The Immunological Effectiveness of Some Common Plants

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Abstract:

Three plant species were picked randomly and their alcoholic extracts have been screened to know their effects on the phagocytic capability and intracellular killing of yeast by human peripheral macrophages. Macrophage cultures were incubated with different concentration of each plant extract: for 15 min., 30 min .and 45 min. The phagocytes activity in *Iresine herbstii* extract was significantly ($p \leq 0.05$) increased with increasing dose and time of incubation. In *Mentha piperita* extract, increasing in dose and time of incubation leads to elevate phagocytic capbility, especially in the dose of 20% and 25% of plant extract, perhaps because the antimicrobial and antiviral activities of this plant, as well as strong antioxidant and antitumor actions. While in *Elettaria cardamomum,* a significant elevation has been observed in phagocytic efficiency when the dose of extract increase to 15%, then decreased in the subsequent doses (20% and 25%), in three periods of time. These findings may suggest that cardamom exert immunomodulatory roles.

Key words: Phagocytosis, *Iresine herbstii, Mentha piperita, Elettaria cardamomum*

Introduction:

Phagocytosis is an innate immunity refers to antigen-nonspecific defense mechanisms that a host uses immediately or within several hours after exposure to an antigen [1]. Phagocytosis is a specific form of endocytosis involving the vesicular internalization of solids such as bacteria, and is, therefore, distinct from other forms of endocytosis such as the vesicular internalization of various liquids.

Phagocytosis is involved in the acquisition of nutrients for some cells, and, in the immune system, it is a major mechanism used to remove pathogens and cell debris. Bacteria, dead tissue cells, and small mineral particles are all examples of objects that may be phagocytosed [2]. Infectious diseases account for high proportion of health problems in the developing countries. Microorganism has developed resistance to many antibiotics and this has created immune clinical problems in the treatment of infectious diseases and because of inadequate availability and high cost of new generation antibiotics, scientists are forced to search for new antimicrobial substances from various sources including medicinal plants [3]. Many of the plants used today were known to the people of ancient culture throughout the world for their preservative and medicinal property [4]. However several plants are used in the form of crude extracts, infusions or plaster to treat common infections without scientific evidence of efficacy [5]. Plants have been used to treat various ailments since the advent of human history, because the herbals have been usually considered to be safe and nontoxic compared to synthetic compounds. So, there are abundant studies about plant pharmacological properties [6,7, 8].

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There has been an increase in people trying to find naturaimmune system boosters. Herbal and homeopathic remedies have been used in traditional medicine for thousands of years to strengthen immune system functioning, acting as immune system tonics to encourage normal and efficient defense against pathogens and routine recovery [9].

Antimicrobial compounds of plant origin may occur in stems, roots, leaves, bark, flowers and fruits of plants [10]. For example: *Iresine herbstii* belongs to the family Amaranthaceae. It is commonly referred to as blood leaf, [11]. *Iresine herbstii* leaves are used as wound healing, anticancer agent [12], post-labor tonic [13], and externally against skin depurative such as eczemas, sores and pimples [11] as well as antimicrobial agents. It contains several bioactive substances and showed different biological activities and is used to treat various diseases. Leaves of *I. diffusa* are used to treat malaria [14]. Red-coloured plants in the family Amaranthaceae are recognized as a rich source of diverse and unique betacyanins such as acetylated and non-acetylated betacyanins. Acylated betacyanins are available with the highest proportion in *I. herbstii* and *Gomphrena globosa* [15] and [16]. We have selected *Iresine herbstii,* based on, these plants are more accessible and affordable [17] and can contribute to new bioactive compounds that are safe and effective. The sesquiterpene *Iresine* is found in high concentration in the plant "herb of the Maya" and the properties include anti-cancer, anti-inflammatory, anti-allergic and antiseptic. Most terpenes are also substances with positive and stimulatory effect on the body in general. These substances are also able to shorten the menstrual period. Isoflavone tlatlancuayin has anti-oxidant, which captures free oxygen radicals, and contributes to cell renewal, and also a powerful antimicrobial agent [18]. The plant is used in astringent, diuretic, spasmolytic, whooping cough and roots in hemicranias [16]. Leaves and flowers are used in decoction, fever, relaxant and kidney problems [19] and also as an antipyretic [11]. Schmidt *et al.* [20], reported that this plant possessed anti-inflammatory, cytotoxic and apoptotic activities and also has very low antioxidant activity [21].

