

PRELIMINARY PHYTOCHEMICAL SCREENING SEPARATION AND IDENTIFICATION OF CERTAIN ACTIVE COMPONENTS FROM SOME PLANT EXTRACTS.

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ABSTRACT

The phytochemical screening of the active constituents of six wild plants were studied in leaves and seeds of both black pepper khella, camphor, geranium, datura and oshar plant extracted using different organic solvents. On the other hand, plant constituents of camphor, datura, oshar and geranium extracted only of both leaves and seeds were separated and identified by using the most proper techniques. Data indicated that nine phytochemical components were separated in various amounts due to plant species and plant organs as well as solvents used in extraction. In general, sterols & triterpenes, phenolic glycosides, alkaloids and carbohydrates & glycosides were found in relatively larger amounts followed by anthraquinone glycosides, tannins which were found in large amounts followed by saponin, flavonoids and cardiac glycosides which were found in traces. TLC of the crude plant extracts reveal the separation of five spots from granuim hexane extract, three spots from both datura and oshar ethanol, (five and one spots) from camphor (leaves and seeds) hexane and (five and three spots) from camphor (leaves and seeds) ethanol crude extracts with R_f values of [(0.11, 0.33, 0.57, 0.71 and 0.89) and (0.29, 0.64 and 0.79) and (0.28, 0.71 and 0.85)], [(0.27, 0.50, 0.70, 0.80 and 0.97) and (0.97), (0.06, 0.16, 0.55, 0.84 and 0.97) and (0.27, 0.33 and 0.93)] respectively. The function groups of the isolated fractions were also identified using IR spectral analysis.

INTRODUCTION

Natural products affecting pests, therefore provide continual inspiration to the agricultural chemicals in their research for new products to control pests and improve field. They may serve as leads for chemical synthesis of structurally or topographically related mimics. The mimics might also have more favorable biological and physical properties than the original natural products.

As a result of human continuous selection over thousand of years for high yield and low toxicity to mammals have lost their chemical defenses against herbivorous pests, e.g. bird, rats, insects...etc. (Fellows, 1979). However, few medical, aromatic and wild plants well known farmers, still maintain these defensive compounds. Furthermore, these compounds, which are the results of million years of development, are biodegradable to non-toxic products and may be specific in their effect against certain pests. Large numbers of compounds having divers biological effects on pests (i.e. killing, attracting, repelling, morphogenetic, feeding, deterring, growth inhibiting and reproduction sterilizing effects) have been isolated and identified from plants which are relative free from pest attack . several bioactive compounds which proved satisfactory in pest control were isolated, identified and evaluated by researchers. i.e. Zedan *et al.* (1994), Bignell and Dunlop (1996) and El-Gengaihi *et al.* (1997).

In a previous work, the authors tested several plants for the avicidal activity against house sparrow *domesticus niloticus* and crested lark, *Galerida cristata*, Abdel-All (1998). Six of this plants showed variable effects against the tested aves. Therefore the aims of the present study are to investigate the occurrence of various phytochemical constituents in this plant extracts. Moreover, the isolation and identification of the most active plant extract components using thin layer chromatography and IR spectral analysis, were studied .

MATERIALS AND METHODS

1- Preliminary phytochemical screening of plant extracts:

Among the tested plants 6 species were proved satisfactory bioactivity against the experimental birds. These plant species were black pepper, khella, camphore (leaves and seeds), geranium, datura and oshar. Extraction was carried out by using hexane and ethanol solvents. The following biochemical constituents were determined.

- 1-1 Steroles and triterpenes: sterols and triterpenes were determined according to the method adopted by Well *et al.* (1964).
- 1-2 Phenolic glycosides: Balbaa (1981) determine phenolic glycosides by the following procedure. Some drops of sulfuric acid were added to 1 ml plant extract, a red colour was produced which disappears on the addition of water.
- 1-3 Tanins: tannins were determined by the method described by Claus (1961).
- 1-4 Anthraquinone glycosides: anthraquinone was calculated according to Balbaa (1981).
- 1-5 Saponin glycosides: saponin glycosides were calculated according to the method mentioned by Well *et al.* (1964).
- 1-6 Flavonoids: flavonoids were determined according to the method adopted by Claus (1961).
- 1-7 Cardic glycosides: cardiac glycosides were determined according to Baljet and schwiez-Aphothztg (1981).
- 1-8 Carbohydrates and / or glycosides: carbohydrate and glycoside were determined by the method adapted by karawya and El-Wahab(1975).

2- Separation and identification of hexane and ethanol crude extracts of camphor (leaves and seeds), geranium, datura and oshar.:

2-1. Thin laver chromatography :

A layer of silica gel GF 254 of 1.5 mm thickness was spread on 20 x 20 cm and 20 x 10 cm glass plates. Geranium hexane extract and (datura and oshar) ethanolic extracts were developed on TLC plates using chloroform : methanol (2:1) developmental system, while, hexane and ethanolic extracts of camphor leaves and seeds were developed using chloroform : acetone : methanol (35 : 15 : 10) system at room temperature. The dry plates were sprayed with the following chromogenic reagents.

- Iodine as a general detection.

- Ferric chloride for phenol and hydroxamic acid (Stahl, 1969).
- Picric acid-iodine, spraying reagent for detection of the alkaloids.
- Sulfuric acid reagent for detection of sterols and / or triterpenes (Stahl, 1969).
- Aluminum chloride for location of flavonoids (Mabry *et al.*, 1970).

