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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACT OF MINT LEAVES AND BASIL LEAVES FOR USING IN WATER PURIFICATION

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ABSTRACT

Aqueous plant extracts have been proven to be effective in reducing microorganisms due to their high concentration of bioactive compounds such as polyphenols and carotenoids which have antimicrobial and antioxidant activity. Plant extracts are indeed concentrated preparations with something like a liquid, solid, or viscous consistency. The present study was focused on the antimicrobial effect of the aqueous extract of mint leaves (*Mentha longifolia)* and the aqueous extract of basil leaves (*Ocimum basilicum)* on bacterial strains (*Escherichia coli*, *Enterococcus* sp., *Bacillus* sp. and *Staphylococcus* sp.) and fungal strain (*Aspergillus niger*, *Candida albicans* and *Penicillium digitatum*). Results show that aqueous extract of mint leaves is more effective than the aqueous extract of basil leaves. Aqueous extract of mint leaves was used with different doses and at different contact time to reduce total coliform and fecal coliform from raw river water.

Key Words: Antifungal, antibacterial**,** aqueous extract, mint leaves, basil leaves.

INTRODUCTION

Humans use water for a variety of different purposes such as reaction, a reactant, temperature regulator, transport means and mechanical support for life activities. Generally, it can be said that water is life, but to create a healthy life, the most important thing is the quality of water **(White, 1992).** Contaminated water is the main cause of diseases because it contains germs as water is a fertile environment for the growth and diversity in different climate conditions. A small percentage of germs cause disease and death in children during their transmission to the mouth through water **(Ashenaf *et al.*, 2018)**.

According to a 2012 assessment by the World Health Organization, water pollution, together with sanitation and a scarcity of water, is responsible for nearly 80 per cent of sickness worldwide **(WHO, 2012)**. Due to the huge and continuous increase in world's population which has

reached 7.9 billion people according to UN estimates in 2021, it is expected that the wastewater will increase to reach more than two million tons of wastewater resulted from agriculture, industry and sewage are dumped daily into water **(UN, 2017).**

The United Nations estimates the production of 359 billion cubic meters of wastewater annually and this quantity is equivalent to 144,000000 Olympic sized swimming pools and approximately 48% of this water is currently dumped untreated **(UN 2017),** which requires intervention to solve this problem in order to preserve human health. As the population density increases and it is expected to reach 9.9 billion people in 2050 according to the UN estimates, acquiring high quality drinking water free of microorganisms such as bacteria, viruses and protozoa from surface and subsurface sources is the most important and biggest challenge.

Many techniques and methods are usually used to remove hazardous microbes such as filtering of various kinds sometimes is used in particular cases. Also ultraviolet treatment technique is sometimes used. Biological treatment techniques and also different chemical treatment techniques such as ozone chlorine and chlorine dioxide are also used. Chlorine is the most extensive commonly used **(Chand *et al*., 2007 and Gagnon *et al.*, 2005).** The aim of this work was to find natural substances with low cost to be used in water treatment.

Mint and basil are antibacterial, antiviral, and fungicidal, because they contain potent antioxidant flavonoids. Therefore, they are added to food to strengthen the immune system, the body resistance to diseases and increase mental activity. Basil and mint are also used to clean the hands and mouth because of their ability to resist microbes and germs in general **(Elansary & Ashmawy, 2013 and El-Kereti *et al*., 2013).**

There are different types of basil and mint cultivated and grown around the world and vary according to different climate conditions. Their oils and extracts are extracted from different parts of the plant because of their economic importance as they are used in many fields for example cooking foods, cosmetic and skin care in addition to pharmaceutics industry, agriculture and pesticides **(Reddy, 2019, Palazzolo *et al.*, 2013and Sabry *et al.*, 2019).** Ancient Egyptian 1000 BC, used mint and basil as an antimicrobial in herbal medicine.

Aqueous extract of mint plant had proved to be effective against a wide range of bacterial strains with different concentrations such as *Bacillus fastidiosus, Staphylococcus aureus, Salmonella choleraesuis,*

Escherichia coli, Pseudomonas aeruginosa and *Klebsiella pneumoniae* **(Al-Sum and Al-Arfaj, 2013)**. Also different doses of aqueous extract of mint plant had proved an antifungal activity against *Penicillium citrinum* (**Panda *et al.*, 2015**).The extract of *Ocimum basilicum* had showed a notable increase in zone of inhibition of the *E. coli* growth **(Muhannad *et al.*, 2014)**.

The current study was focused on using mint and basil as they are widespread in Egypt. The antimicrobial activity of their aqueous extract against different bacterial and fungal species was tested and then using the more effective extract in inactivation of the most common bacteria found in water (total and fecal coliform).

