broad spectrum activity	Puyvelde et al. (1989)
Hyptis suaveolens	Volatile oil	Gram+ and Gram - bacteria	Iwu et al. (1990)
Helichrysum picardii	5,7-dihydroxy-3,8-dimethoxy flavone	Gram+ and Gram - bacteria	Tomas et al. (1991)
Hippocratea welwitschii	Volatile oil	Broad spectrum activity	Iwu et al. (1991)
Hypericum drummondii	Filicinic acid derivatives	Staph. aureus, bacillus subtilis and myocabacterium smegmatis.	Hiranthi et al. (1991)

Table (B) : Continued

Plant species	Active compound	Active against	References
Hebeloma senescens	New farnesane sesquiterpenes	Bacillus subtilis and Staph.aureus	Bocchi et al. (1992)
Indigofera microcarpa	Benzofurans	Against bacteria and fungus	Moraes et al. (1991)
Landsburgia quercifolia	Naphthoquinone deoxylapachol	Against bacteria and fungus	Perry et al. (1991)
Laportea aestuans	Steroids	Against bacteria and fungus	Adebajo et al. (1991)
Ligusticum stewartii	Volatile oil	Gram+ and Gram - bacteria	Meenasyed et al. (1989)
Lindera benzoin	Y-lactones and methyl ketoalkenes	Bacteriocidal	Anderson et al. (1992)
Liver worts	(DMSO) and aqueous fraction	Gram+ and Gram - bacteria	Joshi et al. (1992)
Lactarius deliciosis	Stearic acid ester of sesquiterpene (I)	Antibacterial activity	ANKE et al. (1989)
L. deterrimus	Sesquiterpene aldehyde (lactaroviolin II)	Antibacterial activity	ANKE et al. (1989)
L. sanguifluus	and Alcohol deterrol III	Antibacterial activity	ANKE et al. (1989)
L. flavidulus Imai	Threenew geranylphenols, flavidulols A,B,C	Staph. aureus and other bacteria	ANKE et al. (1989)
Magnolia virginiana	Neolignans	Antibacterial activity	Nitao et al. (1991)
Moringa oleifera	Fresh leaf juice and aqueous extracts	Gram+ and Gram - bacteria	Caceres et al. (1991)
Mentha arvensis	Volatile oil	Gram+ and Gram - bacteria	Singh et al. (1992)
Nigella sativa	Volatile oil	Gram+ and Gram - bacteria	Hanafy and Hatem (1991)
Origanum majorana	Volatile oil	Gram+ and Gram - bacteria	Yadava and Saini (1991)
Perilla frutescens	Perill aldehyde	Gram+ and Gram - bacteria	Kang et al. (1992)
Peganum harmala	Harmine, harmaline and harmalol	Gram+ and Gram - bacteria	Adaay et al. (1989)
Phaeanthus vietnamensis	Isoquinoline alkaloids	Gram+ and Gram - bacteria	Nguyen et al. (1991)
Piper sarmentosum	Phenyl propanoids	E. coli and bacillus subtilis	Masuda et al. (1991)
Pycnocycla aucheriana	Volatile oil	Gram+ and Gram - bacteria	Meena et al. (1989)
Phyllanthus discoideus	Alkaloids, viroallosecurinine and securinine	Gram+ and Gram - bacteria	Mensah et al. (1990)
Prosopis juliflora	Julifloricine	Gram+ and Gram - bacteria	Aqeel et al. (1989)
Psiadia trienervia	Flavonoids	Antibacterial and antifungal	Wang et al. (1989)
Pulicaria Gispa	Volatile oil	Gram+ and Gram - bacteria	Al-Yahya et al. (1989)
Rudbeckia hirta	Thiarubrines	Against bacteria and fungas	Constabel et al. (1989)
Rutales	Limonoids	Bacteriocidal and fungicidal	Champagne et al. (1992)
Rosa rugosa	Carotane sesquiterpenes	Antibacterial action	Hashidoko et al. (1992)
Seseli libanotis	Volatile oil	Gram+ and Gram - bacteria	Meenasyed et al. (1989)
Sanguinaria	Benzo-phenanthridine alkaloids	Antibacterial activity	Rho et al. (1992)
Salsola rosmarinus	Flavonoids	Gram+ and Gram - bacteria	Mahmoud et al. (1989)
Salvia canariensis	Diterpenes	Gram+ and Gram - bacteria	Gonzalez et al. (1989)
S. cardiophylla	Diterpenes	Gram+ and Gram - bacteria	Gonzalez et al. (1989)
S. aegyptiaca	Diterpene quinones	Gram+ and Gram - bacteria	Sabri et al. (1989)
S. texana	Diterpenes	Gram+ and Gram - bacteria	Gonzalez et al. (1989)
Suillus granulatus	Tetraprenyl phenols	Possess antibacterial properties	Tringali et al. (1989)