2-2 Fractionation and isolation .:

About 0.5 gm of crude extract was applied to T.L.C plate in the form of a line at a distance about 2 cm from the lower edge of the plate. The application was done using micropipette by gently moving its end on the plate in the form of straight line. Four plates were exposed separately to each of the following reagents:

- i) Ferric chloride.
- ii) Picric acid-iodine.
- iii) Sulfuric acid.
- iv) Aluminum chloride to visualize the bands of separated fractions and to determine their R_f values.

2-3. Spectroscopy analysis :

the infrared absorption spectrum of the isolated fractions dissolved in acetone was carried out. Infrared apparatus namely (Shimadzu FTIR 8201 PC) was used for this purpose. Interpretation of IR spectra was performed according to Lambert *et al.* (1976).

RESULTS AND DISCUSSION

1- Preliminary screening of the phytochemical components:

data in Table (1) indicate the existence of nine phytochemical components in leaves and seeds of both black pepper, khella, camphor, geranium, datura, and oshar, but in various amounts due to plant species and the tested plant parts as well as solvent of extraction. Generally sterols and triterpenes, phenolic glycosides, alkaloids and carbohydrates & glycosides were found in relatively larger amounts followed by anthraquinone glycosides, tannins were found in saponin, flavonoides and cardiac glycosides were found in trace amounts. This was pronounced with leaves and seeds as well as with both solvents used for extraction. These data indicate that sterols & triterpenes, phenolic glycosides, anthraquinone glycosides, tannins, alkaloids, carbohydrates and glycoside may be responsible for bioactivity on the studied pests.

Similar results were obtained through previous works, Cholchat,(1995) reported that the essential oils from the leaves, branches, flower, buds and mature fruits of *E. globules* depended upon maturity and origin of their collection site.

Menut *et al.*(1995) found that essential oils of leaves of *Eucalyptus* sp. When analyzed by GC and GC / MS contain about 60 monoterpenoids. The major components of the oil are 1,8-cineol, alpha-pinene and p-cymene. Osawa *et al.* (1995) isolated a new cariostatic compound named eucalyptone from leaves of *E.globules*. Bignell and Dunlop (1996) isolated the volatile leaf oils of some south western and southern Australian species of the genus *Eucalyptus* contained alpha-pinene (1.5 – 14 %), 1,8-cineole (0.81 %), p-

cymene (0.6-28%) and aromadendrenl terpinen-4-ol(0.6-24%) as principal leaf oil components.

Aly (1999) separated nine phytochemical components in leaves and seed of both red and spotted gum. Such components were found in different amount according to plant species, plant parts and solvent used.

2- Separation and Identification of isolated components:.

Data in Figs. (1 & 2) indicate the important role of the solvent system in separating the bioactive components from leaves and seeds of both geranium, datura, oshar and camphor. Table (2) and Fig. (1) indicate the separation of different components with different R_f values before and after iodine treatment in geranium, datura and oshar, hexane and ethanol extracts of leaves. Geranium hexane extract gave five spot with R_f values 0.11, 0.33, 0.57, 0.71 and 0.89 respectively. Datura ethanol extract gave three spot with R_f values 0.29, 0.64 and 0.79 respectively, whereas, oshar ethanol extract gave three spot with R_f values 0.28, 0.741 and 0.85 respectively. As for hexane and ethanol extracts of camphor leaves and seeds, data in Table (3) and Fig. (2) indicated the separation of five spots with R_f values 0.27, 0.50, 0.70, 0.80 and 0.97 for camphor leaves of hexane extract, whereas camphor seeds hexane extract gave one spot with R_f value 0.97.

Of camphor leaves ethanol extract gave five spot with R_f values 0.6, 0.16, 0.55, 0.84 and 0.97 respectively, camphor seeds ethanol extract gave three spot with R_f values 0.27, 0.33 and 0.93, respectively. Murata *et al.* (1990) isolated from the leaves of *E. macrocarpa*, a novel antibacterial compound, macrocarpal A and its structure was determined on the basis of an X-ray crystal structure analysis. Adhikari *et al.* (1992) stated that eucalyptol (ciniole) content of the oil *E. camaldulensis* was 15.4 to 77.7 % with an average of 58.7 %.

Nishizawa *et al.* (1992) isolated five Rtase inhibitors, macrocarpals A-E from *E. globules*. Zizira and Benjilalic (1992) studied the chemical composition of the essential oils of fruits and leaves of *E. camaldulensis* trees. They suggested that the major constituents of both fruits and leaves were 1,8-cineole and p-cymene. Wang and Fujimoto (1993) isolated two new triterpene named tercornate A and B from the dried leaves of *Eucalyptus tercornis*.

3- Infra-red spectral analysis :.

The infra-red absorption spectrum of fractions, separated from, geranium, datura, oshar and camphor leaves and seeds in potassium bromide disc were more or less identical Figs. (3 – 19), and they showed the following bands to which assignments were done. Silverstein and Bassler (1967). Hyodo *et al.* (1992) identified the structure of resinosides from *Eucalyptus resinifera* as repellents against the blue mussel, *Mytilus edulis*. Menut *et al.* (1992) identified the essential oils from leaves of *Eucalyptus urophylla* and *E. grandis*. They characterized 43 compounds in the oil and reported that, *E. urophylla* was rich in 1,8-cineole but *E. grandis* was rich in p-cymene and alpha pinene.

