"immunomodulators from natural products"

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ABSTRACT

Immunostimulant plant extractives as well as its isolates constitute an alternative to conventional chemotherapy and prophylaxis of infections. In the present study eight plants (Ecballium elaterium, Luffa cylindrica, Cucurbita maxima, Agaricus bisporous, Pleurotus ostreatus, Oryza sativa (Rica bran), Tropaeolum majus and Echinacea purpurea) were primarily screened for specific and nonspecific immune responses. T. majus and E. elaterium were the most active, in addition to the well known immunostimulant E. purpurea. Tumor Necrosis factor–alfa (TNF-α) and gamma Interferon (INF-γ) production were used as a measure of immunostimulatory activity.

"Cucurbitacin D" and "2-0-β-D-glucopyransoyl cucurbitacin I" of E.elaterium in addition to "5,3'-dihydroxy-7,4'-dimethoxy flavone" and 5,3'-dihydroxy-7,4' dimethoxy flavone – 5-0– glucoside of T. majus were found to significantly stimulate the production of both TNF-α and INF-γ, while , "Androstan" and "4-acetyl-3,5-bis-(methyl thio)-2-pyrrolin-5-Ol" of T. majus stimulated the production of INF-γ only.

INTRODUCTION

Immunostimulation constitutes an attractive alternative to conventional chemotherapy and prophylaxis of infections. That is of prime importance when infectious diseases, persistent infections, chemotherapy-resistant bacteria and viral infections, have to be treated [(1)].
A second field of application is the prophylaxis of opportunistic infections of patients at risk due to lack of suitable medications such as viral infections. Further application is the therapy of malignant diseases where the tumor growth can be inhibited by stimulating specific components of the immune system, such as macrophages or T-killer cells. If chemically defined compounds were available that were capable of specifically stimulating immune system, it might be helpful in the therapy of autoimmune diseases such as rheumatoid arthritis, polyarthritis, chronic active hepatitis, myasthenia gravis, multiple sclerosis, psoriasis and others, immunostimulants having been used predominantly on the basis of empirical clinical observations [(2)].

Tumor necrosis factor alpha (TNF-α,) is an important cytokine involved in systemic inflammation

and the acute phase response. It has a number of actions on various organ systems, generally together with interleukins 1 and 6: Stimulating of the hypothalamic-pituitary-adrenal axis by stimulating the release of corticotropin releasing hormone (CRH), and stimulating the acute phase response of the liver leading to an increase in C-reactive protein and a number of other mediators.

Gamma-Interferon (IFN-□□ is a typical lymphokine, being produced exclusively by activated natural killer cells and T lymphocytes**.** It is an activator of macrophages and thus increases their antitumor activities. If the macrophages are infected by intracellular parasites, it activates macrophages which in turn destroy the parasites. Suppression of intracellular parasites under the influence of IFN-□ takes place in the non-macrophage cells as well. IFN-□ affects differentiation of B-cells immune responses. In the late stages it increases the secretion of the immunoglobulins.

The **aim of the present work** is to demonstrate that in the future, apart from microbial preparations (Gram-positive and Gram-negative bacteria), plant drugs and polyalcohol polymers isolated from fungi might play a role in enhancing either the **nonspecific** and/or **specific** immune response in albino mice model [3-5]. The total plant extractive that prove to be immunostimulant may be considered as prototype, the development of which may lead to novel promising immunostimulant compounds.

MATERIALS & METHODS

1. Plant extract:

200 grams of plants under investigation (*Pleurotus ostreatus, Agaricus bisporus, Rice bran, Tropaeolum majus, Echinacea purpurea, Ecballium elaterium, Luffa cylindrica,* and *Cucurbita maxima*) were extracted by cold maceration in 70% alcohol (three extractions each with 2 liters) and evaporated to dryness under reduced pressure.

2.**Extract preparation for immunostimulant bioassay:**

Dried 70% alcohol extracts of plants under investigation were dissolved in Hank's solution in different concentrations (100, 500, 1000, 1500 and 2000 µg\ml). The extracts were sterilized by filtration through 0.2 µm pore size filters and by addition of 10,000 µg penicillin and 10,000 µg streptomycin antibiotics.

