EFFECT OF SOME INDIGENOUS DIFFERENT PLANT AND ALGAL EXTRACTS AS ANTIMICROBIAL AGENTS AND FOOD PRESERVATIVES

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ABSTRACT

This experiment was conducted to investigate the antimicrobial effect of some plants and algae. Fifteen plants and three algae were used. Extracts were obtained using ethanol: water, hexane, chloroform and methanol. The MIC and MLC were also determined. The results showed that *P. granatum* extracted with ethanol: water gave the highest inhibition zone against tested microorganisms. *Ulva* algae gave the best results when extracted with chloroform followed by hexane. Ethanol: water extracts also gave the highest inhibition against tested fungi followed by hexane and methanol. The MLC against *E. coli* was 16mg/ml with *P. guava*, but reached 256 mg/ml with *R. officinalis*. MIC with *L. mobilis* and *E. globulus* appeared only with 8mg/ml against *A. parasiticus*. Feeding rats on plant and algae extracts had no significant effect on body weight, but there were significant changes in liver enzymes. With the five treatments carried out on Ras cheese, there was no significant effect on the organoleptic properties which means that there extracts could be safely used for human consumption.

Key words: plant and algae extracts, antimicrobial and antifungal effect, MIC & MLC, Ras cheese.

INTRODUCTION

There are an estimated 250,000 to 500,000 species of plants known to man, of which more than 10% are used for medicinal purposes. The curative effects of plants have been extensively documented among different cultures throughout history. Early civilizations, such as the Chinese, Indian or others in the Middle East left documents defining the use of plants as medicinal remedies five thousand years ago. The recent tendency of the general public to reconsider the alternative medicine has attracted the attention of both food industry and research community to generate reliable information regarding the claimed therapeutic effects of medicinal plants **(Cowan 1999 and Li, 2003)**. Clove (*Syzygium aromaticum*), cinnamon (*Cinnamomum zeylanicum*), thyme (*Thymus vulgaris*) oregano (*Origanum vulgare*) and vanilla (*Vanilla planifola, V. pompona, V. tahitensis*) fall into this category, while other spices such as sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), cilantro (*Coriandrum sativum*), tea tree oil (*Melaleuca alternifolia*) and finger root extract (*Boesenbergi apandurata*) are considered lower intensity antimicrobials **(Cowan 1999; Naidu 2000; Casterton *et al.*,2005** and

Davidson 2005). **Pandey** and **Singh, (2011)** found that the methanol extract of clove demonstrated zone of inhibition of 20mm against *E.coli*.

Supreetha *et al.,* (2011) reported that the ethanolic extract of ginger powder has pronounced inhibitory activities against *Candida albicans*.

Voravuthikunchai, *et al., (*2005) reported that *P. granatum* peel extracts have shown antibacterial activity against *E.coli* O157 and methicillin-resistant *Staphylococcus aureus* bacteria. *P. granatum* peel is used to treat infections found in human sexual organs as well as mastitis, acne, folliculitis, pile, allergic dermatitis, tympanitis, scalds, diarrhea, dysentery and as an antioxidant **(Singh, *et al*., 2002)**.

Kadi *et al.,* (2011) investigated the antibacterial activity of ethanol and aqueous extracts of *P. granatum L.* bark. They indicated that the ethanol macerate extract showed strong activity against *Pseudomonas aeruginose* with inhibition zone of 24.4mm.The ethanol decoctate against Gram positive bacteria (*staph. aureus* and *B. stearothermophilus*) also gave a diameter zone of inhibition of 21.1mm and 23.75mm, respectively. **Hassan *et al.,* (2013)** found that ethanol extract of *P. granatum* exhibited clear zone of inhibition against the tested microorganism. Essential oils of cinnamon (*C. cassia*) were found to possess antimicrobial properties in-vitro and shown to inhibit the growth of *B. cereus* **(Kalemba** and **Kunicka, 2003)**.

Rosemary extract has been widely used as a preservative in the food industry, due to its antioxidant activity**.** The antifungal and antimicrobial effects of essential oil of rosemary were extensively reported **(Santoyo *et al.,* 2005).**

The *Eucalyptus* (Myrtaceae) is used to control several diseases derived from microbial infections. The gram positive bacterium such as *Staph. aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning **(Benayache *et al.,* 2001).**

Obiorah*et al.,* (2012) studied the phytochemical and antibacterial characteristics of extracts from leaves of *Cajanus cajan* and *Euglena globulus*. They reported that the preliminary screening showed inhibitory activity against *Staph. aureus* and *B. subtilis* and very slight inhibitory action on *P. aeruginosa* and *Trichophyton rubrum.*

Prashant *et al*. (2006) and **Goud *et al*. (2007)** investigated the methanolic extract of a blue green alga and two green algae which have shown good antimicrobial activity.