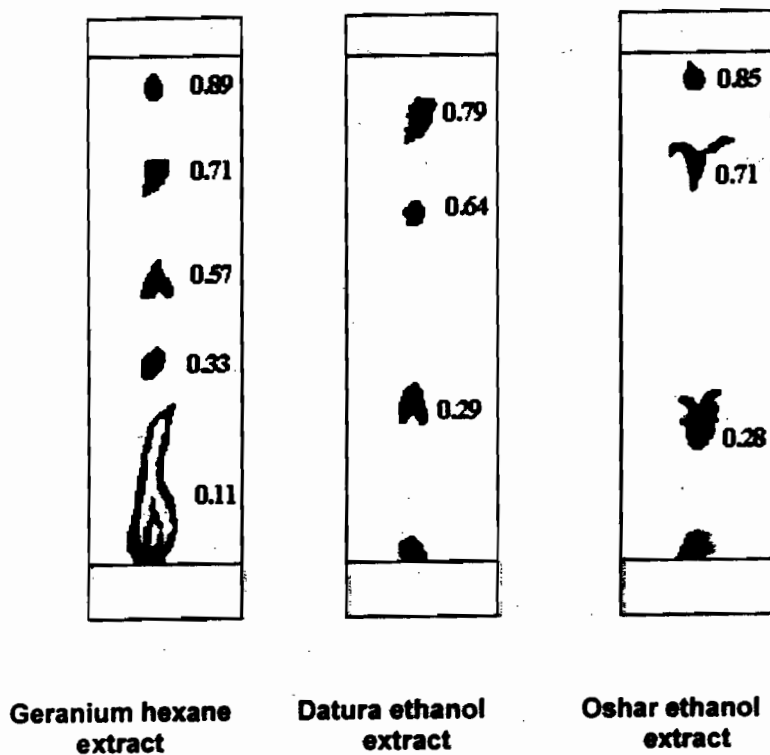


Fig. (1): Silica gel T. L.C of Geranium, Datura and Oshar plant extracts using migrated system chloroform: methanol (2:1) at room temperature .

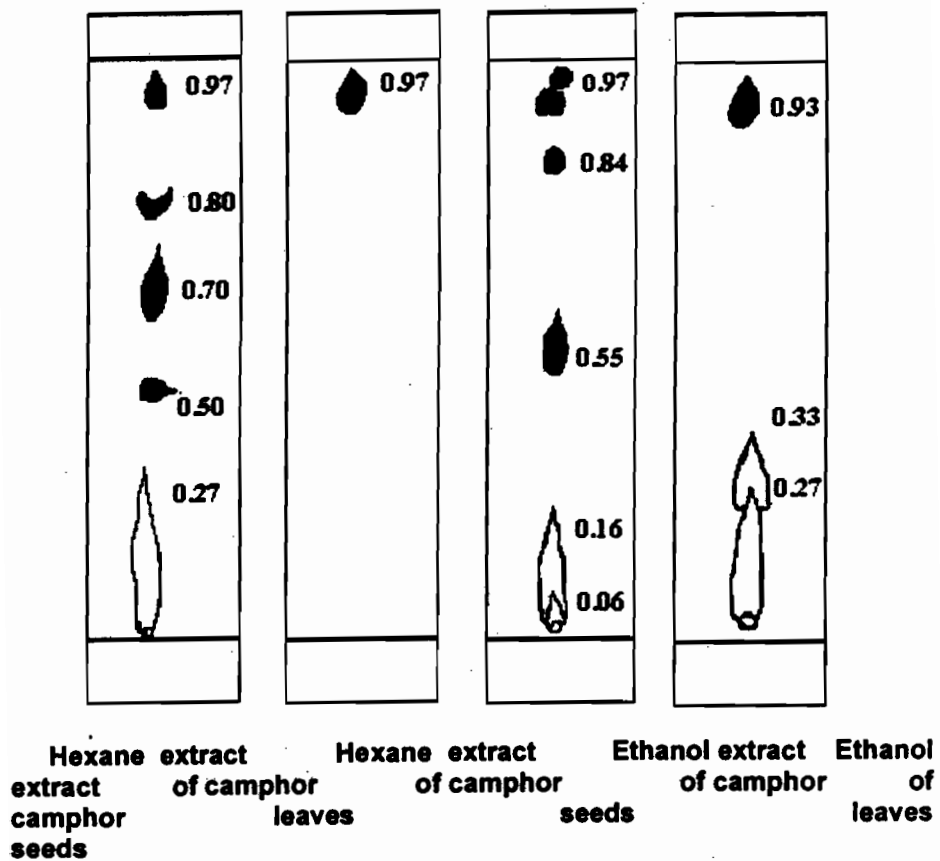


Fig. (2) Silica gel T.L.C of camphor plant extracts using migrated System Chloroform : acetone : methanol (35:15:10) at room temperature.

