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ANTIMICROBIAL ACTIVITIES OF SOME PLANTS USED IN FOLK MEDICINE IN SINAI EGYPT.

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By

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ABSTRACT

Sinai's medicinal plants were screened for their antimicrobial activities against ten different microorganisms. The ethanolic extracts of the shoot system of 41 plant species belonging to 20 families were examined against gram-positive, gram-negative bacteria, a yeast, dermatophytes and a filamentous fungus. 75.6% of the plant species showed activities against the test organisms . Of them 19.5% were active against gram-positive bacteria , 12.3% active against gram-positive and gram-negative bacteria, 12.1% active against gram-positive bacteria and fungi, and 31.7% showed broad spectrum activity.

A preliminary phytochemical screening of *Ricinus communis* shoots showed the presence of tannins; unsaturated sterols and terpenes; alkaloids; flavonoids; glycosides and/or carbohydrates. An antimicrobial agent (Rc 18) was isolated from fresh petiols and stems of *Ricinus communis* L. Its physical, physio-chemical and biological characterstics were studied.

INTRODUCTION

Infectious diseases are of ancient origin. Mankind has a venerable history of use of higher plant extracts for the therapy of such infections. Nothing was reliable known about the nature of infectious diseases untill the 1800s.

The modern antibiotic era were opened with the first clinical trail of penicilin in early 1941. The success with penicilin prompted the search for other antibiotic substances in nature and was also extented to cover the higher plants.

Several individual articles have been published giving the result of large screening studies. The beginning of this new period of intensive search is marked by the work of Osborn (1943), who screened 2300 species from 166 families against *Staphylococcus aureus* and *Echerichia coli*. As a result 63 genera were shown to contain antimicrobial substances which inhibited the growth of at least one of the tested bacteria. Later Huddleson et al. (1944), studied the antimicrobial activities of 23 genera belonging to 15 families against *E. coli*, *S. aureus* and *Brucella abortus*. Hayes (1946) found that 46 plant out of 231 were active against at least one of the test organisms, *S. aureus*, *E. coli*, *Erwinia cartovora* and *Phytomonas tumefaciens*. Carlson et al. (1948) found 115 of 550 plants to have antibacterial activities against *S. aureus* and *E. coli*. Sporoston et al. (1948) found *Impatiens biflora* to be the most active of 73 vermont plants. Atkinson (1949) studied 1200 Australian flowering plants and reported 50 species active against *S. aureus* end 4 active against *Salmonella typhi*. Mariam George and Pandalai (1949) found the aqueous and ethanolic extract of 90 indian plants had antibacterial activity (in vitro); and so on (see for example freerksen and Bonicke, 1951; Hughes, 1952; Tanabe, 1954; Maruzzella and Freundlich, 1959; Celayeta, 1960; Abdou et al, 1972; El-hissy and Ahmed, 1973; Ross et al, 1980; Wat et al, 1980; Mishenkova, et al, 1985; and Nishino et al,, 1970). These screening reports illustrated that it is reasonable to find useful chemotherapeutic agents from higher plants.

In addition, individual papers from time to time describing study of individual plants for antimicrobial activity. These papers are evidence of a continued interest in antimicrobial agents from higher plants.

In this work we wish to report on the antimicrobial activity of plants used in folk medicine in Sinai.

MATERIALS AND METHODS

Plant material

Samples of the shoot system of 41 plant species belonging to 20 families were collected from sites throughout Sinai. These were wild and cultivated plants which are usually used in folk medicine by the Sinai bedouins.

Herbarium specimens have been deposited in the Botany Department's hebarium, Faculty of Science, Suez Canal University.

extraction procedures :

Fresh plant materials were macerated in a mechanical macerator at room temperature with redistilled methanol. Each extract was then filtered and the process repeated until the plant was exhausted .

The total extract from each plant material was concentrated under reduced pressure in a rotary evaporator at 40 ° C. Care being taken to avoid total dryness which led to loss of active material due to polymerisation.