Table (B) : Continued

Plant species	Active compound	Active against	References
Tabernaemontana divaricata	Indol alkaloids	Staph. aureus	Arambewela and Thiliniranatunge (1991)
Thevetia peruviana	Distillate of seed oil	Gram+ and Gram - bacteria	Obasi and Igboechi (1991)
Thymus leptophyllus	Volatile oil	Gram+ and Gram - bacteria	Zafra-Polo et al. (1989)
T. loniflorus Boiss	Volatile oil	Gram+ and Gram - bacteria	Cruz et al. (1989)
Tanacetum densum subsp. sipasiam	Sesquiterpene laitones	Bacillus subtilis and klebsiella pneumonia	Gören et al. (1992)
T. vulgare	Volatile oil	Gram+ and Gram - bacteria	Neszmelyi et al. (1992)
Ziziphoraclino podioides	Phenolic and flavonoid	Gram+ and Gram - bacteria	Oganesyan et al. (1991)

Table (C) : Plants reported to possess antiviral activity

Plant species	Active compound	Active against	References
Anagallis arvensis	Triterpene saponins	Two antiviral activity	Amoros and Girre (1987)
Abrus precatorius	Foundin crude leaf extracts	Tobacco mosaic virus	Beshney and Moghe (1989)
Astragalus membranaceus	Isoastragaloside IV and isoflavone glycoside	Antiviral activity	He and Findlay (1991)
Artemisia parviflora	Sesquiterpene artecanin and the flavone jaceosidin	Vaccinea virus	Saradhavasanth and Purushothman (1989)
A. annua	Sitosterol and stigmasterol	Tobamoviruses	
A. vulgaris	Polyines	Murine cytomegalovirus and sindbis virus	Khan et al. (1991)
Alternanthera brasiliana	Found in crude aqueous extract	Aujeszky virus (ADV) and bovine diarrhoea virus (BVD)	Hudson et al. (1991)
			Koseki et al. (1990)
A. ficoidea	Found in crude aqueous extract	(ADV) and (BVD)	Koseki et al. (1990)
Amaranthus spinosus]	Found in crude aqueous extract	(ADV) and (BVD)	Koseki et al. (1990)
Ageratum conyzoides	Volatil oil	Cowpeamosaic v. (CPMV)& mungbean mosaic virus (MBMV) & bean common mosaic virus (BCMV)	Rao et al. (1986)
Alseis blackiana	Aqueous and ethanolic extract	(Various human virus) Antiviral and virucidal activities	Roming et al. (1992)
Aspidosperma megalocarpon	Aqueous and ethanolic extract	(Various human virus) Antiviral and virucidal activities	Roming et al. (1992)
Castanospermum australe	Sulphated polysaccharides	Antirhino virus and anti-HIV agents	Vlietinck and Berghe (1991)
Cedrela tubiflora	Found in crude leaf extract	Herpes simplex, pseudorables and vesicular stomatits virus	Cordoba et al. (1991)
Cornus capitata	Found in plant extract	Tobamo viruses	Khan et al. (1991)
Collybia maculata	6-Methyl purine 6-Methyl-9-D-D-ribofuremosylpurine 6-Hydroxymethyl-9-13-D-ribofuronosypurine	Antiviral activity	Leonhardt et al. (1987)
Callistemon citrinus	Volatile oil	Cowpea mosaic V. (CPMV) mung	Rao et al. (1986)
Carum copticum	Volatile oil	Bean mosaic V. (MBMV) bean common mosaic virus (BCMV)	Rao et al. (1986)
Clerodendrum inerme	Thuja, carbendazim and resorcinol	Cucumber mosaic virus	Cheema et al. (1991)
Calendula arvensis	Triterpenoid saponins	Vesicular stomatitis virus & rhinovirus	Tommasi et al. (1991)
Datura stramonium	Thuja, carbindazim and resorcinol	Cucumber mosaic virus	Cheema et al. (1991)