3. **Animals:** Albino mice of either sex (20-22 g) were used for the following:-

Preparation of murine (mice) spleen cells:

Spleenocytes were prepared according to conventional procedures, from aseptically removed mouse spleens. The cells were washed three times in RPMI 1640 medium and resuspended in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, 10 mM HEPS, pencillin 100 U / ml, streptomycin 100 µg/ml at a final concentration of 3 x 10^6 cells / ml. Cell viability was evaluated by trypan blue exclusion test.

Lymphocyte cell culture:

Primary lymphoid cell culture was performed by isolating

lymphocytes directly from mice spleens. The lymphocytes then were grown in a chemically defined growth medium RPMI 1640 supplemented with 10% FCS. Cell cultures were then used to study cytokines production involved in the activation, growth and differentiation of various cells involved in the immune response.

Macrophage culture:

Spleen cell suspension was incubated in flat bottomed microtiter plates at 37^{o}C for 7 hours to allow the cells to adhere to the plates then the medium was removed and the adherent cells were washed three times with RPMI 1640. More than 95% adherent cells were macrophage. The cells were cultured with different concentrations of each plant extract for 24 hours. The cells were further assayed for specific and nonspecific immune response.

A battery of assays was used to study the non- specific immune response of stimulated macrophages and lymphocytes, so certain functions of them were assayed including macrophage migration index, phagocytic index and viability percentage test in which the viable cells were counted using freshly filtered trypan blue stain and haemocytometer slide.

Macrophage migration index [Table (1)], Phagocytic index [Table (2)] and Viability percentage [Table (3)] showed the following results:

Table (1): Macrophage migration index of different plant extractives.

Plant / Conc. (µg/ml)	*Pleurotus ostreatus*	*Agaricus bisporus*	*Rice bran Oryza sativa*	*Tropaeolum majus*	*Echinacea purpurea*	*Ecballium elaterium*	*Luffa cylindrica*	*Cucurbita maxima*
100	0.115	0.067	0.050	0.079	0.015	0.067	1.853	0.224
500	0.208	0.187	0.042	0.079	0.018	0.045	1.336	0.208
1000	0.224	0.328	0.042	0.070	0.019	0.059	0.990	0.115
1500	0.093	0.406	0.035	0.059	0.034	0.059	0.531	0.052
2000	0.261	0.531	0.592	0.049	0.026	0.323	0.592	0.095

Table (2): Phagocytic index of different plant extractives.

Plant / Conc. (µg/ml)	*Pleurotus ostreatus*	*Agaricus bisporus*	*Rice bran Oryza sativa*	*Tropaeolum majus*	*Echinacea purpurea*	*Ecballium elaterium*	*Luffa cylindrica*	*Cucurbita maxima*
100	0.59	1.11	0.43	0.22	0.46	0.76	0.23	0.62
500	0.96	0.48	0.62	0.28	0.36	0.58	0.60	0.30
1000	1.65	0.84	0.63	0.37	0.56	1.06	0.95	0.46
1500	1.57	1.39	0.48	0.37	0.92	1.09	0.89	0.47
2000	0.98	1.30	0.37	0.77	1.38	1.12	0.71	1.04

Table (3): Viability percentage of different plant extractives.

Conc. (μg/ml) / Plant	100	500	1000	1500	2000	Control
Pleurotus ostreatus	65%	54%	65%	73%	67%	68%
Agaricus bisporus	30%	42%	39%	56%	37%	48%
Oryza sativa (Rice bran)	39%	37%	33%	30%	19%	40%
Tropaeolum majus	48%	50%	57%	74%	70%	52%
Echinacea purpurea	56%	60%	61%	60%	68%	58%
Ecballium elaterium	54%	63%	57%	62%	68%	54%
Luffa cylindrica	59%	56%	27%	42%	29%	56%
Cucurbita maxima	44%	28%	48%	63%	64%	56%

Results are average from 4 replicates

Non-specific immune response using **"Macrophage migration index"** showed the lowest Macrophage migration index with *Echinacea purpurea* followed by *Ecballium elaterium* and *Tropaeolum majus*.