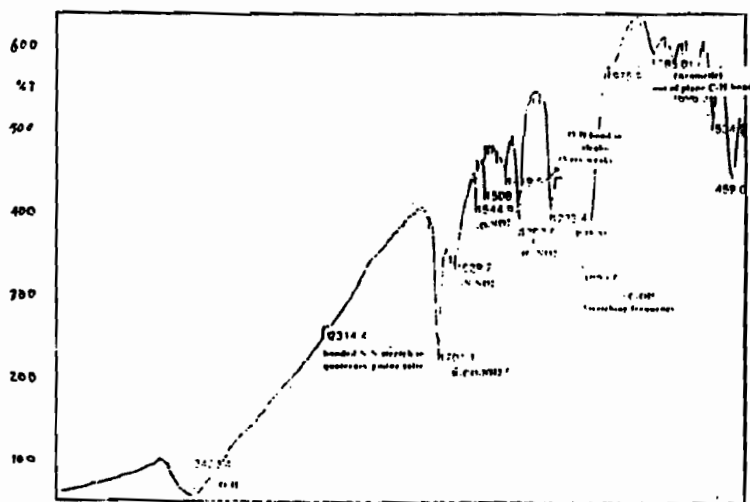


Fig.(3): Infrared spectra of fraction with Rf value 0.27 isolated from camphor (leaves) hexane extract.

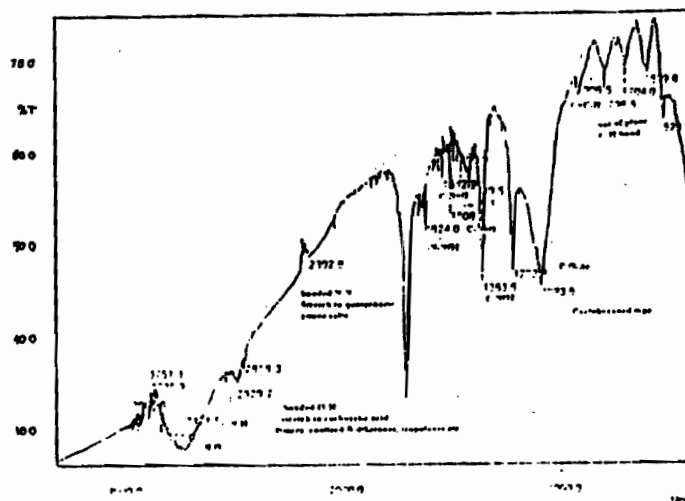


Fig.(4): Infrared spectra of fraction with Rf value 0.5 isolated from camphor (leaves) hexane extract.

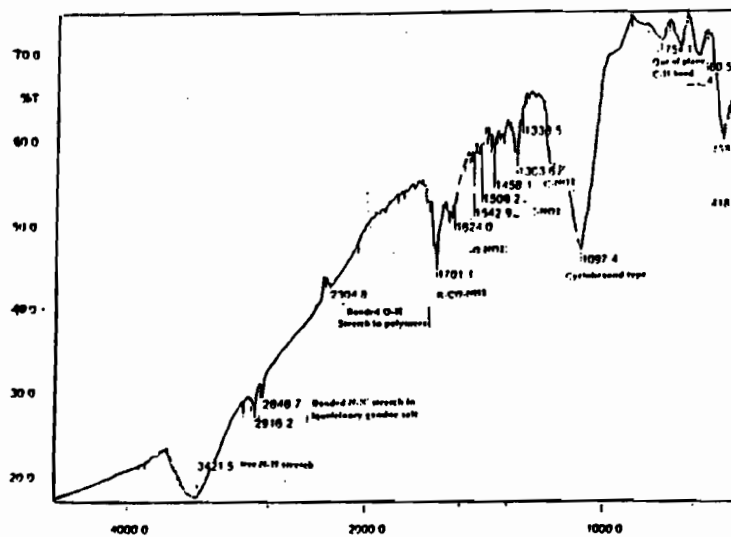


Fig.(5): Infrared spectra of fraction with Rf value 0.70 isolated from camphor (leaves) hexane extract.

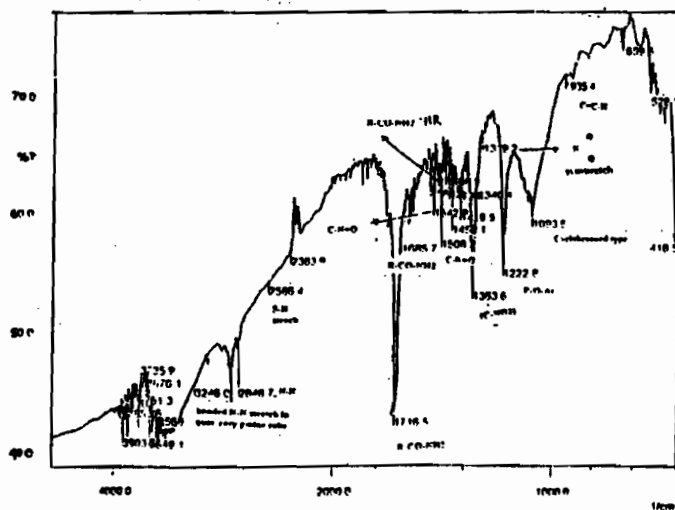


Fig.(6): Infrared spectra of fraction with Rf value 0.80 isolated from camphor (leaves) hexane extract.