Prakash *et al*. (2011) studied the antimicrobial activity of certain fresh water microalgae in south India. They reported that the methanolic extract of *Spirogyra decimina* exhibited the antibacterial activity against *Staph. aureus* and *Proteus mirabitis*. They also found that the ethanol extracts of *S. grantiana* showed the antibacterial activity against three organisms; *E. coli*, *Proteus vulgaris* and *P. mirabilis*

Naik, *et al.*, (2012) indicated that the antimicrobial activity is present in the isolated constituents of *Spirogyra sp*. All the compounds have shown high activity against *Ps. solanacearum*. Moderate activity was seen against *E. coli* and no effect on *C. michiganense*. Among fungi *Fusarium oxysporum* and *Aspergillus niger* were more susceptive to the isolated compounds while *Curvularia sp.* was resistant.

Domettila *et al.*, (2013) performed a phytochemical analysis of some south Indian seaweeds amongst them was *U.lactuca*. They found that both chloroform and petroleum ether extracts gave some compounds could be used as a broad spectrum antimicrobial and bioactive agents.

Algae extracts as food additives and preservatives:

The application of macro algae as mineral feed additives is proposed aiming to increase the bioavailability of microelements from feed additives of biological origin, thereby increasing the microelement content in animal products (meat, eggs, milk) and moreover to improve the feed value and finally to increase the livestock productivity. The use of macro algae as feed additives is an inventive application of biotechnology in animal nutrition. Provision of animal feeds with enhanced nutritional quality improves the sustainability of animal production (**Gavrilescu** and **Roman, 2005)**.

A special attention should be paid to algae, because two-thirds of the biomass on the Earth consists of over 25,000 species of algae. It was suggested that biological material, such as aquatic plants **(Chojnacka, 2007)** or algae **(Michalak** and **Chojnacka 2006)** could be used as a carrier of microelements in animal feeding. Fodders of plant origin are known to be poor in microelements. Algae possess a unique property of binding minerals from aqueous solutions via bio sorption process, which is not metabolically controlled and describes passive binding of metal ions to non-living biomass **(Davis *et al.*, 2003)**.

Out of all sources of microelements, the application of macro algae as mineral feed additives could constitute environmentally friendly and economically beneficial solution. Moreover, macro algae are approved for human and animal consumption by the obligatory law (**Mabeau** and **fleurence 1993)**.

MATERIALS AND METHODS

Screening of natural plant and algae as antimicrobial effects

Fifteen plants were collected from their natural habitats and herbalists shop and tested for their antimicrobial activity. The plants named; *Lupinus termis, Laurus nobilis, Nigella sativa, Psidium guajava, Curcuma domestica, Punica granatum, Zingiber officinale, Cinnamomum verum, Elettaria cardamomim, Matricaria chmomilla, Syzygium aromaticum, Cymbopogon proximus, Eucalyptus globulus, Rosmarinus oficinalis* and *Mentha varidis*. It's worth mentioning that only the leafy parts of the plants were used to extract

the active substances except for pomegranate (*Punica granatum*) where the fruit peel was used.

Three algae; *Ulva lactuca, Spirogyra* sp (Chlorophycea) and *Corallina officinalis* (Rhodophyceae) samples were collected in winter season, the depths of sampling were 1 and 2-3 from Alexandria rocks' surface for Chlorophyta and Rodophyta, respectively. Whereas *Spirogyra* was collected from water drainages in Fayoum.

Preparation of plants and algal extracts:

Grinding the selected plant materials:

All collected plant and algae samples were air dried at room temperature for 72 h. The samples' material was ground in a grinding machine tell homogeneity. Exposure to sunlight was avoided to prevent the loss of active components.

Extraction of selected plant powder:

A portion of 30 grams of the dried ground plant and algae was extracted with 200 ml of the solvent. The filtrate was then concentrated by evaporation at 40 ^{o}C in a rotary evaporator.