The later two plant extractives showed appreciable non specific immunostimulant activity (low Macrophage migration index) comparable to the results displayed by *Echinacea purpurea.*

Rice bran extract showed low macrophage migration index at concentrations (100-1500 μg/ml) but not with conc. 2000 μg/ml).

Regarding the ***non specific*** immune response using **"phagocytic index"**, it was apparent that mushrooms (*pleurotus ostreatus* and *Agaricus bisporous*) in addition to *Ecballium elaterium* especially at high concentrations (1000-2000 μg/ml) had the highest immunostimulant effect.

In relation to **"viability percentage"**, it was apparent, by comparison of each plant extract at different concentrations (100-2000 μg/ml) with its control, that both *Echinacea purpurea* and *Ecballium elaterium* at concentrations (500-2000 μg/ml) had higher viability % than that of control. *Tropaeolum majus* showed significantly high viability % at concentrations (1000-2000 μg/ml) while, *Cucurbita maxima* showed only higher viability % than its control at concentrations 1500-2000 μg/ml.

The three experiments clearly demonstrated that *Ecballium elaterium* and *Tropaeolum majus* had considerable immunostimulant effect in addition to *Echinacea purpurea* (the well known marketed immunostimulant drug). This leads us to investigate the active constituents of *Ecballium elaterium* and *Tropaeolum majus* that possibly have such immunostimulant effect.

Study of immunostimulant activity of different extractives of *Ecballium elaterium & Tropaeolum majus*

One kg of each of *Ecballium elaterium* & *Tropaeolum majus* was subjected to Successive extraction with different solvents of different polarities (petroleum ether, chloroform, ethyl acetate, alcohol 70%, and water) using continuous extraction apparatus.The extraction with each solvent was continued until an aliquot of the last colorless percolate, left no residue when evaporated to dryness. After each extraction, the powder was taken out of the extractor and completely dried before using the next solvent. The solvent was removed completely from each extract, and the residue was dried to a constant weight.

The mean percentages for the different extracts of *E. elaterium* in petroleum ether, chloroform, ethyl acetate, alcohol 70%, and water were 3.85, 12.50, 2.15, 9.25 and 13.10 % respectively and they were 5.23, 8.42, 1.93, 7.26 and 12.60 % respectively for *T. majus*.

The different extractives of *E. elaterium* & *T. majus* were subjected to the following investigation.

Different extractives of *Ecballium elaterium* & *Tropaeolum majus* were dissolved in Hank's solution in different concentrations (100, 500, 1000, 1500 and 2000 µg\ml) and subjected to macrophage migration index, phagocytic index and viability percentage previously mentioned before. The results were presented in tables (4-6).

Table (4): Macrophage migration index of different extractives of *Ecballium elaterium* & *Tropaeolum majus*.

Plant	*Ecballium elaterium*					*Tropaeolum majus*				
Conc. (µg/ml)	*Pet. ether*	*$CHCl_3$*	*EtOAc*	*Alcohol*	*H_2O*	*Pet. ether*	*$CHCl_3$*	*EtOAc*	*Alcohol*	*H_2O*
100	1.336	0.208	0.990	0.328	1.853	0.407	0.672	0.208	0.323	1.330
500	0.990	0.115	0.406	0.226	0.592	0.225	0.453	0.224	0.188	0.990
1000	0.592	0.093	0.225	0.208	0.328	0.114	0.187	0.093	0.094	0.491
1500	0.531	0.067	0.328	0.095	0.337	0.042	0.092	0.087	0.115	0.323
2000	0.406	0.067	0.180	0.052	0.208	0.042	0.045	0.026	0.041	0.209

Table (5): Phagocytic index of different extractives of *Ecballium elaterium* & *Tropaeolum majus*.