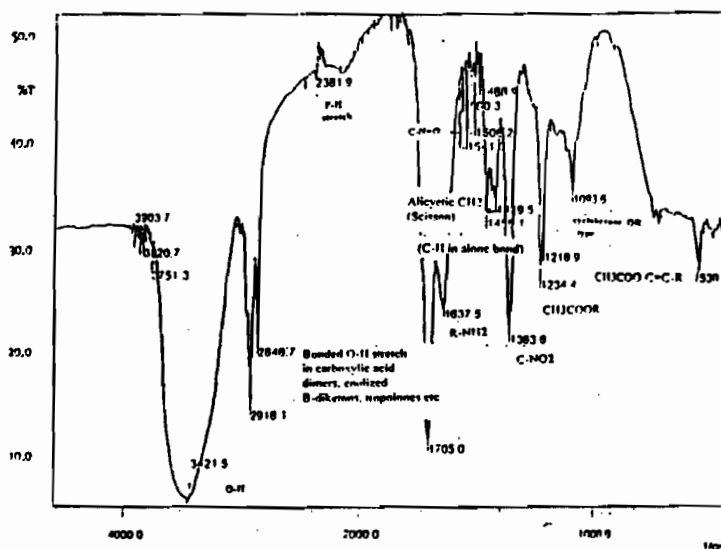


Fig.(7): Infrared spectra of fraction with Rf value 0.97 isolated from camphor (leaves) hexane extract.

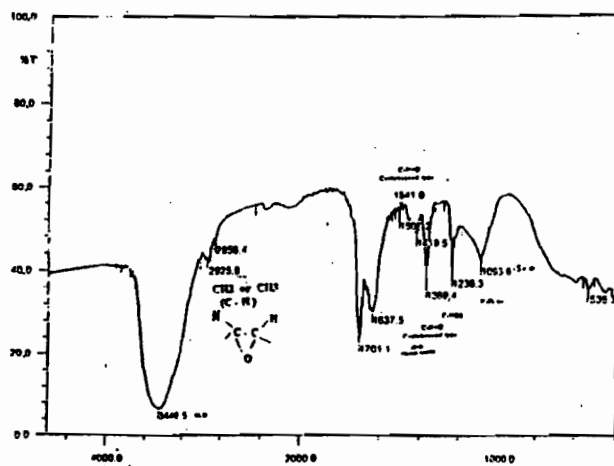


Fig.(8): Infrared spectra of fraction with Rf value 0.97 isolated from camphor (seeds) hexane extract.

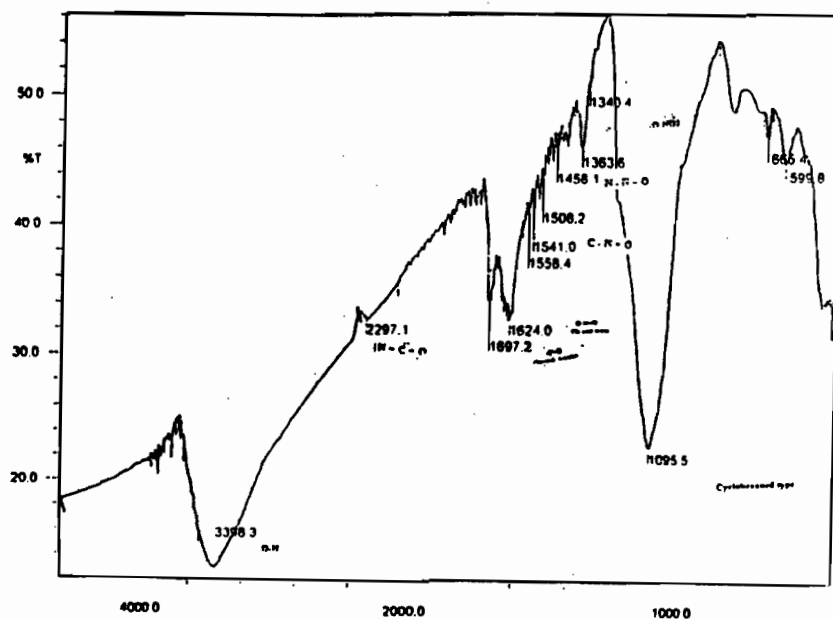


Fig.(9): Infrared spectra of fraction with Rf value 0.6 isolated from camphor (leaves) ethanol extract.

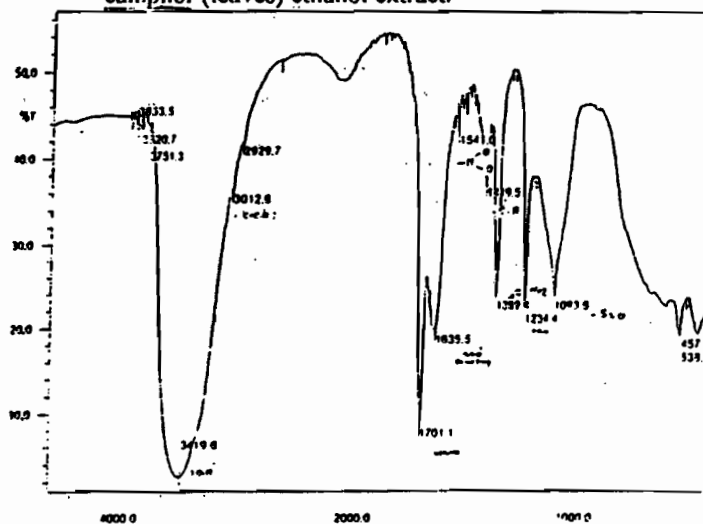


Fig.(10): Infrared spectra of fraction with Rf value 0.16 isolated from camphor (leaves) ethanol extract.