Determination of Antimicrobial Activity:

I-Diffusion Methods:

Disc Diffusion Method:

Crude extracts were tested for antimicrobial activity against gram-positive, gram-negative bacteria, a yeast dermatophytes, and a filamentous fungus as listed (Table 1) Cultures of bacteria were maintained at 27 °C on Nutrient agar slants, dermatophytes and yeasts on Sabouraud agar and filamentous fungi on Czapek's dox agar.

The activity of crude extracts were assayed by dipping 6 mm. diameter paper discs into the test sample, draining then transferring the discs to the surface of an agar plate previously seeded with the test organism.

For quantitative bioassays, known volumes of the test solution measured onto the paper disc with a micropipette. A solvent blank paper disc were included in all bioassays.

Plates were pre-incubated at 4 ° C for an hour to permit maximum diffusion of the substances. After 24-72 hours of incubation at 27 ° C, the diameter of the inhibition zone were measured and used to estimate the minimum inhibitory concentration (MIC).

II- Dilution methods:

Turbidimeter method (Skinner, 1955):

The two-fold serial dilution of the antibiotic were made in 10 ml Nutrient broth, Sabouraud broth and Czapek Dox broth were used for bacteria, yeast, dermatophytes and filaments fungi respectively. The tubes were inoculated with the test organisms and incubated at 27 °C for 24-72 hours then examined. The minimum inhibitory concentration (MIC) was taken as the dilution showing 50% inhibition of turbidity.

Table 1: Organisms used in screening higher plants for antimicrobial activity.

No	Organism	Classification
Bacteria:		
1	*Bacillus subtilis* ATCC 6633	Gram positive
2	*Staphylococcus aureus* ATCC 25923	Gram positive
3	*Klebsella pneumonia* ATCC 13883	Gram negative
4	*Escherichia coli* ATCC 25922	Gram negative
5	*Proteus vulgaris* ATCC 13315	Gram negative
6	*Pseudomonas aeruginosa* ATCC 27853	Gram negative
Fungi:		
7	*Candida albicans* DSM 70014	Yeasts
8	*Microsporum canis*	Dermatophytes
9	*Trichophyton mentagraphytes*	Dermatophytes
10	*Aspergillus niger* ATCC 9642	Filamentous fungi

The bioautography is often the only way to detect antibiotics that have been separated on paper chromatography (Wallhausser, 1969), or thin layer chromatography.

A preliminary phytochemical screening was carried on the plant material of *Ricinus communis* collected from north Sinai. It was tested for volatile oils, tannins, alkaloids and flavonoids according to Balbaa et al. (1981) methodology, unsaturated sterols and triterpenes (Brieskorn et al. 1961); saponins (Balbaa et al, 1981) and glycosides and/or carbohydrates (Vogel, 1978).

The median lethal dose (LD 50) is determined according to Ahmed, (1979) method.

RESULTS AND DISCUSSION

I- Screening the antimicrobial activity of the selected plants:

Plants still provide an important source of the world's pharmaceuticals (Lewis and Elvin Lewis, 1977) and still seems to be a potentially rich source of antimicrobial agents (Betina, 1983).

Only 5-10% of the world's plant resources have been evaluated for pharmacological activity (McCallin et al. 1982) and very few for the production of antimicrobial substances (Betina, 1983).

Sinai's unique flora has a high proportion of medicinal plants and endemic species and has been the subject of a number of chemical studies which have been summarized in Sinai Peninsula Informative Abstracts of Researchers, Studies and News 1960-1980, (1982).

Very few of Sinai's unique flora have been examined from the antimicrobial point of view. There are many studies in Suez Canal antimicrobial point of view. There are many studies in Suez Canal University to determine the active principles of Sinai's medicinal plants through a project number 830511 which concerned the assessment of medicinal plants in Suez Canal zone (Bacha , 1984; Soliman, 1985; Gazara, 1986; and Moustafa, 1986). To complete these studies, we are following folklore medicine and are currently investigating Sinai's medicinal plants for antimicrobial activity.

The chosen plants are usually used in folkloric medicine by the Sinai bedwins. These plants are used medicinally as diuretic, cardiotonic, antidiabetic, antitumor, expectorant, stimulant, anthelmintic, antiseptic, stomachia, antiinflammatory agent, ..etc. They are also used commercially for the production of soaps, lubricants, liqueurs, perfumes, fertilizers, etc.