Another plant: *Mentha piperita* has antimicrobial and antiviral activities, strong antioxidant and antitumor actions, and some antiallergenic potential [22]. Mentha piperita is one of the world's oldest medicinal herbs and used in both Eastern and Western traditions. This plant is a perennial plant in *Lamiaceae* family and contains about 1.2-1.5% essential oils. The chemical composition of the essential oil from peppermint (*Mentha piperita*) was analyzed by GC/FID and GC-MS. The main constituents were menthol (40.7%) and menthone (23.4%). Further components were (+/-)-menthyl acetate, 1,8-cineole, limonene, beta-pinene and beta-caryophyllene [20,23, 24]. *Mentha* (also known as Mint, from Greek *míntha*, Linear B *mi-ta*) is a genus of flowering plants in the family Lamiaceae (mint family). The species are not clearly distinct and an estimate of the number of species varies from 13 to 18. Most Mentha grow best in wet environments and moist soils [25]. Animal studies show *Mentha piperita* to have a relaxation effect on gastrointestinal tissue, analgesic and anesthetic effects in the central and peripheral nervous system, immune system influencing actions and anti cancer potential. Human studies on the gastrointestinal (GI), respiratory tract and analgesic effects of *Mentha piperita* oil and its constituents have been reported [26].

Cardamom (*Elettaria cardamomum*) of the Zingiberacea family is one of the world's very ancient and expensive spices mainly grown in Sir Lanka and South India. Cardamom extract contains a number of volatile oils that provide numerous health benefits such as aiding digestion and improving metabolism. Another of the essential cardamom benefits is that is very helpful in removing toxins from the body. Regular use of cardamom gradually removes the accumulated toxins and improves the blood circulation. In this aspect, cardamom benefits are similar to cinnamon benefits. Although there are no known side effects of cardamom, it should be avoided during pregnancy [27]. The seeds of their ripe fruits are used medicinally, as a spice, and also as a flavoring agent in curries, coffee and cakes, particularly in the Arab countries. Some is used in the manufacture of liqueurs and a relatively small quantity in pharmacy, chiefly in the form of compound tincture of *cardamom* [28]. Cardamom seed yields 4% of volatile oils containing a high proportion of Terpinyl acetate and cincole and small quantities of other monoterpenes, including alcohols and esters [29]. Govindarajan, *et al.* [30] reported the presence of over 150 compounds in cardamom aroma. Many of these compounds are commonly found in cardamom oil [29, 30]. It is also thought to be supportive of the nervous system and could be useful in massage blends addressing sciatica. Finally, *cardamom* contains many vitamins and minerals including: niacin, vitamin C, riboflavin, magnesium and potassium that help ensure optimum health [29]. This study was to determine the possible effects of these three plants: *Iresine herbstii*, *Mentha piperita* and *Elettaria cardamomum* on phagocytic activity in human phagocytes.

Material and Methods:

I. Collection of blood:

Venous blood (5 ml) was collected in heparin tubes, By means of density gradient centrifugation modified by [31]; the lymphocytes were isolated from whole blood.

II. Preparation of heat killed yeast:

Ten grams of yeast (*Saccharomyces cervisiae*) were suspended in a warm (37^{o}C) sterilized physiological saline (one hindered-fifty milliliters), the cell suspension then heated in boiling water bath for sixty minutes, after heating cell suspension was cooled to (37^{o} C) then filtered by tri stratified sterilized gaze. Yeast cells were adjusted to a concentration of 1X 10^{7} cells /ml. distributed into small tubes and freeze at (-20^{o} C) [32].

