Journal homepage www.jzs.univsul.edu.iq
Journal of ZankoySulaimani
Part-A- (Pure and Applied Sciences)

Influence of supplementation three type of Phytogenic plants in diet on growth Performance, intestinal microflora and immunity of Broiler Cobb-500.

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Article info

Original: 14 April 2016
Revised:
5 November 2016
Accepted:
20 November 2016
Published online:
20 June 2017

Key Words:
Medical plants, Growth performance, Immunity, Mortality, and Micro flora.

Abstract

This study was conducted to evaluate the effects of three herbal medical plants, Chamomile flowers (*Matericariachamomilla L)*, Black cumin seeds (*Nigella sativa L)* and Thyme (*Thymus vulgaris*) on growth performance, carcass characteristics, immune system, and intestinal bacterial populations in broiler Cobb-500.

A total of two hundred forty (240) Cobb500, one-day old broiler chicks were randomly divided to four experimental groups (60) chicks/treatment, which divided to (3) replicates (20 Chicks / replicate).

The dietary treatments were: (T1) basal diet as control, (T2) basal diet plus 1kg of Chamomile flowers(*Matericariachamomilla L)* to 100kg diet, (T3) basal diet plus 1kg Black cumin seeds(*Nigella sativa L)* to 100 kg diet, .(T4) basal diet plus 1kg of the Thyme (*Thymus vulgaris*) to 100 kg diet. The experiment was lasted for (42) days. Live body weight (LBW), Weight Gain (WG), Feed Intake (FI), Feed Conversion Ratio (FCR) were measured at the end of feeding program (21, 35 and 42 days) of age. At the end of the experiment carcass characteristics, immunological properties such as antibody titer against Newcastle disease virus as well as relative weight of bursa fabricia, spleen and total mortality rate were measured. The groups fed (T2) Chamomile flowers (*Matericariachamomilla L)* and (T3) Black cumin seeds (*Nigella sativa)* showed significantly ($p>0.05$) higher value for Live body weight (LBW) as compared with (T1, T4) groups. No significant differences were observed in feed conversion ratio (FCR) and total Feed intake (FI) at period (1-42 days). Results showed that total Mortality decreased significantly ($p<0.05$) in all treatment compared with those birds fed control group (T1). The results showed that relative weights of lymphoid organs (spleen, bursa fabrecius) as an indicator of the immune system condition was significant ($P<0.05$) high weight of spleen observed at all treatments with supplementation of dietary herbs compared with control group (T1)..Antibody responses to Newcastle Disease were improved significantly ($p<0.05$) antibody levels increased by treatments (T3, T4)**.** The result shows that treatments (T3, T4) were lead to significantly ($p<0.05$) decreased of total counts of coliform bacterial in jejunum compared with (T1, T2) groups

Introduction

The ban on nutritive antibiotic use in Europe and the increased awareness of the consumers activated a need for natural and safe feed additives to achieve better production results of poultry farm [1]. Alternative

substances and strategies for animal growth promotion are being investigated. In this context, phytogenic and herbal products have received increased attention, because as natural additives they have a high acceptability among consumers [2]. Phytogenic feed additives consist of a wide variety of herbs (flowering, non-woody and non-persistent plants), spices (herbs with an intense smell or taste frequently added to human food) and essential oils. This class of feed additives has recently enjoyed increasing interest, especially for use in swine and poultry [3, 4].

Herbs contain unique anti-oxidants, essential oils, vitamins, phyto-sterols and many other plants derived nutrient substances, which help to provide birds to fight against germs, toxins and enhance immunity level in fact, herbs are medicines in smaller dosages. Recently aromatic plants and their associated essential oils or extracts are being concerned as potentially growth promoters. Documentation of herbal plants is necessary because they are likely to be more important in the future, especially given the escalating costs of drugs and the focus on organic products in most developing countries. In addition, with the development of resistance of pathogens to drugs, herbal medicine might be the alternative to take since herbs tend to be broad spectrum [5].