MATERIALS AND METHODS

Plant material

Mint and basil leaves were taken from growing plants. The leaves were gently rinsed with tap water, subsequently with distilled water to clean them from any dirt before being dried at room temperature. By using grinding machine. The leaves of both mint and basil were transformed to a powder form separately and then kept at room temperature for extraction.

Preparation of the Extracts

Preparing of the extract was done by macerating 20 gm of mint and basil leaves powder in 100 ml sterilized distilled water. Subsequently the extract was filtered twice with whatman filter paper No1 (**Panda *et al.*, 2015**).The aqueous extract was then kept in bottles in the refrigerator at 4°C for further antibacterial and antifungal studies.

Antimicrobial activity assay for bacterial and fungal strains:

The aqueous extracts biological assays were performed on bacterial species (*staphylococcus* sp., *Bacillus* sp., *Enterococcus* sp., and *E. coli*).The bacteria, that were screened, were grown on nutrient agar (NA) media. From 24 hours old culture exactly 0.2 ml from each strain was dispensed into 20 ml of sterile nutrient broth and then incubated from 3 to 5 hours to make a standard culture of 10^6 CFU/ml **(Al-Sum and Al-Arfaj, 2013)**.Mueller Hinton agar (MHA) medium was utilized in all bioassays for disc diffusion method. For the fungal strains the aqueous extract was investigated against the fungal species (*Aspergillus niger*, *Candida albicans* and *Penicillium digitatum*).The antibacterial and antifungal properties of plant extract were determined using the agar disc diffusion method, and all tests were carried out in duplicate for each strain. Distilled water was used as a control. Bacterial strains were

incubated at 37°C for 24 hours while fungal strains were incubated at 28 °C for 5-7 days.

Water treatment by plant extract

Raw water samples (from River Nile) of 1L were poured into 2L conical flasks for this assay. From the extract solution doses of 40, 80, 120, 160, and 200 ml were added to the water samples in different conical flasks and mixed for special desired contact time (15,30,45,60, 75 and 90 min) on a magnetic stirring plate. Total and fecal coliforms were assessed by using the membrane filter technique, as they are the most frequent bacterial strains identified in water, according to Standard Methods 9222B and 9222D **(APHA, 2017)**. The findings were matched to a 1L sample of the same surface raw water sample as a control sample and for greater accuracy each test was carried out in triplicate. The multiple-tube fermentation technique was also used to confirm total coliform and fecal coliform colonies according to Standard Method 9221 **(APHA, 2017).** Membrane filter technique was filtering the water sample (with or without extract) via a specific membrane (0.45 µm) capable of catching the bacteria in the water sample. This membrane was then incubated after being placed on selective medium at 35 ± 0.5 °C for total coliform and 44.5 ± 0.2 °C for fecal coliform. By counting the colonies that had been grown, the bacteria that resulted were recorded. mFC and mENDO media, respectively, were applied to identify fecal and total coliform colonies.

RESULTS AND DISCUSSION

Leaf extracts from basil and mint leaves were studied for their antibacterial and antifungal activities. The results are graded into levels that are represented by plus and minus signs, as the fungal hypha extension method was used and also the zones of inhibition were measured by using the disc diffusion method for detecting the antimicrobial activity as demonstrated in **(Table 1).** Our findings show that some plant extracts had a broad range of activity by producing distinct zones of inhibition, whereas others had insignificant zones of inhibition and had weak activity against the strains. Negative results for some aqueous extract imply that the extract did not have any active compounds, or that if they did, they were either in very trace amounts or had ceased their antimicrobial activity. It may be deduced from the literature review that in certain circumstances, isolated peptides were ineffective, and the activity observed was due to the presence of additional active molecules. **(Harborne, 1992).**

Table (1): Grading system for antibacterial and antifungal assay:

Sr. No.	Mathematical sign	Zone of inhibition (mm)	Interpretation
1	-	0	No or poor antimicrobial activity
2	+	1-15	Activity present
3	++	16-20	Strong activity
4	+++	21-35	Very strong activity

Evaluation for the antibacterial activity of the aqueous extracts :

The four selected bacterial strains are chosen according to standard methods and from literature review as they were found in surface water for example *staphylococcus* sp. and *bacillus* sp. which were found in stream water **(Olagoke *et al*., 2018)**. For *Enterococcus* sp.,it is used as an indicator for the presence or absence of fecal contamination (**Canadian guidance,2020)** and also for *E.coli* sp. as its found in surface water due to animal or human contamination. The results of their antibacterial activity are given in **Table 2.**The aqueous extract of basil leaves has strong antibacterial activity against *E. coli* and *staphylococcus* sp. but has small antibacterial activity against *Bacillus sp*. and *Enterococcus* sp. On the other hand, the extract of mint plant possesses a very strong antibacterial activity against *E. coli* and *Bacillus* sp. and strong activity against *Enterococcus sp.* and *staphylococcus* sp.