III. Plant samples preparation:

Healthy, disease free (fresh) leafs and stems of *Iresine herbstii*, *Mintha piperta* and seeds of *Elettaria cardamomum*, were collected, washed properly in the tap water followed by detergent water and finally rinsed with distilled water until no foreign material remained (damaged leaves were removed). The fresh plant materials were left to dry in a closed room (25-28ºC) for approximately five days. The dried plant parts were pulverized to obtain a powder by using sterile electrical blender. The powdered samples were stored in air tight container, protected from sunlight for further use [33].

IV. Alcoholic extracts:

Twenty grams of powdered materials of each of the three plants were continuously extracted with eighty ml of solvent like ethyl alcohol (99%) [34]. For successive solvent extraction based on polarity using soxhlet extraction apparatus at the boiling point of the respective solvents for 3-4 h or until the color of the extracted solvent become clear. Extracts were concentrated under reduced pressure

using rotary evaporator and they were poured into a pre-weighed vial, further dried in a desiccating chamber until a constant dry weight was obtained. The extract vials were stored at 4ºC for further studies [33].

VI.

0.25 ml Cells + 0.05 ml killed yeast suspension + 0.1 ml normal salin + 0.1 ml different concentration of plant extracts (5%, 10%, 15%, 20% and 25%) which mixed before as in guidelines for laboratories and field testing from WHO [35].

All were incubated for different periods (15min., 30min. and 45min).

The phagocytic activity was determined, as in [36] by counting the number of leukocytes (neutrophils and macrophages) that phagocytes yeast cells as in this equation:

Phagocytosis Index (%) = Number of phagocytotic Cells/ Total Count X 100

V. Statistical Analysis

The results were analyzed using the computer program SPSS (Statistical package for Social Sciences) version 13. Their data were presented in terms of means ± standard errors (S.E), and differences between means were assessed by ANOVA and LSD tests.

Results and Discussion:

Table (1): Phagocytic activity levels with different concentrations of *Iresine herbstii* extracts and control in three periods of time.

Concentration (%)	Number	Time per minutes Phagocytic Index (Mean ± S.E)		
		15min.	30 min.	45 min.
Control	4	38.75 ± 0.75^{D}	45.75 ± 0.85^{C}	45.50 ± 1.32^{E}
5	4	57.75 ± 1.03^{C}	63.50 ± 1.32^{B}	63.00 ± 1.22^{D}
10	4	59.75 ± 0.63^{C}	66.25 ± 0.48^{B}	67.00 ± 0.91^{C}
15	4	63.00 ± 0.71^{B}	66.50 ± 1.32^{B}	70.25 ± 1.43^{B}
20	4	69.25 ± 1.25^{A}	70.00 ± 0.91^{A}	73.00 ± 0.81^{B}
25	4	71.25 ± 1.03^{A}	72.50 ± 0.86^{A}	76.25 ± 0.75^{A}

***Different letters: Significant difference ($P \leq 0.05$) between mean values within the columns**.

Phagocytes activity of monocytes using *iresine herbstii* extract was significantly ($p \leq 0.05$) increased with increasing dose and time of incubation, (Table -1). After 15minits of incubation, phagocytic percentages were increased gradually (57.75%, 59.75%, 63.00%, 69.25% and 71.25%) using plant extract concentrations (5, 10, 15, 20, 25%, respectively), as compared to control group (38.75 %). While, when incubated it for 30 minutes, phagocytic percentages also was elevated (63.50%, 66.25%, 66.50%, 70.00% and 72.50%) in extract concentrations (5, 10, 15, 20, 25%, respectively), as compared to control group (45.75 %). When the incubation period extended to 45 minits, phagocytosis results were showed increased levels (63.00%, 67.00%,70.25%,73.00% and 76.25%) in extract concentrations (5, 10, 15, 20, 25%, respectively), as compared to control group (45.50 %). Such findings may highlight the importance of *Iresine herbstii* extract in defense against pathogens which in agreement with [11] and [20], whom reported that this plant possessed anti-inflammatory, cytotoxic and apoptotic activities and also has very low antioxidant activity [21].