Chamomile flowers (*Matericaria chamomilla L*), is packed with numerous health benefiting phyto-nutrients. It contain up to one percent of an essential oil with azulene ,bisabolo, flavonoid, glycosides and fatty acids, These substance give anti-inflammatory, antiseptic, carminative, diaphoretic, sedative properties[6,7]. Thyme (*Thymus Vulgaris*) is one of the herbs that provide substantial amount of flavonoids which have health promoting properties, as antioxidants, anti-inflammatory, antitumor agents , Volatile oil from thyme (*Thymus vulgaris*)was assessed for antibacterial and antiviral activity as inhibitors of microbial growth and contains minerals and vitamins which are essential for overall wellness. Thyme is one of the popular among culinary herb plants, originally is native to the southern Europe and Mediterranean regions [8, 9, and 10].

Other medical plant used as feed additives are black cumin seed. Black cumin seed *(Nigella sativa L.)* is also known as black seed and grows in Asian and Mediterranean countries. The seed has been reported to have many biological properties including anti-parasitic and antibacterial activity [11].

The seeds of N. sativa contain volatile oil (0.5-1.6%), fixed oil (35.6-41.6%), protein and amino acids (22.7%).So *(N. sativa L*.) seeds appear to be a multi-purpose feed growth promoter and may be promising in improving broiler performance [12].

Therefore, this study was carried out to evaluate and compare the possible growth enhancer and health benefit effects of three medicinal plants, in broiler chicks. The plants were selected for their potential benefits to birds' appetite, digestion, immunity and antibacterial activities.

Material and method

The study was conducted at a poultry hall of the animal resources department of the Agriculture College, Salahadin University/ Erbil/ Kurdistan Region. Experiment lasted for 42 days from (01/04 /2014 to 12/05/2014).

A total of two hundred forty (240) Cobb500, one-day old broiler chicks were randomly divided to four experimental groups (60) chicks/treatment, which divided to (3) replicates (20 Chicks / replicate).

Chicks were raised in floor pens with wood shaving litter. Birds were fed a three phase-feeding program, Starter, grower and finisher diets were presented from (0 to 21days) as starter, (22 to 35 days) as grower and (36 to 42 days) as finisher . Feed and water were provided ad-libitum throughout the experiment. All diets were presented to the birds as crumbles. The composition and nutrients content of the basal diets are shown in Table 1.

Table 1. Composition Percentage and calculated analysis of the experimental diets.

Ingredients	Starter (1-21) days	Grower (22-35) days	Finisher (36-42) days
Corn	30	30	30
Wheat	27.5	29	35
Soybean meal	28	26.5	20
Protein concentrate *	10	10	10
Sunflower (oil)	3	3	3.5
Lysine	0.15	0.15	0.15
Methionine	0.05	0.05	0.05
Enzyme **	0.10	0.10	0.10
Limestone	0.40	0.35	0.35
Calcium phosphate	0.35	0.35	0.35
Salt	0.30	0.30	0.30
Mineral and Vitamin premix ***	0.1	0.15	0.15
Choline chloride	0.05	0.05	0.05
	Calculated Value		
ME , Kcal / kg	3000	3044	3125
CP , %	23	21.8	19.90
Calcium , %	0.85	0.75	0.75
Available Phosphorus , %	0.75	0.72	0.72
Lysine , %	1.45	1.29	1.18
Methionine , %	0.71	0.66	0.60

* Protein concentration using for experiment produced by Holland Company , WAFI Holland contain 40% CP , 210000 kg NE , 5% Fat , 2% CF , 6.5% Ca , 2.5% P , 3.85% Lys. , 3.7 Meth. and 4% cys.

** Beta-glucanase, xylanase, protease.

*** B12 , B2 , B1 , K , E , D3 , A , Niacin , Pantothenic acid , Folic acid , Biotin , Choline , Ca , P , Na , Mn , Fe , Zn , Cu , I , Co , Se .

The diets were formulated to exceed the National Research Council NRC, 1994[13] requirements. A basal diet with no additives considered as control (T1), and three experimental treatments were formulated by supplementation of 1kg of herbal plants to (100 kg) diet. The supplemented herbal plants were prepared with equal ratio as follow:

The dietary treatments were: (T1) basal diet as control; (T2) basal diet plus (1 kg) of Chamomile flowers (*Matericaria chamomilla*) to (100 kg) diet , (T3) basal diet plus (1 kg) Black cumin seeds(*Nigella sativa* L.) to (100 kg)diet, (T4)) basal diet plus (1 kg) of the Thyme (*Thymus vulgaris*) to (100 kg) diet.