Microorganisms

Different pure and selected strains of bacteria and Fungi strains were used in this study. All of them were obtained or isolated locally from (Agric. Fac. Fayoum). *Staphylococcus aureus* ATCC 8095, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 13753, *Listeria monocytogenes* ATCC 15313, *Salmonella typhimurium, Candida albicans, Asperagillus parasiticus* ASU612, and *Penicillium roquefortii***.**

Antimicrobial susceptibility studies:

Inhibition of microbial growth was tested by using the paper disc agar diffusion method, while the MIC was determined by the dilution method.

Bacterial broth dilution method for the determination of MIC and MLC for both plants and algae:

The MIC and MLC were determined according to the method of **Ellen *et al.*, (1994).**

Fungal method for determination of the MIC and MLC of antimicrobial plant and algae agents:

The inhibition percentage of radial growth of the test fungus by tested extracts was calculated according to **Tripathi *et al*., (2008).**

Mycelial inhibition (%) = [(cdc-cdt) /cdc] × 100

Where:

cdc = colony diameter of control. cdt = colony diameter of treatment.

Feed experiment on rats (Biological Assay):

Sixty five male albino rats with average body weight of 100-105g were obtained from Faculty of Science, Fayoum and were acclimated to animal house conditions for one week prior to experiment. They were housed in groups of five each in universal polypropylene cages at room temperature and

at 12h/day photoperiod. Animals were fed on stander laboratory chow and water *ad libitum* till the end of the experiment (six weeks).

Experimental design

The rats were divided into thirteen experimental groups of five rats each, as follows:

-Group I	(control) fed with normal basal diet daily.
-Group II	fed on a diet containing 10% grinded *Eucalyptus globulus daily.*
-Group III	fed on a diet containing 20% grinded *Eucalyptus globulus daily.*
-Group IV	fed on a diet containing 40% grinded *Eucalyptus globulus daily*.
-Group V	fed on a diet containing 10% grinded *Ulva lactuca daily.*
-Group VI	fed on a diet containing 20% grinded *Ulva lactuca daily.*
-Group VII	fed on a diet containing 40% grinded *Ulva lactuca* daily.
-Group VIII	fed on a diet containing 10% grinded *Corallina officinalis daily.*
-Group IX	fed on a diet containing 20% grinded *Corallina officinalis daily.*
-Group X	fed on a diet containing 40% grinded *Corallina officinalis daily.*
-Group XI	fed on a diet containing 10% grinded *Spirogyra sp daily.*
-Group XII	fed on a diet containing 20% grinded *Spirogyra sp daily.*
-Group XIII	fed on a diet containing 40% grinded *Spirogyra sp daily*.

Body weight (BW): Body weight (BW) was recorded at weekly intervals during the experimental period (six week).

Body weight gain (BWG): Body weight gain (BWG) was calculated by subtracting the initial body weight (BW_0) at the beginning of each period from the final body weight (BW_6) at the end of the experiment as follows.
($BWG = BW_6 – BW_0$).

At the end of experimental period (6 week) animals were fasted overnight, and blood sample were collected in tubes containing gel called vacutainer and left at room temperature. Plasma was obtained by centrifugation at 3000 rpm for 15min and then stored at (-20^{o}C) until analysis. Liver, Spleen and kidney organs were excised, rinsed in chilled solution, and then stored in formalin solution.

Liver functions

The concentration of serum glutamic pyruvic (GPT) and glutamic oxaloacetic (GOT) transaminase were colorimetrically determined using transaminase kit according to the method of **Reitman** and **Frankel (1957).**

Kidney functions

Creatinine, bilirubin and urea were determined according to the methods previously described previously **(Doumas *et al*., 1971).**

Manufacture of Ras cheese

Five treatments were carried out for the manufacture of Ras cheese as described by **Hofi *et al.* (1970)**. Each treatment was made using 30 Kg fresh cows' milk (3.5% fat). Samples of the resultant cheese were taken for chemical analysis and microbiological examinations when fresh, 30, 60 and 90 days during the ripening period.

Microbiological analysis

The total viable counts (TVC) of five cm depth of Ras cheese samples were determined using LB agar media. The plates were incubated at 32°C for 48 h **(IDF, 1991).**

Fungi counts of five cm depth Ras cheese samples were determined on PDA, the plates were incubated at 25 - 30°C for 5 days **(Oxoid, 1990).**

Coliform bacterial counts Ras cheese samples determined on MacConkey agar media, the plates were incubated at 37°C for 24 h **(Oxoid, 1990).** Five cm depth Ras cheese, samples were detected on Eosin MacConkey broth media, the tubes were incubated at 44.5°C for 24 h to determine *E. coli* **(Oxoid, 1990).**

Organoleptic properties

All resultant cheese samples were evaluated for organoleptic properties by 12 panels from students and members of Microbiol. Dept. Agric, Fayoum Univ.