Plant / Conc. (µg/ml)	*Ecballium elaterium*					*Tropaeolum majus*				
	Pet. ether	***$CHCl_3$***	***EtOAc***	***Alcohol***	***H_2O***	***Pet. ether***	***$CHCl_3$***	***EtOAc***	***Alcohol***	***H_2O***
100	0.37	0.78	0.22	0.46	0.189	0.47	0.24	0.46	0.60	0.23
500	0.48	**0.99**	0.27	0.58	0.56	0.58	0.52	0.38	0.89	0.38
1000	0.79	**0.98**	0.36	0.92	0.48	0.79	0.82	0.56	0.71	0.46
1500	0.93	**1.57**	0.48	**0.97**	0.58	**0.93**	**0.96**	**0.92**	**0.95**	0.53
2000	0.89	**1.38**	0.65	**1.04**	0.78	**1.44**	**1.08**	**1.38**	**1.04**	0.64

Table (6): Viability percentage of different extractives of *Ecballium elaterium* & *Tropaeolum majus*.

Plant / Conc. (µg/ml)	*Ecballium elaterium*					*Tropaeolum majus*				
	Pet. ether	***$CHCl_3$***	***EtOAc***	***Alcohol***	***H_2O***	***Pet. ether***	***$CHCl_3$***	***EtOAc***	***Alcohol***	***H_2O***
100	35%	48%	38%	53%	29%	51%	48%	53%	31%	28%
500	39%	57%	39%	**62%**	41%	56%	51%	51%	48%	39%
1000	40%	**68%**	48%	**68%**	30%	**62%**	**58%**	**59%**	**61%**	53%
1500	44%	**70%**	53%	**73%**	39%	**68%**	**63%**	**64%**	**69%**	54%
2000	**59%**	**74%**	49%	**69%**	44%	**73%**	**70%**	**74%**	**72%**	**58%**
Control	48%	48%	48%	48%	48%	48%	48%	48%	48%	48%

Results are average from 4 replicates

Non-specific immune response using **"Macrophage migration index"** showed the lowest Macrophage migration index with chloroform and alcohol extracts of *Ecballium elaterium* at concentrations 1500 & 2000 µg/ml and pet. ether, chloroform, ethyl acetate and alcohol extracts of *Tropaeolum majus* nearly at the same concentrations.

They showed appreciable non specific immunostimulant activity (Low Macrophage migration index) comparable to the results displayed by the control.

Regarding the non specific immune response using **"phagocytic index"**, it was apparent that chloroform extract of *E. elaterium* at concentrations 500 & 2000 µg/ml and in addition to alcohol extract at concentrations 1500 & 2000 µg/ml together with pet.ether, chloroform, ethyl acetate and alcohol extracts

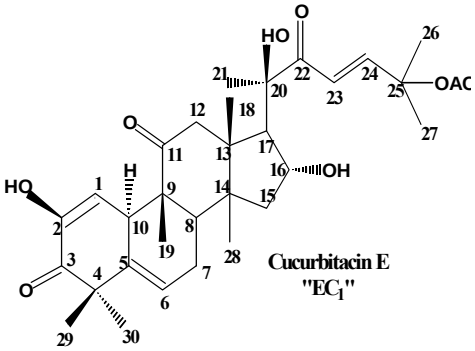
Tropaeolum majus at concentrations 1500 & 2000 µg/ml were the best as immunostimulants.

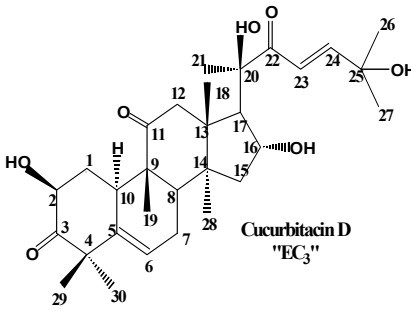
In determination of **"viability percentage"**, it was apparent, by comparison of each plant extract at different concentrations (100-2000 µg/ml) with control, that both chloroform extract at concentrations 1500 & 2000 µg/ml and alcohol extract at concentrations 500-2000 µg/ml of *E. elaterium* had higher viability % than that of control. Pet. ether, chloroform, ethyl acetate and alcohol extracts of *T. majus* showed significantly high viability % at concentrations 1000-2000 µg/ml.