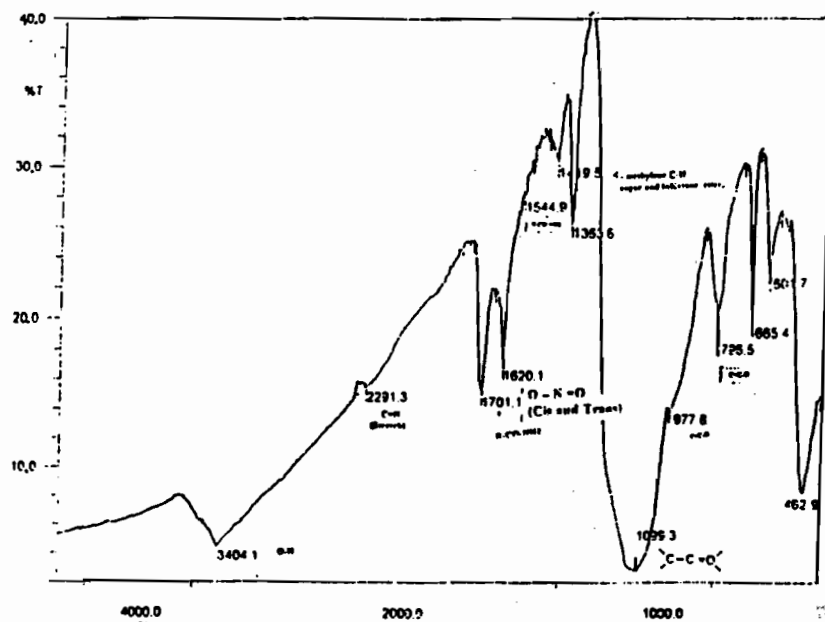


Fig.(11): Infrared spectra of fraction with Rf value 0.55 isolated from camphor (leaves) ethanol extract.

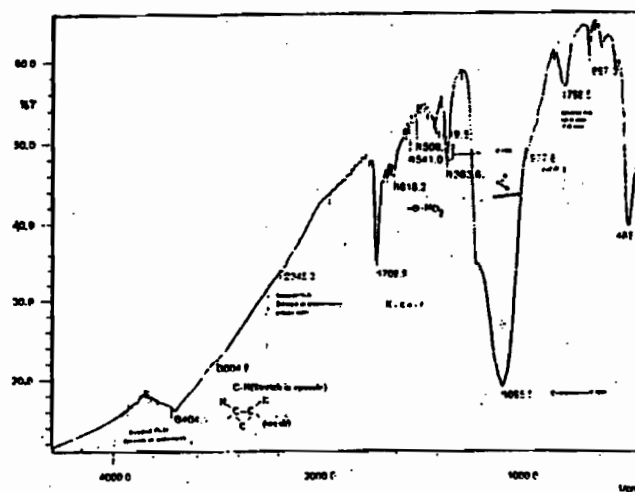


Fig.(12): Infrared spectra of fraction with Rf value 0.84 isolated from camphor (leaves) ethanol extract.

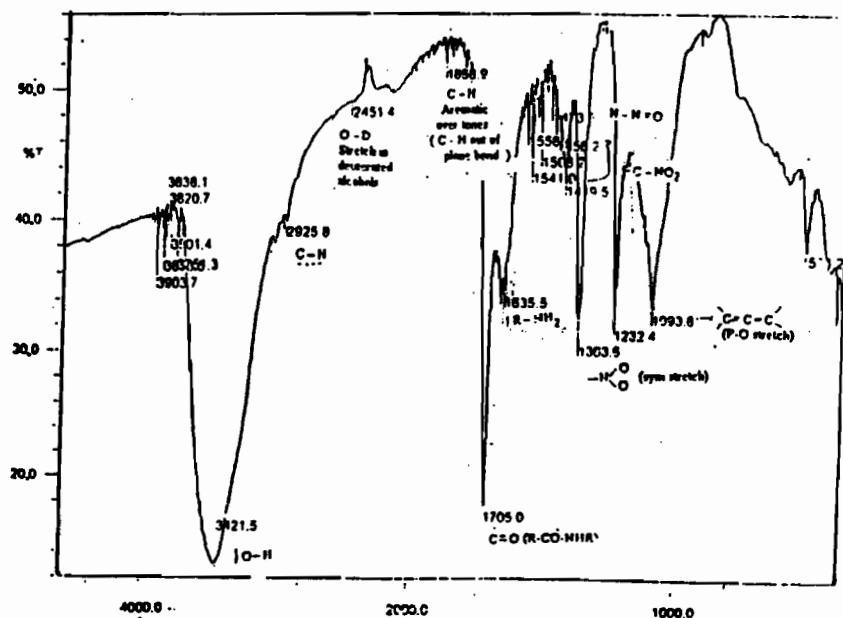


Fig.(13): Infrared spectra of fraction with Rf value 0.97 isolated from camphor (leaves) ethanol extract.

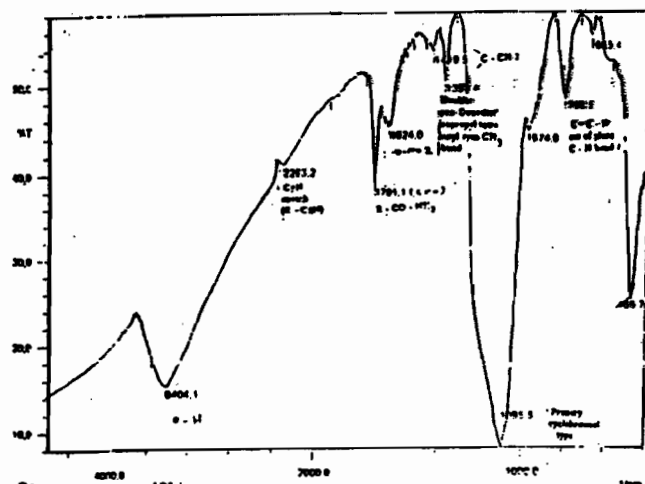


Fig.(14): Infrared spectra of fraction with Rf value 0.27 isolated from camphor (seeds) ethanol extract.