Table (2): Phagocytic activity levels in five concentrations of *Mintha piperta* extracts in three periods of time

Concentration (%)	Number	Time per minutes Phagocytic index (Mean ± S.E)		
		15 min.	30 min.	30 min.
Control	4	38.75 ± 0.75^{E}	45.75 ± 0.85^{C}	45.50 ± 1.32^{C}
5	4	50.25 ± 2.06^{C}	61.75 ± 0.75^{B}	56.75 ± 2.14^{B}
10	4	43.00 ± 0.71^{D}	63.50 ± 1.19^{B}	67.00 ± 1.41^{A}
15	4	47.00 ± 1.76^{C}	64.75 ± 1.38^{B}	65.25 ± 0.85^{A}
20	4	58.75 ± 1.11^{B}	64.23 ± 0.75^{B}	65.50 ± 0.65^{A}
25	4	63.00 ± 0.82^{A}	70.50 ± 1.85^{A}	69.00 ± 0.71^{A}

***Different letters: Significant difference ($P \leq 0.05$) between mean values within the columns.**

Table-2, demonstrates phagocytic activity of monoctytes using *Mentha piperita* extract which was significantly ($p \leq 0.05$) increased with increasing time of incubation. In the first period (15 minutes), the phagocytosis using plant extract concentrations (5, 10, 15, 20, 25%) were (50.25%, 43.00%, 47.00%, 58.75% and 63.00% respectively) as compared to control group (38.75 %), whereas at the second period of incubation (30 minutes) phagocytosis percentages were (61.75%, 63.50%, 64.75%, 64.23% and 70.50% respectively) as compared to control group (45.75%),While after 45 minutes incubation with the same extract concentrations results were (56.75%, 67.00%, 65.25%, 65.50% and 69.00% respectively) when compared with control group(45.50%). Increasing in dose and time of incubation leads to elevate phagocytic capbility, especially in the dose of 20% and 25% of plant extract. Mentha is widely used as one of the important spices and traditional herbs in the world. Many reports have confirmed that the Mentha or its extracts has some pharmacological activities including, anti-tumor and anti-oxidation.

Studies have shown that *Mentha piperta* possess an anti-inflammatory effect against both acute and chronic models of inflammation [37]. *In vitro* data*:* Peppermint oil and menthol have moderate antibacterial effects against both Gram-positive and Gram- negative bacteria. Peppermint extracts are bacteriostatic against *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Menthol is bactericidal against *Staphylococcus pyogenes, S. aureus, Streptococcus pyogenes, Serratia marcescens, Escherichia coli,* and *Mycobacterium avium* [38].

It is well known that many diseases/disorders, that have immunomodulated components, can be modified by administration of biological compounds that activate key pathways in the immune system. They strengthen the defense and immune mechanisms of the body and can be used for stimulating the non-specific immune responsiveness in both the human and veterinary medical practice [39].That is compatible with our findings in increasing phagocytosis, which considered as non-specific immune response.

Table 3: Phagocytic activity levels in five concentrations of *Elettaria cardamomum* extracts in three periods of time.