Table (2): Antibacterial activity of aqueous extracts of mint and basil leaves:

Extract of	*staphylococcus* sp.	*Bacillus* sp.	*Enterococcus* sp.	*E. coli* sp.
Basil leaves	++	+	+	++
Mint leaves	++	+++	++	+++

Evaluation for the antifungal activity of the aqueous extracts:

From literature review, a screening for the presence or absence of different fungal strains in tap, ground and surface water observed that *A.niger* is found in tap, ground and surface water while *C. albicans* and *P. digitatum* were found only in surface water **(Monika *et al*.,2017)**. These three fungal strains were chosen to investigate the effect of the aqueous extracts on their activity. The results are presented in **(Table 3).**The aqueous extract of basil leaves has no antifungal activity against *A. niger*, but it has a slight effect on *P. digitatum* and a strong effect on *C. albicans*., but on the other hand the aqueous extract of mint leaves possesses a strong antifungal activity against *A. niger and P. digitatum* and a very strong antifungal activity against *C. albicans.*

Table (3): Antifungal activity of aqueous extracts of mint and basil leaves:

Extract of	*A.niger*	*C. albicans*	*P. digitatum*
Basil leaves	-	++	+
Mint leaves	++	+++	++

Water treatment by plant extract

Effect of extract dose

Total coliforms were inactivated more by aqueous extract of mint leaves than fecal coliforms as shown in **Figure 1**. Within one hour of contact time, overall coliform inactivation increased as the extract dose increased from 40 ml/L to 200ml/L. Because fecal coliforms are a subgroup of total coliforms, the observation that total coliforms were inactivated more than fecal coliforms shows that the active chemical components inside the extract was preferring to impact total coliform bacteria that aren't from the fecal coliform group.

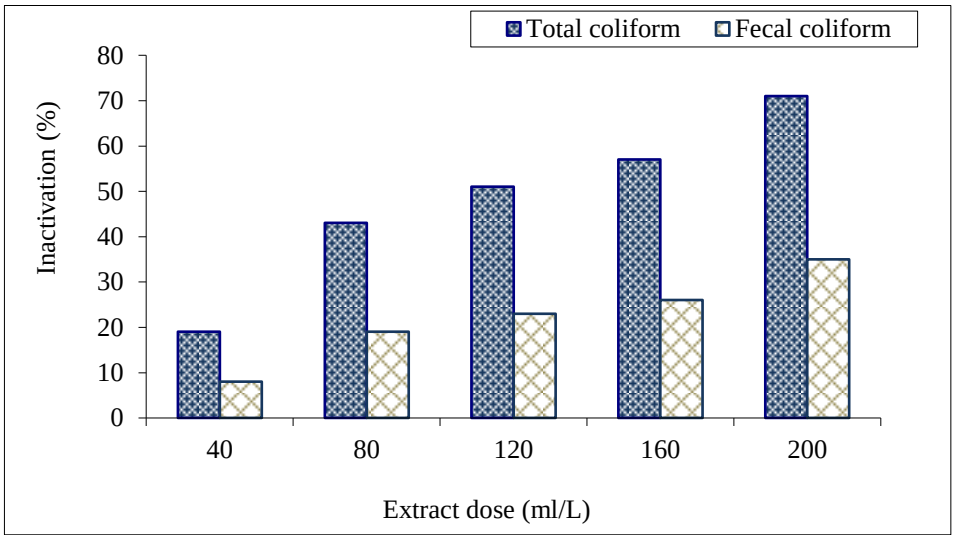

Figure (1): Effect of mint aqueous extract dose on total and fecal coliform within one hour.

Effect of contact time

The effect of the mint leaves extract at a fixed dose of 40 ml/L and contact times varying from 15 to 90 minutes is represented in **Figure 2**. Total coliform inactivation was observed to be greater than fecal

coliform inactivation on multiple occasions. The correlation between coliform bacteria inactivation and contact time was not stable. This may be due to that antibacterial chemical components were quickly depleted. Form literature review it was found that some natural organic compounds or natural ingredients like suspended particles inside the water sample may make neutralization for a percentage of the active components of the extract. However, it is impossible to tell for certain how much is used in bacteria inactivation versus how much is neutralized through other natural water elements at this time. For understanding the neutralizing effect more experiments with various doses of natural organic compounds could be performed.