The three experiments suggested that chloroform and alcohol extracts of *E. elaterium* and pet.ether, chloroform, ethyl acetate and alcohol extracts of *T. majus* had considerable immunostimulant effect. This led us to investigate the active constituent of *Ecballium elaterium* and *Tropaeolum majus* that possibly to have such immunostimulant effect.

Investigation of the chloroform extract of *Ecballium elaterium* fruits (A. Rich.) F. Cucurbitaceae resulted in isolation of two compounds named **"EC_1" and "EC_3"**.

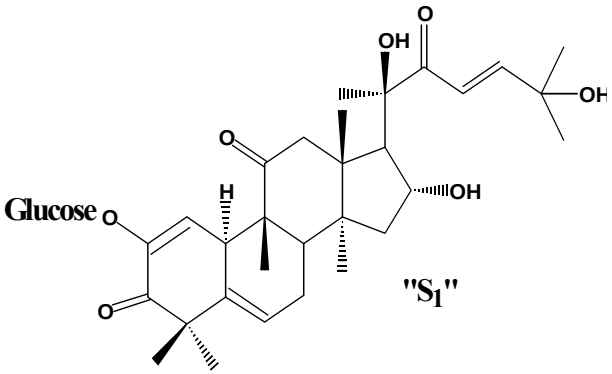
Compounds "EC_1" and "EC_3" gave positive test for cucurbitacins by spraying with vanillin/phosphoric acid reagent to give red and brown spots[6]. Its spectral data was identical to published data of **"cucurbitacin E"** and **"cucurbitacin D"** respectively[7-10].





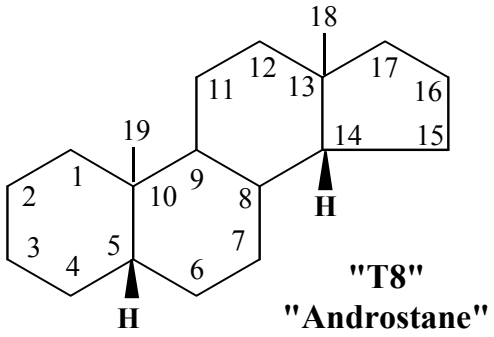
Investigation of the methanol extract of *Ecballium elaterium* fruits (A. Rich.) F. Cucurbitaceae resulted in isolation of one compound named **"S_1".**

"2-O-β-D glucopyranosyl-cucurbitacin I"



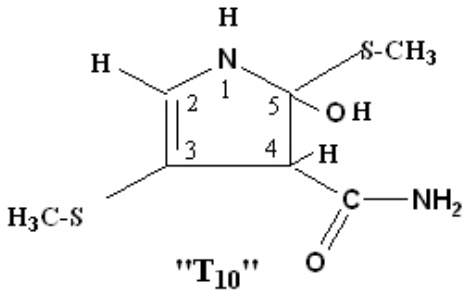
Investigation of the petroleum ether extract of *Tropaeolum majus* herb L. F. Tropaeolaceae resulted in isolation of two compounds named **"T_8"** and **"T_{10}".**

Compound "T_8" isolated from petroleum ether extract of *Tropaeolum majus* herb gave positive Leiberman-Burchard test for steroids and/or triterpenes. It was identified as **"Androstane".**



Compound "T_{10}" responded negatively to Leiberman-Burchard test but gave faint orange Dragendorff's test for nitrogenous substances.

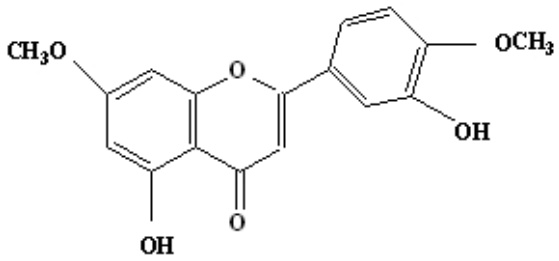
Compound **"T_{10}"** was identified as a sulphur-compound **"4-acetyl- 3, 5-bis-(methyl thio)-2-pyrrolin-5-Ol"**



"T_{10}"

Investigation of the chloroform extract of *Tropaeolum majus* herb L. F. Tropaeolaceae resulted in isolation of one compound named "**TC_4**".