Average of live Body Weight (LBW), Body weight gain (BWG) and Feed Intake (FI), Feed Conversion Ratio (FCR) at days21, 35 and 42 were measured. Two birds from each replicate were randomly selected, slaughtered and eviscerated to record carcass ratio, breast, tights, wing, gizzard and liver yield; Immune organs (Spleen and Bursa of Fabrecius) were measured on a relative carcass weight basis.

Mortality were recorded when occurred and calculated from the records of dead birds against total number of birds and expressed as percentage.

The chicks were vaccinated three times by spray route against Newcastle Disease (ND) at the age (1, 15, 30 days) the type of vaccine was(Clone 30) at the first day and(Lasota) at 15 and 30 days of age. Blood samples were collected from the jugular vein during slathering for Detection of antibody titer against Newcastle Disease Virus (NDV) in serum of immunized chickens was performed by enzyme linked immunosorbent assay (ELISA).

The micro flora account were done for total counts of aerobic bacteria and coliform bacteria (*E.coli*) in digestive duct after postmortem, the MacConkey agars used to culture Coliforms bacteria (*E.coli)* and Nutrient agar was used to culture total aerobic bacteria the samples were collected from the chickens jejunum in small intestine, each samples was measured (1 g) and distilled in (9 ml) water. A serial dilution up to 10/10 was spared on nutrient ager plates and Mackonky ager plates from each of 10/4, 10/5, 10/6 dilutions, plates were incubates at 37°C for 24 hours, and bacterial population enumerated with aid colony forming unit(cfu/g) Fowled and Oso 2004 [14].

All data were analyzed using the CRD (Completely Randomized Design) of SAS (SAS Institute, 1998 [15]). Duncan's multiple range tests were used to compare differences among treatment means [16].

Results and Discussion

The influence of supplementation different type of Phytogenic plant in diet on live body weight(g), weight gain(g), feed intake(g) and Feed conversion ratio (g feed/g body weight gain) during feeding programs (Starter, Grower, and Finisher) for broiler chickens are summarized in Table (2).

As the results showed that average live body weight (LBW) there are no significant differences between T1, T2, T3 and T4 in 21 days. 1n 35 and 42 days there are no significant differences between T2, T3 and T4 meanwhile T2, T3 were significantly higher ($p<0.05$) from the control group (T1).

These results agree with that obtained by [7] who reported that adding Chamomile flowers at a level of 1 kg/100kg diet, increased significantly ($p<0.05$) live body weight (LBW) as compared with the control group at the 42 days of age. This may be due to active compounds that are presented in chamomile flowers. It could be inhibit the excessive growth of a harmful intestinal microorganism, which may affect positively on poultry health and productivity, our result is in support with that obtained by [17].

The results showed that no significant differences observed in bodyweight gain (BWG), among treatments group in whole phases and our results agree with those obtained by [9,18] who reported that adding thyme or black cumin seeds either alone or in combination to low energy diets gave not significant differences($p<0.05$) in body weight gain during whole experimental period.

The results shows that dietary inclusion of medical plants affect significantly $p>0.05$on the feed intake (FI) at starter and grower period, feed intake was significantly ($p<0.05$) increased by supplementation of black cumin seeds, thyme and chamomile comparatively with chicks feed control group. Meanwhile feed intake for the control group at the finisher period (36-42) days, was significantly ($P<0.05$) higher than treatments groups with supplementation of medical herbs.

The results shows that there was no significant ($P>0.05$) effect of Phytogenic plants supplementation on feed conversion ratio (FCR) through the whole experimental period. This results agree with that reported by [19] who found that diets with 4% grounded black cumin resulted in less feed intake and no significant ($P>0.05$) effect of black cumin supplementation on feed conversion ratio (FCR), meanwhile controversy results were reported by [20] reduced FCR was observed in broiler chicks fed diet supplemented with 1 and 2 % black seeds.

Table 2. Influence of supplementation three type of phytogenic plants in diet on live body weight(LBW), weight gain(WG) feed intake(FI)and Feed conversion ratio(FCR)during feeding program (Starter, Grower, and Finisher)of broiler cob-500.