Antimicrobial activities of these plants were examined by extracting their shoot system with ethanol and testing them against gram-positive, gram-negative bacteria, yeasts, dermatophytes,and a filamentous fungus. The results proved that :

1. None of the extracts were active against the filamentous fungus *Aspergillus niger* ATCC 9642.
2. 24.4% of the species were inactive.
3. 75.6% of the species were active against the test organism.
4. 19.5% of the species were active against gram-positive bacteria.
5. The species active against gram-positive and gram-negative bacteria represented 12.3%.
6. The species active against gram-positive bacteria and fungi represented 12.1%.
7. The species showing broad spectrum activity represented 31.7%.

8. Consequently, the species showing broad spectrum activity represented the highest percentage among the other groups.

Reviewing the above results in relation to available literature on the antimicrobial activity of higher plants, it could be concluded that: this study showed that 25 plant species are recorded for the first time to show an''microbial activity against at least one of the test organisms. These 25 plant species are marked with an asterisk.(Table 2).

II-Isolation, purification and characterization of the antimicrobial agent (Rc18).

Ricicnus communis L. was selected for further studies, since it is widely distributed in north Sinai,. It is tolerant to different severe environmental conditions. Although the plant has many medicinal use, there are no systematic study for its importance as producer of antimicrobial agents. So, it was found reasonable to select this particular plant for isolating its antimicrobial principle and studying its characters.

The preliminary phytochemical screening of the shoot system of *Ricinus communis* L. shows the presence of tannins, unsaturated sterols, terpenes, alkaloids, flavonoids, glycosides, and\or carbohydrates negative results were obtained for volatile oils and saponins.

The results revealed that the antimicrobial activities of *Ricinus communis* L. extracts were detected only in fresh stems, petioles and flowers. Only the miscible solvents succeeded to extract the antimicrobial principle. The highest microbial activity of the plant was reported in spring for all parts of seedling and adult plant namely stem, leaf, petiole and flower (Histogram 1)

Table (2): Antimicrobial activity of the selected plant extracts.

Plant species	Organism number table (1)										Remarks
	1	2	3	4	5	6	7	8	9	10	
Apocynaceae:											
1* Nerium oleander L.	++	++	-	-	-	-	-	-	-	-	a
2* Vinca rosea L.	++	++	++	-	-	-	-	-	-	-	a
Capparaceae:											
1 Capparis spinosa L.	-	-	-	-	-	-	-	-	-	-	i
Chenopodiaceae:											
1* Arthrocnemum glaucum Del.	+++	+++	+++	+++	+++	++	+++	+++	+++	-	a
2 Chenopodium murale L.	-	-	-	-	-	-	-	-	-	-	i
3 Cornulaca monacantha Del.	-	-	-	-	-	-	-	-	-	-	i
4 Halocnemum strobilaceum (Pall.) M. Bieb.	-	-	-	-	-	-	-	-	-	-	i
Cleomaceae:											
1* Cleome droserifolia (Forssk.) Del.	++	++	++	++	++	++	++	++	++	-	a
Compositae:											
1 Artemisia herba alba Asso	+++	+++	-	-	-	-	++	++	-	-	a
2* Artemisia judaica L.	+++	+++	-	-	-	-	++	++	-	-	a
3 Artemisia monosperma Del.	+++	+++	+++	+++	++	++	+++	++	++	-	a
4* Conyza dioscoridis Del.	++	++	-	++	+	+	+	+	-	-	a
5* Echinops spinosa L.	+	+	-	-	-	-	+	-	-	-	a
6* Santolina chamaecyparissus L.	++	++	++	++	++	++	++	++	++	-	a
7* Senecio desfontainei Druce	+	+	+	-	-	-	+	-	-	-	a
8* Sonchus oleraceus L.	++	++	++	++	++	++	++	++	++	-	a
Convolvulaceae:											
1 Convolvulus arvensis L.	-	-	-	-	-	-	-	-	-	-	i
Euphorbiaceae:											
1* Ricinus communis L.	+++	+++	+++	+++	++	+++	+++	+++	+++	-	a
Gramineae:											
1 Phragmites australis (Cav.) Trin. ex steudel	-	-	-	-	-	-	-	-	-	-	i

* Species showimg antimicrobial activity for the first time.