Ras cheese samples were evaluated according to the score card sheet of **Hofi *et al.* (1991)** at ages of fresh, 30, 60 and 90 d. Score card with 60 points for flavor, 30 points for body and texture and 10 points for appearance was used.

Statistical analysis

Data (except FFA analysis) were analyzed using General Linear Models (GLM) procedure of SPSS software **(SPSS, 10)**. Duncan's multiple range tests was used to compare between the means **(Duncan, 1955).**

RESULTSAND DISCUSSIONS

Survey of some plant and algae containing antimicrobials substances:

The ultimate goal of this work is to obtain, as much as possible, natural substances isolated or separated from natural plants and algae available in our surrounding and may be used in the preservation of some foods avoiding the spread usage of some chemicals, as preservatives which could cause several health problems. In order to realize this goal it was of utmost importance to select plants and algae which never cause changes in the main properties of the food (flavor, odor, color consistency, appearance ….etc.) and are also not toxic to the user.

Effect of various plant and algae extracts on tested bacterial strains:

The effect of different extracts using ethanol: water, hexane, chloroform and methanol on tested microorganisms; *Staph. aureus*, *B. subtilis, L. monocytogenes* and *S. typhimurium* has been investigated.

With regard to ethanol: water extract, results in **Table (1)** indicate that the best results were with the *P.granatum* extract against all test microorganisms where the inhibition zone with *Staph. aureus* reached 30mm followed by *E.coli*, *B. subtilis* and *L. monocytogenes*; 26, 24 and 20mm, respectively. Where *Sal. typhimurium* exhibited some resistance (17mm).

The results are in agreement with the findings of **Choi *et al.*, (2011)** who reported that the ethanol extract had insignificant effects on mortality and number of viable *S. typhimurium*, but added that the extract had potential to provide an effective treatment for *Salmonellosis*. Similar results were also reported by **Kadi *et al.*, (2011)** and **Pai *et al.*, (2011)** against *Staph. aureus, B. stearothermophilus* and *A. hydrophila*.

The rosemary extract followed *P. granatum* in the effect against the tested microorganisms. The best results were with *staph. aureus* (25mm) followed by *B. subtilis, E. coli, L. monocytogenes, S. typhimiurium* ;(20, 17, 16 and 12mm inhibition zone, respectively **(Table 1).**

Similar results were reported by **Frankel, *et al.*, (1996)** who showed that Rosemary extract has been widely used as a preservative in the food industry, due to its antioxidant activity.

The ethanol: water extract of E. *globulus* Showed its highest inhibition effect against both *staph.aureus* and *E.coli* (25 and 22mm), respectively. Where a weaker effect was observed for both *B. subtilis* and *S. typhimurium*(15 and 12mm), respectively and there was no effect against *L. monocytogenes* From **Table (1)** it could also be shown that the other plants gave higher effects against *Staph .aureus* , *B. subtilis* and *E .coli* but lower ones against both *S. typhimurium* and *L. momocytogens*, respectively.

The results in **Table (1)** showed that the best results were obtained with *Spirogyra sp*. Against *L. monocytogens* (25 mm) with an equal effect against both *E. coli* and *B. subtilis* (13mm),and no effect against both *Staph. aureus* and *S. typhimurium*. The previous results are in agreement with **Prakash *et al.* (2011)** who found that the ethanol extracts of *S. grantiana* showed the antibacterial activity against three organisms; *E .coli*, *proteusvulgaris* and *P. mirabilis* with the zone of inhibition of 9, 10 and 9mm, respectively. With *U. lactuca*, there was a moderate effect against all tested microorganisms except *S. typhimurium* where it gave an inhibition zone of 15, 13, 13 and 12 mm against *E .coli*, *L. monocytogenes*, *Staph. aureus* and *B. subtilis*, respectively. These results are in agreement with the findings of **Hanaa, *et al.*, (2008)** who reported that the antibacterial activity of *Ulva* organic extracts are apparently related to their lipophelic and phenolic contents, in particular steroids fatty substances and reported that the *Ulva* organic extracts of showed potential antimicrobial activity against all tested microorganisms with MIC value ranged from 350 to 400 µg/ml (ppm). When compared with standard antibiotic chloramphenicol (MIC was 20 µg/ml). Similar results were also reported that by **Ibraheem, *et al.*,(2012)** who indicated the capability of ethanol: water to release the antimicrobial agents because of the high polarity added to ethanol by mixing with water to release such substances which part of them could be released by water and others by alcohol.