Live body weight (g)				
Feeding period(days)	**(T1)control**	**(T2)Chamomile flowers**	**(T3) Black cumin seeds**	**(T4) Thyme**
Starter (0-21)days	629.17 ± 3.88 a	638.67 ± 5.34 a	638.67 ± 1.42 a	650.67 ± 12.9 a
Grower(22-35)days	1574.67 ± 12.2 b	1678.33 ± 13.8 a	1652.67 ± 17.1 a	1624.0 ± 16.2 ab
finisher(36-42)day	2600.0 ± 15.4 b	2716.67 ± 14.9 a	2700.0 ± 15.2 a	2650.0 ± 12.8 ab
BWG(g)				

Feeding period(days)	(T1) control	(T2) Chamomile flowers	(T3) Black cumin seeds	(T4) Thyme
(0-21)days	584.17 ± 3.88 a	593.67 ± 5.34 a	593.67 ± 1.42 a	605.58 ± 12.95 a
(22-35)days	945.50 ± 28.7 a	1039.66 ± 27.8 a	1014.0 ± 26.48 a	973.42 ± 16.5 a
(36-42)days	1025.33 ± 19.8 a	1038.33 ± 13.5 a	1047.33 ± 29.4 a	1026.0 ± 18.5 a
		FI(g)		
Feeding period(days)	(T1) control	(T2) Chamomile flowers	(T3) Black cumin seeds	(T4)Thyme
Starter (0-21)	854.71 ± 23.04 b	853.22 ± 3.38 b	869.53 ± 20.54 a	872.38 ±17.28 a
Grower (22-35)	1464.72 ± 26.00 b	1549.95 ± 10.45 a	1572.53 ± 16.49 a	1571.42 ±11.56 a
Finisher (36-42)	1734.83 ± 30.03 a	1582.30 ± 33.25 d	1589.71 ± 12.45 c	1615.12 ± 10.77 b
Total Feed intake (g)1-42 day	4054.26 ± 21.03 a	3985.17 ± 23.83 a	4031.77 ± 14.03 a	4058.92 ± 22.00 a
		FCR (g feed/g body weight gain)		
Feeding Period(days)	(T1) control	(T2)Chamomile flowers	(T3) Black cumin seeds	(T4) Thyme
Starter (0-21);	1.46 ± 0.11 a	1.44 ± 0.09 a	1.46 ± 0.22 a	1.44 ± 0.16 a
Grower (22-35)	1.55 ± 0.33 a	1.49 ± 0.12 a	1.55 ± 0.18 a	1.61 ± 0.07 a
Finisher (36-42)	1.70 ± 0.13 a	1.52 ± 0.10 a	1.52 ± 0.15 a	1.57 ± 0.12 a
Feed Conversion ratio 1-42 day	1.57 ± 0.10 a	1.48 ± 0.13 a	1.51 ± 0.23 a	1.54 ± 0.33 a

Means with different superscripts in the same row differ significantly ($p<0.05$) Means ± Standard error. (T1) basal diet as control; (T2) basal diet plus (1 kg) Chamomile flowers (T3) basal diet plus (1 kg) Black cumin seeds.(T4) basal diet plus (1 kg) of the Thyme

Table .3 shows the Influence of supplementation different type of phytogenic plant in diet on dressing percentage, carcass parts ratio and eatable visceral ratio (Liver, Gizzard and Heart) in broiler chicks at 42 day-old. There were no significantly ($P>0.05$) differences among treatments in dressing percentage while there are improvement in dressing percentage in T2, T4 compared with T1, T3. Carcass cut ratio (back) and relative weight of heart in the different treatments group showed a significant increase as compared with that in control groups, while relative weight of liver and gizzard were unaffected by the different treatments group.

These results agree with that obtained by [10,18and 20] who showed that dressing percentage of broiler was significantly higher by supplementing different levels of herbal feed additives or herbal plant extracts. It may be due to the stimulatory effects of herbal plants on pancreatic secretions such as digestive enzymes which help to digest and absorb more amino acids form the digestive tract and thereby improve carcass traits as reported by [21].The results informed by [22, 23] disagree with recent study, who informed that adding different herbal plants or spices to broiler diets had no effect on carcass characteristics.

Table 3. Influence of supplementation three Phytogenic plants in diet on dressing percentage, carcass parts ratio and eatable visceral ratio in broilercobb-500 at 42 day-old.