+++ highly active

++ active

+ weakly active

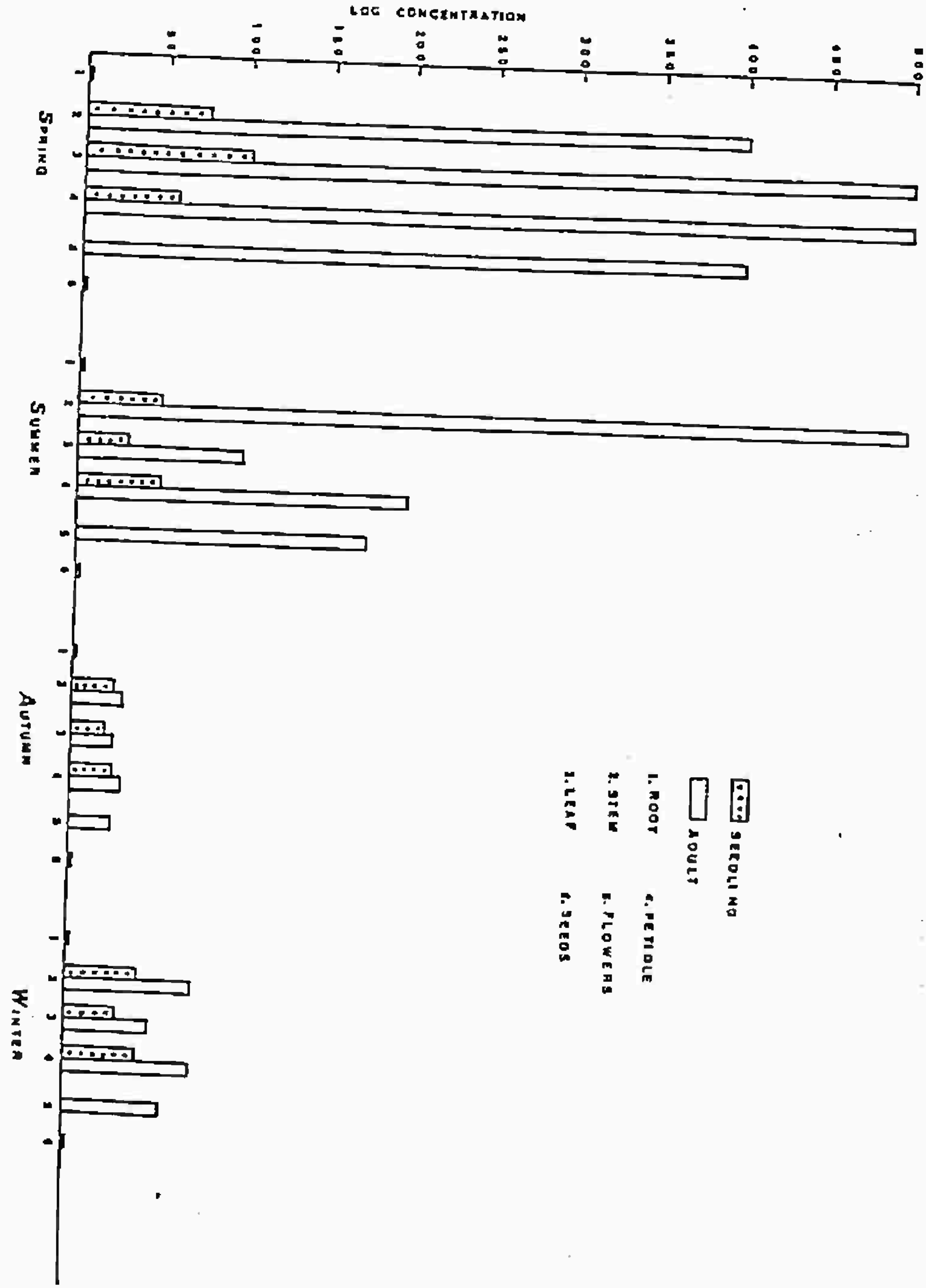
- inactive

a : active

i : inactive

Table 2 (contd.)