For the hexane extracts, it could be noticed from **Table (1)** that the best results were obtained with Rosemary followed by *Syzygium aromaticum*, *Cymbopogon proximus* and *Elettaria cardamomim*, respectively. The results are in agreement with **Witkowska *et al*., (2013)** who found that the ethanol and hexane extracts of oregano, clove, sage, rosemary and celery showed relatively strong antimicrobial activities against all bacteria tested.

The hexane extract of *C. proximus* also showed high inhibition activity but only against three of tested microorganisms. The inhibition zones were 18, 16 and 15mm against *Staph. aureus*, *E. coli* and *B. subtilis*, respectively. The *Elettaria* hexane extract shows lower activity the abovementioned three plants where it gave inhibition zones of 16, 15 and 15mm against *B. subtilis*, *Staph. aureus* and *E.coli*, respectively.

Only the algal hexane extract of *Ulva* showed inhibitory effect where the extract gave inhibition zones of 20, 15 and 14 mm for *E.coli*, *Listeria* and *Staph*, respectively. The results are in agreement with **Alang, *et al*., (2009)** who reported that the Green alga *U. lactuca* Linnaeus was successively extracted with n-hexane.

The tested plants were extracted by chloroform and tested for other antibacterial activity against the selected bacterial strain. Results in **Table (1)** showed that *Elettaria* chloroform extract among tested plants where the inhibition zones were 23, 20 and 18 mm against *B. subtilis*, *L. monocytogenes* and *E. coli*, respectively with no effect on both *Staph. aureus* and *Salmonella typhimurium*. The second plants were *Syzygium aromaticum* which showed its highest effect against *L. monocytogenes* followed by *Bacillus* and *Staph*. 23, 17 and 15 mm inhibition zones. Then came *Cinnamomum* to give effects against *Listeria*, *Staph.aureus*and *B. subtilis*(20, 20, and 18 mm), respectively. With algal, the chloroform extract gave the best results with *Ulva* algal against *Bacillus*, *listeria* and *Staph.* 20, 18 and 14mm, respectively with no effect against neither *E.coli* nor *Salmonella* **(Table 1).** Regarding the methanol extract of different plants, the best results were obtained with Eucalyptus followed by *Cympogon*, *Matricariachamomilla*, *S. aromaticum* and *Elettaria* especially against *L. monocytogenes*, *B. subtilis* and *Staph.aureus* , with no effect on *E. coli* or *Salmonella*. The *Ulva* algal extract was active only against *Staph* and *E. coli*. These results are in agreement with **Kolanjinathan** and **Stella (2011)** who found that the methanolic extract was the best amongst the five organic solvents used.

In general, the ethanol: water extract gave the best results against the tested microorganisms followed by hexane, chloroform and methanol, respectively. This could due to the polarity and the power of extraction of these used solvents.

Table (1): Inhibition zones (mm)* of various plants and algae and using different solvents against different bacterial strains.