Compound "TC_4", isolated from chloroform extract of *Tropaeolum majus* herb gave faint yellow color in visible light, yellowish blue in U.V., faint yellow in U.V./NH_3 and yellow color after spraying with $AlCl_3$ spray reagent indicating the flavonoid nature. Compound "**TC_4**" was identified as **"5, 3'-dihydoxy-7, 4'-dimethoxy flavone" [(13)].**

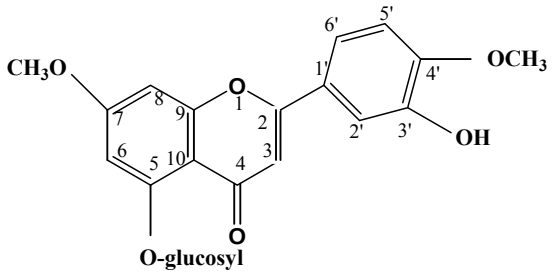


"TC_4"

"5,3'-dihydroxy-7-4'-dimethoxy flavone ":

Investigation of flavonoids and other phenolic constituents of *Tropaeolum majus* herb L. F. Tropaeolaceae resulted in isolation of one compound named "**TE_4**".

Compound "TE_4", isolated from ethyl acetate extracts of *Tropaeolum majus* showed faint yellow color in visible light, yellowish blue in U.V. light & in U.V./NH_3 and yellow color by spraying with $AlCl_3$ spray reagent indicating the flavonoid nature. Compound **"TE_4"** was identified as **"5, 3'-dihydroxy-7, 4'-diemthoxy flavone-5-O-glucoside".**



"TE_4"

"5, 3'-dihydroxy-7, 4'-dimethoxy flavone-5-O-glucoside

Study of immunostimulant activity by the isolated compounds:

Tumor Necrosis factor–alpha (TNF-α) and Gamma Interferon (INF-γ) production [14], using CytElisa™ technique, were used as a measure of immunostimulant activity by seven compounds that were isolated in appreciable amounts (**EC_1, EC_3 & S_1** from *Ecballium elaterium* and **T_8, T_{10}, TC_4 & TE_4** from *Tropaeolum majus)* in comparison to marketed drug *Echinacea purpurea*. Three concentrations, (0.5, 50 and 500 µg/ml), of isolated compounds were used and procedure was carried out according manufacturer procedure and results obtained were presented in table (٧).

Table (7): Concentrations of produced cytokines "TNF-α and INF-γ" produced after treatment with isolated compounds:

Isolated compound	Conc. of Isolated compound (µg/ml)	Conc. of TNF.α (pg/ml)			Conc. of INF-γ (pg/ml)		
		Lymphocyte		macrophage	After 24 hr.	After 48 hr.	After 72 hr.
		After 24 hr.	After 48 hr.	After 24 hr.			
EC_1	0.5	5.95	-	٦.٢٠	٢٧.٩٠	٧٨.٢٢	٧٦.٢٢
	50	١١.٩٩	-	-	٧٤.١١	٢٠.٨٦	٩.٥٣
	500	-	٢٨.٨٧	-	٦٠.٥٥	٣٧.٧٩	١٤.٧٣
EC_3	0.5	١١.٨٤	-	٣٥.٢٧	٣٧١.٨٠	٦٠.٥٥	٥٣.٢٠
	٥٠	٢٣.٣٧	٣٦٧.٥٦	٢.٥٧	٦٢٧.١١	١٣٥٠.٠٠	٢٠.٠٥
	٥٠٠	٠.١٩	٦٧.٧٨	-	٢٤.٩٠	٢٥٨.٦١	٤٠.٢٦
S_1	٠.٥	٧٧.٨٥	٨٥.٦٥	٢٣.٣٧	٨٣.٠٨	١١٤.٩٢	٢٨.١٣
	٥٠	٥.٠٨	١٨٢.٣٧	-	٣٢.١٨	٩٦.١٨	٢٠.٠٥
	٥٠٠	-	-	٣.٨٦	-	٩.٥٣	٧٦.٩٢
T_8	٠.٥	٣.٩٥	-	٤٠.٧٨	١٣١٠.٠٠	٢٠٤٠.٠٠	٤٦١.٨٣
	٥٠	١.٠٧	-	٤.٨٣	١٠٠.٨٤	١٦٤.١٧	٤٠٢.٠٦
	٥٠٠	٠.٠٦	٤٩.٣٤	١٥.٧٣	٦٧.٧٨	٧٠.١٢	٢٤٩.٠٣
T_{10}	٠.٥	١.١٩	٢٦.٣٣	١٤.٠٢	٥٨٥.٤٦	١٨٨٠.٠٠	١١٤٠.٠٠
	٥٠	٦.٢٠	٢٧.٥٨	٣٨.٣١	١١٢٠.٠٠	٢٠٩٠.٠٠	٨٩٦.١٣
	٥٠٠	٢.١٩	-	٧.٤٦	٧٥.٣٠	٣٦٥.٥٦	٣٣.٧٩
TC_4	٠.٥	٨.٤٦	٤٥٨.١٢	٢٩.٥٦	٣٦.٢٢	١٤٢.٠٧	١٣٧.٢١
	٥٠	٨.٣٣	٩.٤٦	-	٧٣.٣٦	٩.٥٣	٨٩.٨٩
	٥٠٠	-	٦.٨٣	-	-	٥٨.٨٦	١٣١.١٠
TE_4	٠.٥	١١.٣٤	٩٠.٧٨	٨٩٨.٦٢	١٤٥٠.٠٠	١٧٢٠.٠٠	١١٠٠.٠٠
	٥٠	٢.٥٤	٢٥.٤٣	٧٧٦.٢٦	١١٢٠.٠٠	١٨٢٠.٠٠	٣٤٨.٧٢
	٥٠٠	١٠.٢١	١٠٠.٩١	٢٠.٦١	٣١٣.١٦	٥٢٣.٢٤	٩٢٩.٣٥
Echinacea extract	٠.٥	-	٣٨.١٢	١١.٥٩	٥٠٤.٠٥	١٧٤٠.٠٠	٩٤.١٠
	٥٠	-	١.٠٧	٢.٩٥	١٦٤.٤٣	١٧٤.١٦	٥٨.٨٦
	٥٠٠	٦.٧١	٤.٠٨	-	٢٠.٨٦	-	-

DISCUSSION

Evaluation of isolated compounds from *Tropaeolum majus* and *Ecballium elaterium*, relative to the reference plant *Echinacea purpurea*, for specific immune response to produce cytokines (TNF-α and INF-γ) clearly demonstrated good inductive effect for production of TNF-α by lymphocytes displayed by compounds "**EC_3**" (50 µg/ml), "**S_1**" (0.5 µg/ml), "**TC_4**" (0.5 µg/ml) and "**TE_4**" (0.5 and 500 µg/ml) after 48 hrs producing 367.36, 85.65, 182.37, 458.12, 90.78, and 100.91 Pg/ml respectively while, only compound **"TE_4"** displayed good inductive effect for production of TNF-α by macrophages producing 898.62 and 776.26 Pg/ml at concentrations 0.5 and 50 µg/ml of compound **"TE_4"** respectively.

Regarding the production of INF-γ after treatment of macrophages for 24, 48 and 72 hours it was apparent that compounds **"EC_3"** and **"S_1"** displayed good inductive effect after

24 and 48 but not after 72 hours while, compounds**"T_8", "T_{10}" and "TE_4"** were powerful inductive for the production of INF-γ after 24, 48 and 72 hours resulting in production of high amount of such cytokine reached 2090.00 Pg/ml with compound **"T_{10}"**.

It was clear that the non acetylated form of the cucurbitacin compound **"EC_3"** was more active that the acetylated form **"EC_1".**

Compound **"T_8"** which is a steroidal compound displayed a powerful effect for production of INF-γ. Also compound **"T_{10}"** which is a sulfur compound showed the highest level of INF-γ (2090.00 pg/ml) production suggesting the possible effect of natural sulfur compounds as immunostimulant drugs.