Table (C) : Continued

Plant species	Active compound	Active against	References
Echinops bannaticus	Polyines	Murine cytomegalovirus and sindbis virus	Hundson et al. (1991)
Glycyrrhiza glabra	Saponin glycyrrhizin	Antirhino virus and anti-HIV agents	Vlietinck et al. (1991)
Hypericum	Naphthobian thrones hypericin and pseudohypericin	Antirhino virus and anti-HIV agents	Vlietinck et al. (1991)
Hybanthus prunifolius	Found aqueous and ethanolic extract	Antiviral and virucidal activities	Roming et al. (1992)
Iresine herbstii	Found in crude aqueous extract	Aujeszkyvirus (ADV) and bovine diarrhoea v.(BVD)	Koseki et al. (1990)
Jacaranda jasminoides	Found in crude aqueous extract	Aujeszkyvirus (ADV) and bovine diarrhoea v.(BVD)	Koseki et al. (1990)
Kunzea ericoides	Phloroglucinol	Herpes simplex type I and polio type I virus	Bloor (1992)
K. sinclairii	Phloroglucinol	Herpes simplex type I and polio type I virus	Bloor (1992)
Lawsonia inermis	Found in plant extract	Tobamo viruses	Kan et al. (1991)
Lophira alata	Tetraflavonoids	EB virus	Tih et al. (1992)
Leucanthemum vulgare	Polyines	Murine cytomegalovirus and sindbis virus	Hundson et al. (1991)
Mirabilis Jalapa	Found in crude aqueous extract	Aujeszkyvirus (ADV) and bovine diarrhoea v.(BVD)	Koseki et al. (1990)
Magnolia officinalis	Neolignans	Epstein Barr virus activation	Konoshima et al. (1990)
Ocimum sanctum	Volatile oil	Cowpeamosaric v. (CPMV)& mungbean mosauc virus (MBMV) & bean common mosaic virus (BCMV)	Rao et al. (1986)
Ouratea lucens	Aqueous and ethanolic extract	(Various human virus) Antiviral and virucidal activities	Roming et al. (1992)
Prunella vulgaris	Sulphated polysaccharides	Antirhinovirus and Anti-HIV agent	Vlietinck and Berghe (1991)
Piper cordulatum	Aqueous and ethanolic extract	Various human virus	Roming et al. (1992)
Peperomia pellucida	Volatile oil	(CPMV) (MBMV) (BCMV)	Rao et al. (1986)
Peganum harmala	Aqueous seed extract	DNA-containing herpes virus horminis type I (HSV-1)	Rashan et al. (1989)
Phyllanthusamarus	Hydrolysable tannin	Hepatitis B virus	Foo and Wongg (1992)
Santolina chamaecyparissus	Polyines	Murine cytomegalovirus and sindbis virus	Hundson et al. (1991)
Tetragastris panamensis	Aqueous and ethanolic extract	Various human virus	Roming et al. (1992)
Trichilia cipo	Aqueous and ethanolic extract	Various human virus	Roming et al. (1992)
Tripterygium wilfordii	Tripterifordin	Anti-HIV replication activity	Chen et al. (1992)
T. wilfordii	Salaspermic acid	HIV reverse transcriptase & HIV replication	Chen et al. (1992)
Vicoa indica	7 triterpenes	Rainkhet disease virus	Chowdhury et al. (1990)
Viola yedoensis	Sulphated polysaccharides	Antirhinovirus and Anti-HIV agent	Vlietinck and Berghe (1991)
Vernonia amygdalina	3-methylquercetin	Antiviral activity	Laekeman et al. (1986)