Concentration (%)	Number	Time per minutes Phagocytic Index (Mean ± S.E)		
		15 min.	30 min.	45 min.
control	4	38.75 ± 0.75^{D}	45.75 ± 0.85^{D}	45.50 ± 1.32^{D}
5	4	42.75 ± 2.29^{CD}	42.25 ± 2.66^{D}	43.50 ± 1.94^{D}
10	4	43.75 ± 2.32^{BCD}	56.00 ± 2.16^{C}	66.7 ± 1.49^{B}
15	4	67.25 ± 1.11^{A}	72.75 ± 1.03^{A}	89.25 ± 1.11^{A}
20	4	47.25 ± 1.25^{B}	63.00 ± 1.08^{B}	52.25 ± 2.50^{C}
25	4	45.25 ± 2.78^{B}	42.75 ± 1.31^{D}	44.25 ± 1.11^{D}

***Different letters: Significant difference ($P \leq 0.05$) between mean values within the columns.**

In the first period of incubation with extract concentration (15minits), phagocytic percentages were (42.75%, 43.75%, 67.25%, 47.25% and 45.25%) using the concentrations (5, 10, 15, 20, 25%, respectively), as compared to control group (38.75 %). But, after incubation for 30 minutes, percentages were (42.25%, 56.00%, 72.75%, 63.00% and 42.75%) using concentrations (5, 10, 15, 20, 25%, respectively), as compared to control group (45.75 %). While phagocytic percentages were (43.50%, 66.7%, 89.25%, 52.25% and 44.25%) using concentrations (5, 10, 15, 20, 25%, respectively), as compared to control group (45.50%). Here we observed that there was a significant elevation in phagocytic efficiency when the dose of extract increased to 15%, then decreased in the subsequent doses (20% and 25%), in three periods of time.

The findings indicated that immune defense may be diminished in high concentration of cardamom and exert immunomodulatory roles, as suggested by Majdalawieh and Carr [40], whom also confirmed that nitric oxide production by macrophages is significantly augmented and reduced by black pepper and cardamom, respectively and they suggested that black pepper and cardamom exert immunomodulatory roles and antitumor activities, and hence they manifest themselves as natural agents that can promote the maintenance of a healthy immune system. Generally, our results agreed with Acharya, *et al.* [41] that Cardamom oil is also noted for its antiseptic properties, and may stimulate phagocytic action of the immune system.

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الفعاليـــة المناعية لبعض النبـــاتات الشائعة

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الخلاصة:

تم تحضير المستخلص الكحولي (بتراكيز مختلفة) لبعض الأنواع من النباتات الشائعة مثل: دم العاشق *Iresine herbstii* والنعناع *Mentha piperita* اضافة الى نبات الهيل *Elettaria cardamomum* وفحصت فاعليتها في عملية البلعمة باستخدام خميرة الخبز المعروفة *Saccharomyces cervisiae* ، حيث تم حضن الخلايا العدلى (متعددة أشكال النوى) للانسان مع تلك المستخلصات لفترات متعددة (15 دقيقة، 30 دقيقة و45 دقيقة) وقد حدد التأثير المحتمل لتلك النباتات في فعالية الالتهام وعلى معدل ابتلاع الخميرة من قبل الخلايا البلعمية ، اذ لوحظ زيادة معنوية ($p\leq0.05$) في مستوى البلعمة باستخدام مستخلص نبات (دم العاشق) بزيادة الوقت وزيادة التركيز، وقد يعزى السبب في ذلك لكون النبات يمتلك خصائص سمية ومضادة للالتهاب. أما فيما يخص نبات (النعناع)، فقد ارتفعت مستويات البلعمة أيضا بزيادة الوقت والتركيز ولا سيما عند استخدام النبات بتركيز 20% و25%. ومحتمل ان يعود ذلك للدور الفعال لهذا النبات كمضاد للبكتريا والفايروسات ومضاد قوي للأكسدة والاورام. وبالنسبة لنبات الهيل، فقد لوحظ ارتفاع معنوي في فعالية البلعمة باستخدام المستخلص بتركيز 15% ثم حصل انخفاض في مستويات الالتهام للخلايا البلعمية في الجرع اللاحقة 20% و25% على مدى الفترات الزمنية الثلاثة (15قيقة، 30 دقيقة و45 دقيقة). وتفسر تلك النتائج ، على كون نبات الهيل يمتلك دورا مهما كمعدل مناعي immunomodulator.