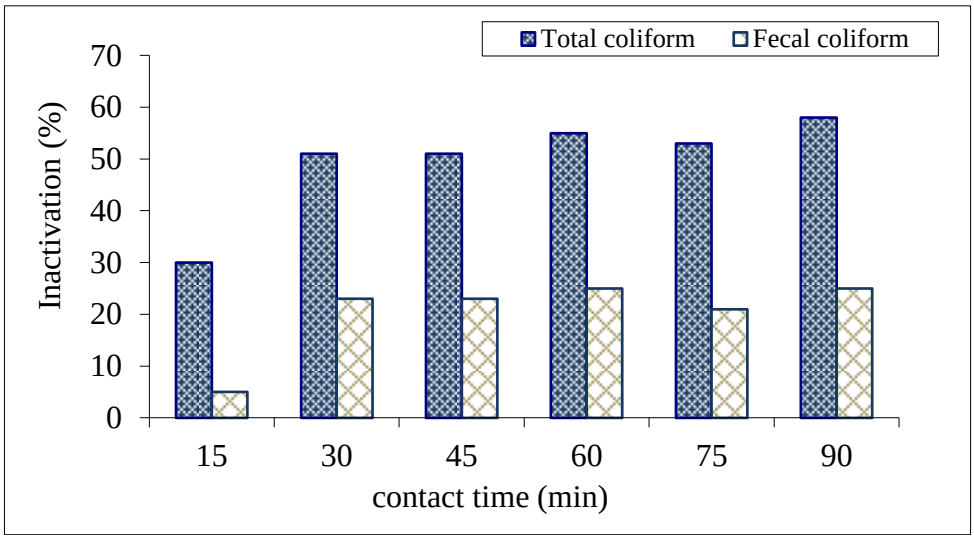

Figure (2): Effect of different contact time on total and fecal coliform by 40ml/l mint extract dose

The findings of this investigation revealed that mint aqueous extract has antibacterial characteristics against coliform bacteria, but this activity did not make total inactivation for coliforms which is an essential part for treatment of water to be used in drinking according to the guidelines of **WHO, (2017).** It has been shown that when essential oils and aqueous plant extract are employed as food preservatives in the presence of organic matter, their antimicrobial property is decreased **(Kanika *et al.*, 2017)**. According to a study, the presence of organic materials in water affects the effectiveness of aqueous extracts disinfection of water **(Robert *et al.*, 2009**). Organic compounds can

protect bacteria by hydrostatically binding to cell membranes or neutralise active components in plant extracts by connecting to active sites **(Hammer *et al*., 1999).**The shielding effect of suspended particles has also been found to decrease the effectiveness of many other disinfectants, such as essential oils or aqueous extracts of some plants**(Winward *et al.*, 2008).** So, combination of a pre-filtration process like sand or activated carbon with the disinfection of aqueous extract of mint could improve the reduction of the coliforms and thus can be used in drinking water.

CONCLUSIONS

From this study it can be concluded that both aqueous extract of natural herbs (mint and basil leaves) have antimicrobial activities against a wide spectrum of microorganisms but mint extracts exceeds basil extracts as it shows more antimicrobial activity against some species. However, these extracts have antibacterial activity, their effectiveness for removal of bacteria from drinking water was not sufficient to guarantee their use to clean drinking water alone as it needs to be preceded by a pre-treatment process like filtration process such as sand or activated carbon filtration which is also a simple and low cost technique. Using aqueous extract of mint leaves to eliminate harmful bacteria in water has many advantages as it's a low-cost, natural material with no negative effects on human health or even the environment.

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تقييم النشاط المضاد الميكروبى للمستخلص المائي لاوراق النعناع و اوراق الريحان لتنقية المياه

محمد على السعيد ، رحاب جمال حسن

المركز القومى لبحوث االسكان و البناء

المستخلصات النباتية تعتبر فعالة في الحد من الكائنات الحية الدقيقة بسبب محتواها من المركبات النشطة بيولوجيا مثل البوليفينول والكاروتينات التي لها نشاط مضاد للميكروبات ومضادات الأكسدة . ركزت دراستنا على التأثير المضاد للميكروبى لاوراق النعناع و اوراق الريحان على السلالات البكتيرية (*Escherichia coli* و *Enterococcus* sp. و *Bacillus* و *Staphylococcus sp.*) والسلالات الفطرية (*Aspergillus niger* و *Candida albicans* و *Penicillium Digitatum*). أظهرت النتائج أن المستخلص المائي لأوراق النعناع أكثر فاعلية من المستخلص المائي لأوراق الريحان. يستخدم المستخلص المائي لأوراق النعناع بجرعات مختلفة وفي أوقات تلامس مختلفة لتقليل بكتريا القولون القولوني الكلي والبكتيريا القولونية البرازية من المياه الخام.