MATERIALS AND METHODS

Plant Materials :

Nigella sativa L seeds, *Alpinia officinarum* Hance rhizomes, *Peganum harmala* L. seeds, and *Ambrosia maritma* L. leaves were obtained from herbalist shops.
Carthamus tinctorius L. "seed" were obtained from birds shops

These plants are verified by Dr. Samia S. Hafez, Lecturer of Pharmacognosy, Faculty of Pharmacy, Zagazig University.

Chemicals :

Ethanol 95% diethyl ether, light petroleum 40-60%.

Equipment :

Glass wares and filter papers (Whatmann No.1), bottles with metalic screw caps, sterile cotton swabs, aluminium foil, rotatory evaporator (Buchi r.p.m 111) and ground stoppered flasks .

Bacteriological media :

Mueller Hinton agar	(Becton-Dickinson)
Mueller Hinton broth	(Becton-Dickinson)
MacConkey agar	(Prolabo)

Nutrient agar	(Oxoid)
Blood agar	(10% blood on neutrient agar)

Bacterial strain :

The following strains were isolated from the following samples after complete bacteriologic identification :

E. coli	From	Stool
Strept. faecalis	From	Stool
Staph. aureus	From	Abscess
Pseudomonas aeruginosa	From	Septic wound
Sal. typhimurium	From	Central Laboratory of Ministry of Health Cairo

Antibiotic Sensitivity Discs :

Antibiotic sensitivity discs were obtained from commercial sources demonstrated in Table (D). This Table were obtained from (Oxoid)

Table (D)

Commercial name of antibiotic		Concentration for disc	Scientific Name	Zone of inhibition (mm)		
				≥ Sensitive	≤ Resistance	Intermediate
Ampicillin	AM	10 µg	Ampicillin	14	11	12-13
Duricef	C.F.	30	Cefadroxyl			
Cefatrexyl	C.F.	30	Cephapirin			
Garamycin	G.M.	10	Gentamicin	13	12	-
Negram	N.A.	30	Nalidixic Acid	19	13	14-18
Neomycin	N.	30		17	12	13-16
Amikin	A.K.	30	Amikacin	17	14	15-16
Chloramphenicol	C	30		18	12	13-17
Cefobid	CFP	75	Cefoperazone	21	15	16-20
Amoxil	AMX	25	Amoxycillin	17	14	15-16
Norxin	NOR	10	Norfloxacin	17	12	13-16
Ceftriaxone	CRO	30	Ceftriaxone	21	13	14-20
Trimethaprin-sulphamethoxazole	SXt	1.25+23.75	Trimethaprin-sulphumetoxasole	16	10	11-15
Sulphonamides	S_3	300		17	12	13-16

All authentic antibiotic discs were obtained from Pasteur Lab. A.R.E. except ceftriaxone from (Oxoid) England and trimethoprim- sulphamethoxanzole (SXt) from Biomerieux.

Methods

1- Identification of organisms :

A- Microscopic examination

B- Cultural characters

C- Biochemical reaction

2- Sequential exhaustive extract method of crude plant material :

Principles :

The aim of this experiment was to evaluate the ability of sequential exhaustive extraction with light petroleum followed by ether followed by ethanol with water to detect the presence of any antibacterial substances found in plants under study.

This experiment was carried out to clarify the organic solvent which is suitable for extraction of suspected antibacterial substances of the five plants (*Nigella sativa* L., *Peganum harmala* L., *Carthamus tinctorius* L., *Ambrosia maritima*L. and *Alpinia officinarum* Hance).