In addition compound **"TE_4"** as a flavonoid glycoside displayed higher immunostimlant effects than its flavonoid aglycone **"TC_4"** suggesting that the sugar moiety of the glycoside might play an important rule in cytokine production.

As a conclusion, Cucurbitacin D **"EC_3"** and 2-0-β-D-glucopyransoyl cucurbitacin I **"S_1"** of *E.elaterium* in addition to 5,3'-dihydroxy-7,4'-dimethoxy flavone**"TC_4"** and 5,3'-dihydroxy-7,4' dimethoxy flavone – 5-0– glucoside **"TE_4"** of *T. majus* were found to significantly stimulate the production of both TNF-α and INF-γ, while, Androstan **"T_8"** and 4-acetyl-3,5-bis-(methyl thio)-2-pyrrolin-5-Ol **"T_{10}"** of *T. majus* stimulated the production of INF-γ only.

Cucurbitacins, steroidal compounds, sulfur compounds and flavonoids might play an important rule in immunostimulation.

RECOMMENDATIONS

We need to pay more attention to our Egyptian flora as it presents more convenient and in some instances superior to the already established and marketed drug formulations.

Promising new compounds that were isolated from *Ecballium elaterium* and *Tropaeolum majus* extractives should be subjected to extensive clinical and pharmacokinetic studies to support their presentation in drug market.

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معَدِلات المناعة من مصادر طبيعية

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تمثل محفزات المناعة من خلاصات النباتات والمواد المفصولة منها بديلا هاما للعلاج الكميائى التقليدى والوقائى من العدوى. ويعتبر هذا ذو اهمية قصوى خاصة عند الحاجة لعلاج الامراض المعدية، الامراض المتوطنة، و البكتيريا والفيروسات المقاومة للعلاج الكميائي بالاضافة الى الوقاية من العدوى الانتهازية خاصة لبعض المرضى ذوى الحالات الحرجة. هذا اضافة الى استخدامه فى علاج الاورام الخبيثة عندما يمكن ان نقلل من نمو الورم الخبيث بتنشيط بعض العوامل الحفازة للجهاز المناعى للجسم .

فى هذه الدراسة تم اختيار ثمان نباتات هم : القثاء البرى ، اللوف المصرى ، والقرع الاستانبولى ونوعين من عيش الغراب وقشر الارز ونبات ابو خنجر بالاضافة الى نبات الاشنسيا بربوريا. وقد بينت التجارب الاولية لتحفيز المناعة المتخصصة ان نبات ابو خنجر ونبات القثاء البرى بالاضافة لنبات الاشنسيا بربوريا (المعروف كمحفز للمناعه) هم الأكثر تأثيرا كمحفز للمناعة. وقد دعمهذا لفصل والتعرف على المركبات الفعالة فى نبات ابو خنجر والقثاء البرى. وقد كان قياس كمية ال (TNF-α) ، (INF-γ) االمنتجة بعد المعاملة بهذه المركبات المفصولة من هذين النباتين يعتبر دليلا على تحفيزهم للمناعة.

وقد تبين ان مركب " كوكربيتاسين دى" ، " ٢– او– دى– جلوكوبيرانوزيل كوكربيتاسين آى" من نبات القثاء البرى بالاضافة لمركب " ٥، ٣'– داى هيدروكسى–٧، ٤'– داى ميثوكسى فلافون" ، " ٥، ٣'– داى هيدروكسى–٧، ٤'– داى ميثوكسى فلافون – ٥– او–جلوكوسيد" من نبات ابو خنجر ذوى تاثير قوى كمحفز لانتاج (TNF-α) ، (INF-γ) على حد سواء . بينما كان مركب "اندروستان" ، مركب "٤– خلات– ٣، ٥– بز(ميثيل ثيو) ٢– – بيرولين– ٥– اول" من نبات ابو خنجر ذو تأثير محفز لانتاج (INF-γ) فقط . وهذا يبين تأثير هذه المركبات كمحفز للمناعة.