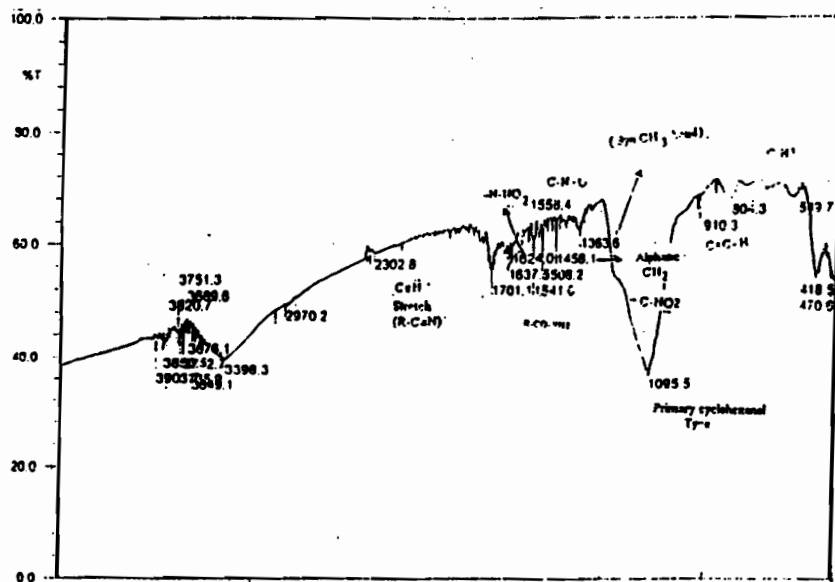


Fig.(15): Infrared spectra of fraction with Rf value 0.33 isolated from camphor (seeds) ethanol extract.

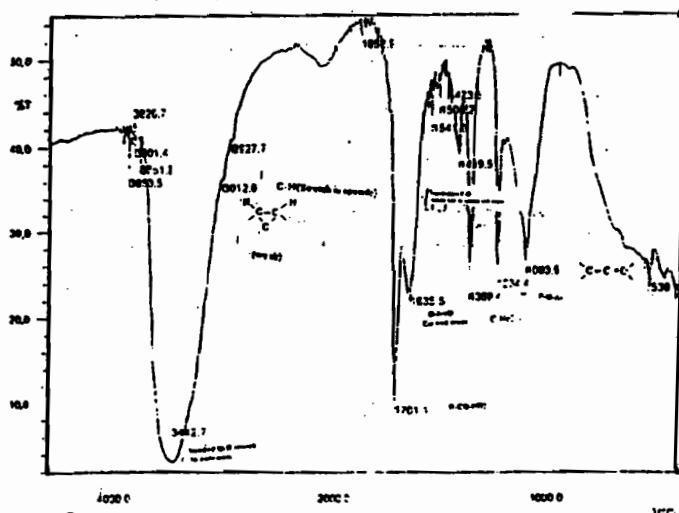


Fig.(16): Infrared spectra of fraction with Rf value 0.93 isolated from camphor (seeds) ethanol extract.

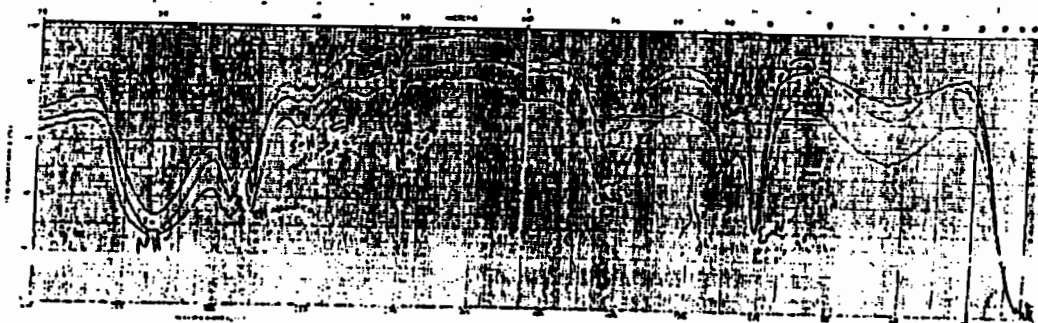


Fig.(17): Infrared spectra of fraction with Rf value 0.11, 0.31 and 0.57 isolated from Geranium hexane extract.

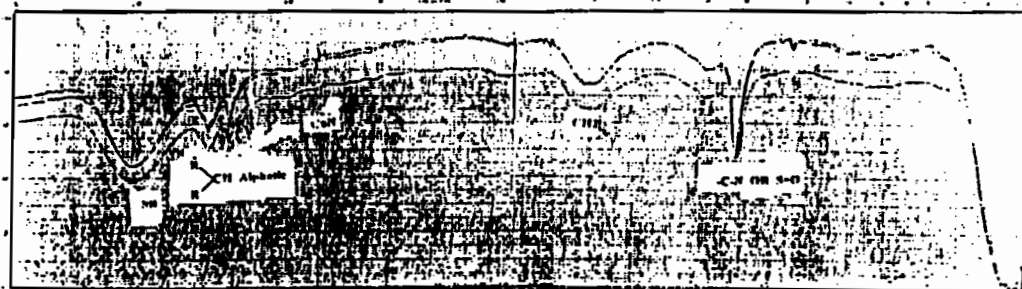


Fig.(18): Infrared spectra of fraction with Rf value 0.28, 0.71 and 0.85 isolated from Oshar ethanol extract.