Preparation of extracts of Nigella sativa L. :

The following method was used to prepare different extracts from *Nigella sativa* L.. light Pet. extract, diethyl ether extfact and aqueous alcoholic extract *Nigella sativa* L. were prepared as follow :

* The air dried powdered plant material (500 gm) was extracted with

ethanol 95% almost till exhaustion. This extract was evaporated and give a total residue of (7.31 gm). This residue was partitioned between aqueous alcohol (1:1) and light Pet. (6x200 ml). i.e. (the aqueous alcoholic extract was extracted with 6 times of light Pet. each with 200 ml). The light Pet. fractions were combined and concentrated and afford a total residue (2.50 gm) the remaining aqueous ethanolic portion was extracted with diethyl ether (6x200 ml) and concentrated and afford yield with residue (2.01/gm). The residue remained aqueous ethanolic extract evaporated to give (2.80/gm).

* The same experimental procedure was repeated with other plants under study : (*Peganum harmala* L., *Ambrosia maritima* L., *Alpinia officinarum* Hance and *Carthamus tinctorius* L.), the different colours and weights of different extracts were demonstrated in table (E) and (F)

Table (E)

Different colours of extracts after evaporation

Plants / extracts	Light Pet.	Diethyl ether	Aqueous alcohol
Nigella sativa L.	Yellowish brown	Yellowish brown	Dark yellowish brown
Peganum harmala L.	Red colour	Redish yellow	Redish brown
Ambrosia maritima L.	Dark green	Yellowish green	Greenish brown
Alpinia officinarum Hance	Faint brown	Yellowish brown	Brownish orange
Carthamus tinctorius L.	Dark yellow	Pale yellow	Yellow

Table (F)

Different weights of extracts after evaporation

Plants / extracts	Light Pet.	Diethyl ether	Aqueous alcohol	Total extract
Nigella sativa L.	2.50 g.	2.01 g.	2.80 g.	7.31 g.
Peganum harmala L.	3.90 g.	2.58 g.	4.47 g.	9.95 g.
*Ambrosia maritima*L.	3.52 g.	3.27 g.	2.90 g.	9.69 g.
Alpinia officinarum Hance	3.46 g.	1.86 g.	4.92 g.	10.24 g.
Carthamus tinctorius L.	3.07 g.	2.49 g.	4.56 g.	10.12 g.

3- Disc diffusion sensitivity testing :

Disc diffusion technique was carried out as described by ***Cruickshank et al. (1975) and Finegold and Martin (1982)*** as follows :

A- Preparation of antibacterial sensitivity discs :

Sensitivity discs were prepared as described by ***Cruikshank et al. (1975)*** as follows :

* Filter paper discs (England by Whatman Limited) were made manually, from Whatmann No. 1, as (6mm) in diameter.
* Batches of 100 discs were placed in screw capped bottles which loosely capped and sterilized in the oven at 140°C for 60 minutes then they were allowed to cool to room temperature.

B- Semiquantitative evaluation of the antibacterial activity of extracts of different plants under test :

Double fold dilutions of each extract (Light Pet., ether, Aqueous alcohol) of different plants were prepared at a concentration of (80, 40, 20, 10 mg/ml solvent) as follow :

- 2 gm of extract + 25 ml of solvent
- 1 gm of extract + 25 ml of solvent
- 0.5 gm of extract + 25 ml of solvent
- 0.25 gm of extract + 25 ml of solvent
- 1 ml aliquot of these dilutions was adsorbed on bottles containing batches of 100 filter paper discs.
- Bottles were placed in water bath at 50°C with occasional shaking to mix discs to allow even distribution of extract between discs till the extract is completely absorbed by discs and the disc become completely dry (Complete evaporation of solvent)
- Each filter paper contain 100, 200, 400, 800 µg/disc.

C- Preparation of bacterial inoculum :

* One or two colonies of tested organism were transferred using a sterile loop to a tube containing 5 ml of Mueller-Hinton broth.
* The broth was incubated at 37°C for overnight
* Inoculated broth was diluted 1/100 in case of *Staph. aureus* and *Strept. faecalis* by using sterile saline. In case of *Sal. typhimurium*

and *Ps. auroginosa*, and *E. coli* the inoculated broth was diluted 1/1000.