Characteristics	ontrol	(T2) Chamomile flowers	(T3);Black cumin seeds	(T4) Thyme
Dressing	79.92 ± 0.95 a	82.45 ± 2.00 a	79.63 ± 3.16 a	83.03 ± 0.02 a

percentage				
Breast	44.42 ± 0.69 a	45.94 ± 0.86 a	47.12 ± 0.73 a	47.83 ± 1.75 a
Tights	28.08 ± 0.81 a	26.01 ± 0.98 a	26.26 ± 0.35 a	25.58 ± 0.82 a
Back	11.34 ± 0.14 ab	11.26 ± 0.35 ab	10.55 ± 0.29 b	12.17 ± 0.05 a
Wing	11.23 ± 0.09 a	11.68 ± 0.87 a	10.83 ± 0.39 a	10.96 ± 0.23 a
Neck	4.49 ± 0.08 a	5.10 ± 0.09 a	5.24 ± 0.08 a	3.42 ± 1.71 a
Liver	2.62 ± 0.28 a	2.90 ± 0.23 a	2.63 ± 0.07 a	3.41 ± 0.42 a
Gizzard	2.25 ± 0.34 a	2.25 ± 0.26 a	2.39 ± 0.28 a	2.92 ± 0.04 a
Heart	0.51 ± 0.02 b	0.63 ± 0.06 a	0.66 ± 0.08 a	0.65 ± 0.07 a

Means with different small superscripts in the same row differ significantly ($p<0.05$) Means ± Standard error. (T1) basal diet as control; (T2) basal diet plus (1 kg) Chamomile flowers , (T3) basal diet plus (1 kg) Black cumin seeds , (T4) basal diet plus (1 kg) of the Thyme.

In table (4) shows the relative weights of lymphoid organs (spleen, bursa fabrecius) as an indicator of the immune system condition. Immune organs weight (spleen and bursa fabrecius) was measured on a relative carcass weight basis. The highest weight of spleen with significant ($P<0.05$) differences observed at the (treatments T2, T3 and T4) with addition of herbal plants (chamomile flowers, black cumin seeds and thyme) compared to the (T1) control group while there was no significant ($P>0.05$) differences observed about the weight of bursa fabrecius (immune organ) in all treatments groups but there was improvement in the weight of bursa fabrecius (T3, T4) compared with T1, T2. These results agree with [24] who reported that the Beneficial effects of herbal supplementation may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, antiviral and antioxidant actions. Flavonoids, glucosinolates and other plant metabolites may affect the physiological and chemical function of the digestive tract. Herbs that are rich in such flavonoids as thyme (*Thymus vulgaris*) extend the activity of vitamin C, act as antioxidants and may therefore enhance the immune function. Similar results were typically recorded by [25, 26] those found that Black seed supplementation caused a significant ($P<0.05$) increase in the weight of lymphoid organs.

In table (4) shows there were significant effects of medicinal plants supplementation on Newcastle disease virus (NDV) antibody titer, The statistical analysis of the data showed that supplementation of herbal products of thyme and black cumin seeds in diet showed a significant increase of ($p < 0.05$) antibody titer against NDV at day 42, and highest antibody titers were belonged to the group fed thyme(T4) followed by black cumin seeds (T3) and there was no significant differences between (T2) chamomile group and (T1)control group. A similar results was obtained by [27] who informed that the highest antibody titers were seen significantly ($p<0.05$) in chicks fed basal diet supplemented with mixture of 1% thyme plus 1% garlic and chicks fed basal diet supplemented with 1% garlic then chicks fed basal diet supplemented with 1% thyme respectively as compared with control group, this improvement in antibody titer against ND viruses could be due to their relationship between ND vaccine and immunomodulation feed additive (medicinal plant) or present of active ingredients in thyme and garlic such as thymol, carvacrol, allicin could have positive effects on performance and growth of broilers also antibacterial, anticoccidial, antifungal, and antioxidant effects. Our results were in agreement with that reported by [21, 28, 29, 30] who stated that these medical plants improving health status and performance of broiler, in addition to improve antibody titer against ND virus. Furthermore, these results agree with [31] who reported that the black Cumin seeds (*Nigella sativa*) could be the most suitable alternative to antibiotics in poultry nutrition. A Black Cumin seed

(*Nigella sativa*) not only promote bird's health and production performance, but also plays a significant role as a natural antioxidant and immuno-stimulant.