Juncaceae:											
1 Juncus rigidus C.A.Mey	-	-	-	-	-	-	-	-	-	-	i
Labiatae:											
1* Mentha microphylla C.Koch.	++	++	-	-	-	-	-	-	-	-	a
2 M.piperita L.	++	++	++	++	++	++	++	-	-	-	a
3 Ocimum basilicum L.	++	++	-	-	-	-	-	-	-	-	a
4* Origanum syriacum L.	++	++	-	-	-	-	-	-	-	-	a
5* Phlomis aurea Decne	++	++	-	-	-	-	-	-	-	-	a
6* Stachys aegyptiaca Pers.	++	++	-	-	-	-	-	-	-	-	a
7 Teucrium polium L.	++	++	++	++	++	++	++	++	++	-	a
Leguminosae:											
1* Acacia nilotica (L.) Willd. ex. Del.	+	-	-	-	-	-	-	-	-	-	a
2 Melilotus indica (L.) All.	-	-	-	-	-	-	-	-	-	-	i
Myrtaceae:											
1* Eucalyptus globulus labill.	++	++	++	++	++	++	++	++	++	-	a
Nitrariaceae:											
1* Nitraria retusa (Forssk.) Asch.	++	++	-	++	+	+	++	++	++	-	a
Orobanchaceae:											
1* Cistanche phelypaea (L.) Cout.	++	++	++	++	+	+	++	++	-	-	a
Resedaceae:											
1* Ochradenus baccatus Del.	++	++	+	+	+	+	++	-	-	-	a
Solanaceae:											
1 Datura stramonium L.	++	-	-	-	-	-	-	-	-	-	a
2 Hyoscyamus muticus L.	-	-	-	-	-	-	-	-	-	-	i
Tamaricaceae:											
1* Tamarix aphylla (L.) Karst.	+	-	-	-	-	-	-	-	-	-	a
Thymelaeaceae:											
1 Thymelaea hirsuta (L.) Endl.	-	-	-	-	-	-	-	-	-	-	i
Umbelliferae:											
1* Ammi majus L.	++	++	-	-	-	-	-	-	-	-	a
Zygophyllaceae:											
1* Peganum harmala L.	++	++	-	++	-	-	-	-	-	-	a
2* Zygyphyllum album L.f.	[illegible]	+	-	-	-	-	-	-	-	-	a
3* Z. coccineum L.	+	+	+	-	-	-	-	-	-	-	a



HISTOGRAM(1)

SEASONAL DISTRIBUTION OF R.c.18 IN DIFFERENT PARTS OF <u>RICINUS</u> <u>COMMUNIS</u> L.

Extraction and purification of the active principle:

The systematic fractionation scheme of extraction and purification of the active principle is illustrated in Fig. 1.

The isolated pale yellew powder was subjected to further purification by thin layer chromatography using silica gel G plates and ethyl acetate: methanol (2:1) as solvent system. The pure active principle (Rc18)was obtained as pale yellow crystals (m. p. 203-205 ° C). It is soluble in acetone, butanol, ethanol, ethyl acetate and water, but insoluble in petroleum-ether, ether or chloroform. The ultraviolet absorption spectrum of the antimicrobial agent (Rc 18) in methanol exhibits two maximum peaks at λ *max* 255 and 313 nm. (Fig.2). These two regions are in agreement with those of flavones and flavonoids. The infra-red spectrum showed 1 characteristic peaks at 530, 1110, 1660 3480 cm^{-1}. (Fig.3).

The purity of these crystals was ensured by bidimensional chromatographic technique using different solvent systems.

The absorption at 530 cm was assigned to benzene ring, the strong absorption at 1110 cm^{-1} to a single bond stretching; the peak at 1660 cm to C=C, C=N, C=O and that 3480 cm^{-1} to NH or OH stretching (Williams and Fleming 1980). Since the elemental analysis showed the absence of nitrogen, the peak at 1660 cm^{-1} may be due to C=C or C=O and that at 3480 cm^{-1} may be due to OH stretching only which characterize the phenolic compounds.