D- Inoculation of the test plate :

* A sterile cotton swab was dipped into diluted bacterial suspension.
* The excess fluid was removed by rotating the swab with firm pressure against the inside of the tube above fluid level.
* The swab was then used to streak the dried surface of a Mueller-Hinton plate (in three different planes by rotating the plate) approximately 60 degrees each time to ensure an even distribution of the inoculum.
* The plates were replaced and the inoculated plates were allowed to remain on a flate level undisturbed for 5-10 minutes to allow the adsorption of excess moisture then discs were applied.

E- Placement of discs :

With fine pointed forceps, the selected disc (impregnated with extract of plant) were placed on the surface of the inoculated plate and pressed firmly into the agar to ensure complete contact with the agar. Adequate spacing between different discs (at least 24 mm. apart from center to center of every two discs) and between discs and away from the wall of petri-dish (at least 15 mm. away) was done. The same method were used for placement of authentic antibiotic discs.

F- Reading of the results :

After incubation (18 hours), the diameter of zones of inhibition of bacterial growth produced by diffusion of the antibacterial agent from discs into the surrounding medium was measured using a transparent plastic ruler.

G- Interaction between the ether extract of Nigella sativa L., Aqueous alcoholic extract of Peganum harmala L. and Light Pet. extract of Alpinia officinarum Hance with some antibiotic drugs :

* Disc diffusion techniques was used for this study as described by ***Krogstad and Moellering (1979).***
* Two types of discs were used one containing ether extract of *Nigella sativa* L. (800 µg/disc) and the other disc containing an antibiotic (Commercial disc).
* The two types of discs were placed on the surface of Mueller-Hinton agar plates inoculated with different types of organisms (*E. coli, Sal. typhimurum, Ps. aeruginosa, Strept. faecalis* and *Staph. aureus*).
* The distance between the two discs was slightly greater than the sum of the radii of the zones of inhibition when tested separately.

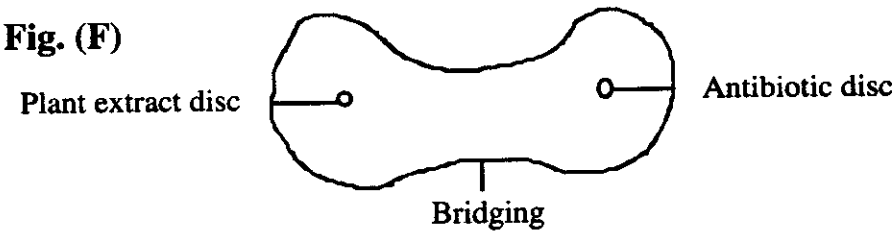
Plates were incubated at 37°C for 18 hours.

* Bacterial growth at the area between the two discs was examined and results were interpreted as shown in Figures as Fig. F, G, H.

* The above method was repeated with using aqueous alcoholic extract of *Peganum harmala* L. (800 µg/disc) and Light Pet. extract of *Alpinia officinarum* Hance (800 µg/disc).

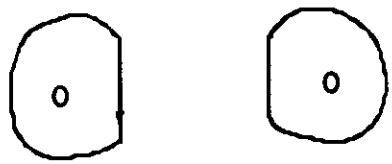
I - Antibacterial synergism when bridging between the zones of inhibition was observed

Fig. (F)



II - Antibacterial antagonism when truncation was observed or

Fig. (G)



Truncation (Antagonism)

III - Additive antibacterial activity when two independent circular zone of inhibition were observed *Krogstod and Moellering (1979)*

Fig. (H)

Two independent circles (Additive)

<u>The antibacterial activity of different boiled extract of Nigella sativa L.</u>

The light Pet. extract Benzene extract, Alcoholic extract and Aqueous extract of *Nigella sativa* L. which were kindly prepared in

National Research Centre with help of Dr. Awatif Khattab. Extracts were tested at 400., 800, 1600, 3200 µg/disc, on Mueller Hinton agar plate inoculated with three different organisms(*Staph. aureus, Sal. typhimurium* and *Ps. aeruginosa*), using the technique as in non boiled of *Nigella sativa* L.