Table (4) are summarized the Micro flora identification in each treatment (total aerobic bacteria, and coliform bacteria.

The study showed that treatment (T3) black cumin seeds group had the most influence on the total number of aerobic bacteria at 42 day of age in the digestive system, meanwhile the differences were not significant (P>0.05) compared to the other experiment groups.

Concerning the effect of different medical plant on coliform populations, the total of coliform bacteria decreased significantly (P < 0.05) in (T3) black cumin seeds compared with other treatment and control group however in (T2) chamomile group and (T4) Thyme group there is decreasing in the number of coliform bacteria compared with control group. Our results agree with that reported by those authors indicated that medical plant are well known for their antimicrobial activity which dependent on the physico-chemical characteristics of the thyme plant compounds and bacterial strains and their effects on invitro against important pathogens but also against fungi [31,32, 33]. The results obtained by [34] agree with our study who reported that adding black cumin seed and seed extract showed decrease tendency in total coliform bacteria count in the caecal intestine in broiler.

Our results differ with that obtained by [35] who observed that the microbiological counts of the ileum with the supplementing of (medicinal plants) did not alter the population of Lactobacilli and coliform bacteria in the ileum. However, a slight numerical reduction, but not significant (P>0.05), was observed in Coliform bacteria when the birds compared with the control.

Mortality rates were represented in table (4) the results of the cumulative mean mortality shows significantly (P < 0.05) differences exist between the birds fed diet supplemented with medical plant and those fed control diet. At the end of the experiment (42)days of age the mortality rate for the control group(T= 6.39%) which was significantly (P < 0.05) higher than those fed diet containing chamomile (T2=4.34%)), diet containing Black cumin seed (T3=4.00%) and diet containing thyme (T4=3.97%) this results agree with that obtained by [36] who reported that the total mortality rate for birds fed herbal plant (lemongrass) was found to be significantly (P < 0.05) lower than the mortality rate in control group and this due to the ability of the medical plant to control the microbial activates and thus reduce infection, strength immune system and reduce mortality rate.

Table (4) Influence of supplementation three Phytogenic plants in diet on the immune organ, immune response, bacteria count in digestive duct and mortality in broiler cobb-500 at 42 day-old

Parameters	**.Treatments**			
	T1	**T2**	**T3**	**T4**
Percentage of (Immune organ)/Live body weight				
Spleen	0.10 ±0.01 b	0.12± 0.08 a	0.13±0.01 a	**0.12±0.01 a**
ursa fabrecius	0.169 ± 0.02 a	0.165 ±0.011 a	0.170 ±0.026 a	**0.177 ±0.018 a**
Intestinal micro flora in digestive duct (jejunum) (log cfu/g)				
No. of total Aerobic bacteria (log cfu/g).	6,46 ± 7,33 a	5,99 ± 4.67 a	7,12± 1.44 a	**6,21 ± 0.18 a**
No. of coliform bacteria (log cfu/g).	6,77 ±0.29 b	5.19 ± 6.89 b	4.39± 1.85 a	**5.48 ± 3.11 b**
Antibody titer				
ELISA For NDV	3375 ±30.94 b	3047 ±64.33 b	6502 ±57.78 a	**7746 ±19.58 a**
Total mortality% (1-42 days)	6.39 ±0.33 a	4.34 ± 0.45 b	4.00 ± 0.39 b	**3.97 ± 0.29 b**

Means with different small superscripts in the same row differ significantly ($p<0.05$) Means ± Standard error. (T1) basal diet as control, (T2) basal diet plus (1 kg) Chamomile flowers, (T3) basal diet plus (1 kg) Black cumin seeds, (T4) basal diet plus (1 kg) of the Thyme. NDV= Newcastle disease virus, cfu= colony forming unit

Conclusion

In the present study, positive results in live body weight, carcass weight, significance decreasing of mortality rate were obtained when cobb-500 chicks fed a diet supplemented with Photogenic plant at the level of one kg / 100kg of diet, This effect could be attributed to enhancement of digestive enzymes secretion, activation of immune response and antibacterial, antiviral and antioxidant actions.

However, more experiment is needed to clarify the effect of different medicinal Plants, herbal extracts and active substances of extracts, a combination between medical plants in different percentages to show the real effect of them.

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