These physical and physico chemical characteristics lead to the conclusion that the antimicrobial agent Rc 18 isolated from *Ricinus communis* L. may be a flavonoid .

This is in agreement with the fact that numerous flavonoids of plant

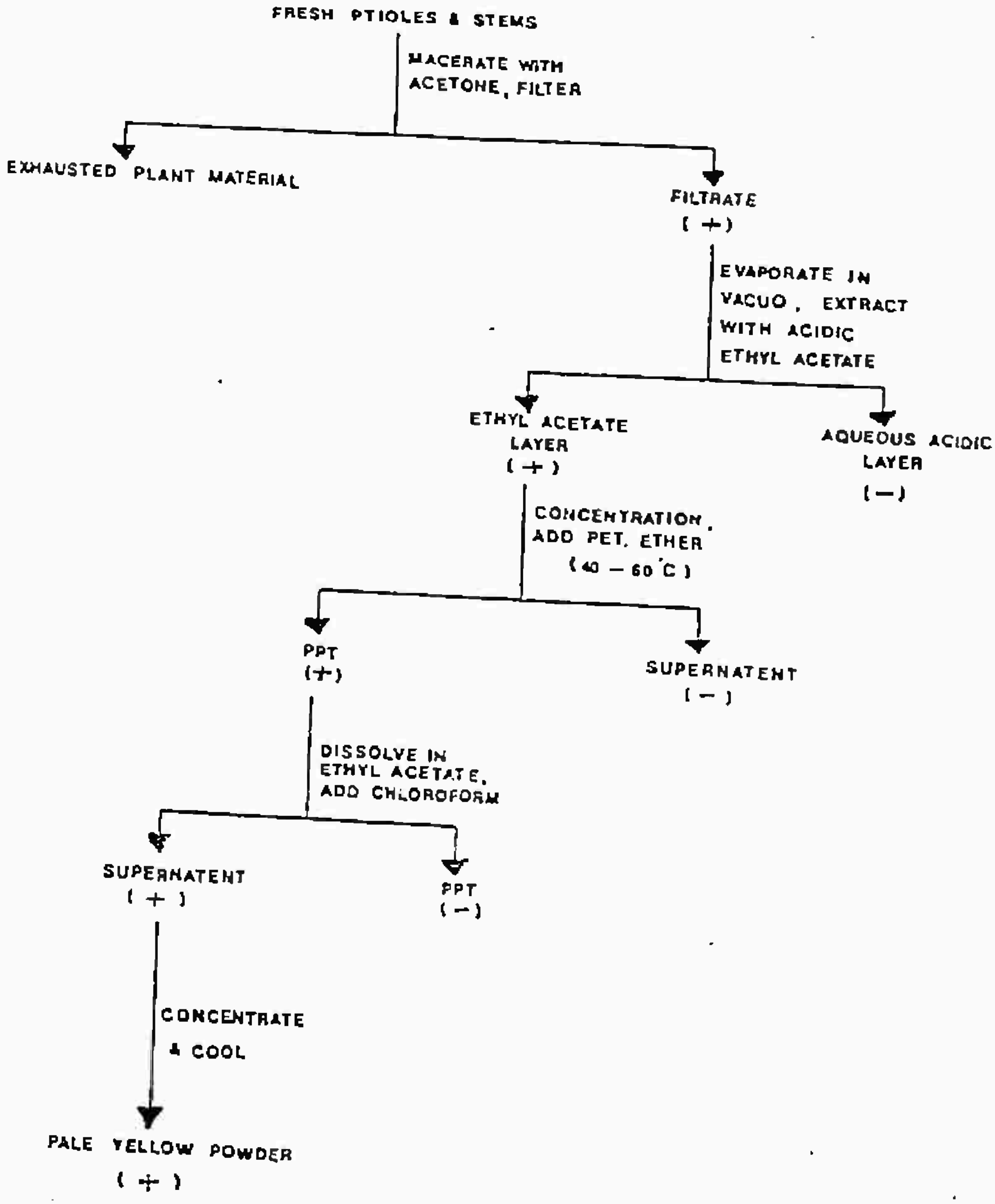


FIG (1) SCHEMATIC DIAGRAM FOR ISOLATION OF ANTIMICROBIAL AGENT FROM RICINUS COMMUNIS L.