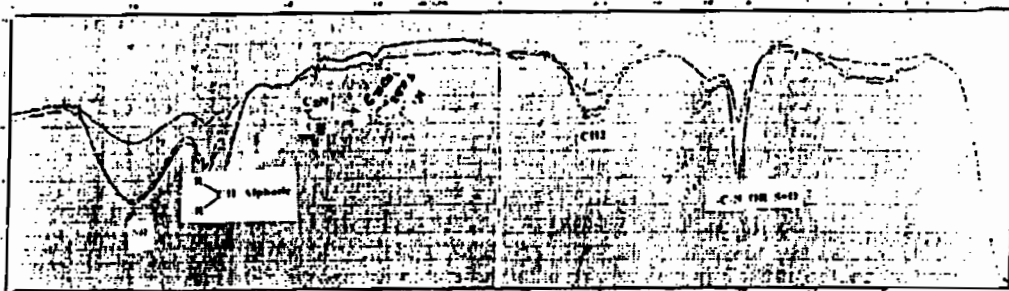


Fig.(19): Infrared spectra of fraction with Rf value 0.29, 0.64 and 0.79 isolated from Datura ethanol extract.

Table (1) : Preliminary phytochemical screening for some hexane and ethanol plant extracts

Plant extracts	Hexane plant extracts					Ethanol plant extracts					
	Black pepper	Khella	Camphor leaves	Camphor seeds	Geranium	Black pepper	Khella	Camphor leaves	Camphor seeds	Datura Leaves	Oshar leaves
Sterols & Triterpens	+++	+++	+++	+++	+++	±	+	+++	+++	±	±
Phenolic glycosodes	±	+++	±	±	+++	±	+	±	±	±	++
Tannins	±	+	++	±	+	±	++	++	+++	++	+
Anthraquinone glycosides	++	±	+++	+++	+	++	±	++	++	+	++
Saponin	±	+	±	±	±	±	±	+++	++	±	+
Flavonoids	±	++	±	±	±	±	++	±	±	±	++
Cardiac glycosides	±	±	±	±	+	±	±	±	±	+	+++
Alkaloids	±	±	+++	±	+++	±	+	+++	+++	++	+++
Carbohydrates & glycosides	+++	+	+	+++	++	+	++	+	++	++	+++

± Traces

+

++ Present in large amount

+++ Present in relatively larger amounts

Table (2) TLC of geranium, datura and oshar crude plant extracts.

Plant extracts	No. of spots	R _F value *
Geranium hexane extract	1	0.11
	2	0.33
	3	0.57
	4	0.71
	5	0.89
Datura ethanol extract	1	0.29
	2	0.64
	3	0.79
Oshar ethanol extract	1	0.28
	2	0.71
	3	0.85

* Solvent system : chloroform : methanol (2:1)

Table (3) T.L.C of camphor leaves and seeds crude plant extracts.

Plant extracts	No. of spots	R _F value *
Camphor leaves hexane extract	1	0.27
	2	0.50
	3	0.70
	4	0.80
	5	0.97
Camphor seeds Hexane extract	1	0.97
Camphor leaves ethanolic extract	1	0.06
	2	0.16
	3	0.55
	4	0.84
	5	0.97
Camphor seeds ethanolic extract	1	0.27
	2	0.33
	3	0.93

* Solvent system : chloroform : acetone : methanol (35:15:10) .

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الفصل والتعرف الأولى للمكونات الكيميائية لبعض المستخلصات النباتية

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- ** معهد بحوث وقاية النباتات - مركز البحوث الزراعية- الدقى - الجيزة - مصر

تناولت هذه الدراسة فصل المكونات الكيميائية الداخلية وبذور المستخلصات النباتية لكل من الفلفل الأسود - الخلة - الكافور - العتر - الداتورا - العشار وكذلك الفصل الكروماتوجرافى للمكونات بالاضافة الى تعريف المجموعات الفعالة باستخدام أجهزة القياس الاسبكتروفوتومترية لكل من أوراق وبذور مستخلصات الكافور - الداتورا - العشار - العتر فقط .

أظهرت النتائج التى أسفرت عنها الدراسة فصل تسعة مركبات كيميائية من هذه المستخلصات ووجدت هذه المكونات بكميات مختلفة تبعا لنوع النبات والجزء المستخلص وكذلك نوع المذيب حيث اتضح بوجه عام أن الأسترولات والتربينات، الفينولات، الكالوميروز، كربوهيدرات، وجليكوسيد توجد بكميات عالية جدا يليها أنثراكينونات، جليكوسيد، التانينات موجودة بكميات عالية أما بالنسبة للصابونين، الفلافونويدات، الكاردك جليكوسيد فيوجد منهم آثار قليلة، وثبت أن نظام المذيب المستخدم فى الفصل له دور كبير فى فصل المكونات الحيوية الموجودة فى المستخلصات النباتية المختبرة باستخدام الفصل الكروماتوجرافى وأجهزة القياس الاسبكتروفوتومترية (IR) فى التعرف على المجاميع الفعالة والمسئولة من الفعل الابادى لهذه المستخلصات.