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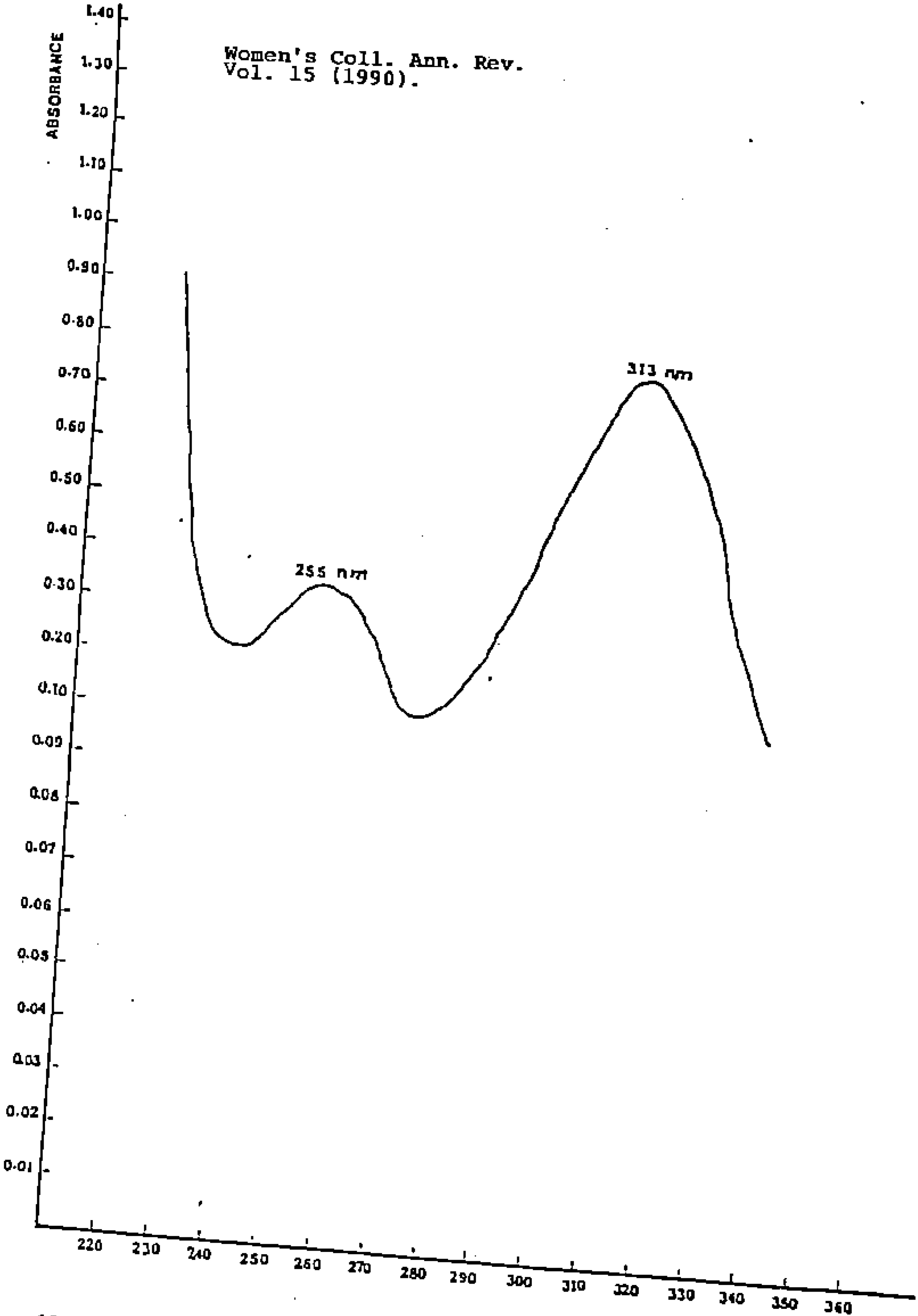

fig.'2' ULTRAVIOLET ABSORPTION SPECTRUM OF R.c.18

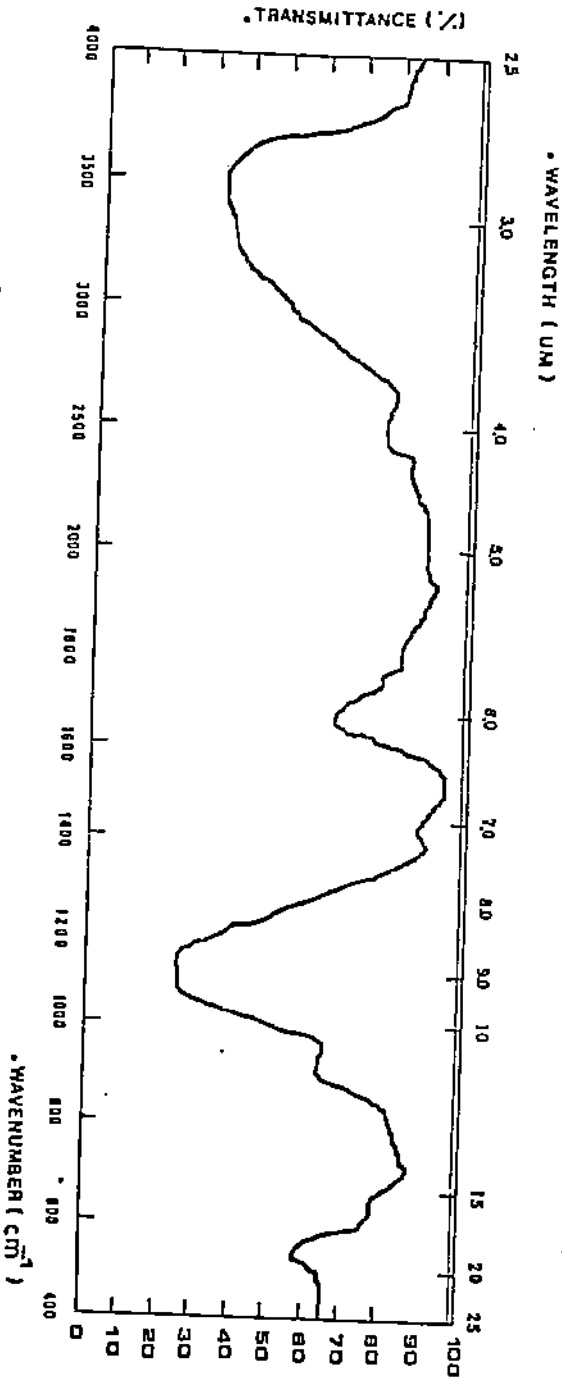

fig. "3"

INFRA–RED SPECTRUM OF R. C. 18

origin exhibited strong antibacterial and antifungal activities. (Freerksen and Bonike 1951, and Kuhnan 1976).

The antimicrobial activity of Rc 18 acting against a variety of microorganisms (MIC) showed strong inhibitory activity against gram-positive bacteria and yeasts at concentration of 6.25μg. /ml. It also showed activity against gram- negative bacteria and dermatophytes in the range of 6.25- 25μg./ml. On the other hand, no important activity was detected on filamentous fungi (Table 3).

Determination of LD 50 of Rc 18 revealed that this antimicrobial agent is nontoxic when applied in concentrated dose as high as 350 mg./kg.of body weight.

Table (3): Antimicrobial activity of the antimicrobial agent Rc 18 .

Bacteria / Fungi	Minimum inhibitory concentration (μg/ml)
Gram-negative bacteria:	
Escherichia coli ATCC 25922	12.50
Klebsella pneumoniae ATCC 13883	06.25
Proteus vulgaris ATCC 13315	12.50
Pseudomonas aeruginosa ATCC 27853	25.00
Gram-positive bacteria:	
Bacillus subtilis ATCC 6633	06.25
Staphyllococcus aureus ATCC 25923	06.25
Fungi:	
Aspergillus niger ATCC 9642	> 100
Candida albicans DSM 70014	06.25
Microsporum canis	25.00
Trichophyton mentagrophytes	25.00

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