Samples	Different extracts tested against different bacterial strains																			
	Ethanol : Water					Hexane					Chloroform					Methanol				
	St.	*B.*	*List.*	*Sal.*	*E.*	*St.*	*B.*	*List.*	*Sal.*	*E.*	*St.*	*B.*	*List.*	*Sal.*	*E.*	*St.*	*B.*	*List.*	*Sal.*	*E.*
Lupinus termis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Laurus nobilis	15	20	16	-	12	16	-	-	-	-	-	-	-	-	-	-	16	-	-	-
Nigella sativa	12	13	-	-	13	-	-	-	-	-	-	-	-	-	-	-	15	-	-	-
Psidium guajava	16	12	-	-	-	-	-	-	-	-	15	18	15	-	-	13	15	16	-	-
Curcuma domestica	-	-	-	-	-	-	-	-	-	-	-	-	17	-	-	-	-	-	-	-
Punica granatum	30	24	20	17	26	-	-	-	-	-	-	-	-	-	-	15	12	15	-	-
Zingiber officinale	13	15	-	-	15	-	-	-	-	-	-	15	20	-	-	-	-	-	-	-
Cinnamom umverum	15	13	-	-	12	-	-	-	-	15	20	18	20	-	-	15	-	12	-	-
Elettaria cardamomim	15	21	16	-	13	15	16	-	-	15	-	23	20	-	18	16	14	12	-	-
Matricaria chmomilla	16	17	16	-	15	-	-	-	-	-	-	-	-	-	15	15	-	18	-	-
Syzygium aromaticum	20	20	12	14	15	20	20	16	15	20	15	17	23	-	-	15	12	16	-	-
Cymbopogon proximus	15	13	12	-	14	18	15	-	-	16	-	-	12	-	-	14	-	20	-	-
Eucalyptus globulus	25	15	-	12	22	-	-	-	-	-	-	-	15	-	-	15	15	20	-	-
Rosmarinus oficinalis	25	20	16	12	17	26	24	22	-	20	15	12	13	-	-	15	15	13	-	-
Menthavaridis	15	14	12	-	13	-	-	-	-	-	12	15	17	-	-	-	-	-	-	-
Spirogyra sp	-	13	25	-	13	-		-	-	-	-	-	-	-	-	-	-	-	-	-
Corallina officinalis	-	12	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ulva lactuca	13	12	13	-	15	14	-	15	-	20	14	20	18	-	-	12	-	-	-	15
control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
*Distance between the edge of the disk and the end of the inhibition zone (mm).																				

List: Listeria monocytogenes. *B.: Bacillus subtilis.* St.: *Staphylococcus aureus.* *Sal: Salmonella typhimurium.* *E.:Escherichia coli*

Effect of different plant and algal extracts against tested fungal:-

The effect of plant and algal extracts carried out using ethanol: water, hexane, chloroform and methanol against *C. albicans*, *A. parasiticus* and *P. roqueforti* was investigated.

Results in **Table (2)** show the effect of ethanol: water extract on different tested fungi. It could be observed that *P. granatum* showed the highest effect against *C.albicans* (25mm) followed by *E. globulus* (23m) *Z.officinale* and *S.aromaticum* (20mm) and *R.officinales* (16mm). The same extracts showed a clear effect against *A.parasiticus* except for *Eucalyptus*. Only *S.aromaticum*, *E.globulus* and *R.officinalis* showed activity against *P.requeforti* and delayed its growth.

For algal extracts, *Ulva* algae was the best among other tested algal extracts where it gave an inhibition zone of 18mm against *C. albicans* and stopped the growth of the other tested fungal. *C. officinalis* stopped the growth of both *A. parasiticus* and *P. roqueforti* with no effect on *C. albicans* where Spirogyra sp. has no effect on all tested fungi.

Regarding the hexane extract of the tested plants, the highest effect was detected with *R. officinalis* followed by *S. aromaticum*, *C. verum* and *C. proximus* against *C. albicans* (22 ,20 ,15 and 15mm). All tested plants had no effect against the other two tested fungi.

Similarly, *Ulva* hexane extract only showed activity against *C. albicans* (20mm) with no effect on the other two tested fungi. The other two algae showed no effect against all tested fungi.

For chloroform extract, results in **Table (2)** indicated that only *C. proximus* showed activity against *C. albicans* (17mm) and stopped the growth of *A. parasiticus*. *S.aromaticum, E.glopulus* and *R.officinalis* also stopped the growth of *A. parasiticus* with no effect on the other tested fungi. For algae, only *Ulva lactuca* showed activity against *A.parasiticus*.When the methanol extract of the tested plants was used against fungi, only five plants showed activity against *C. albicans*.

The highest effect was clear with *C.verum* (25mm inhibition zone) and the lowest (12mm) was with *E. cardamomum, M. chamomilla* and *C. proxiumus*. *P. granatum* stopped the growth of *A. parasiticus* with no effect on the other fungi. *S. aromaticum* showed moderate effect on *C. albicans* (15mm), but stopped the growth *A. parasiticus* with no effect on *P. roqueforti.* Also, *E. globulus* and R. *officinalis* stopped only the growth of *A. parasiticus* with no effect on the other two tested fungi.

The methanol algal extract of *U. lactuca* was the only one which showed activity against *A. parasiticus* with no effect on the other fungi. Also, the other two algae showed no effect on the tested fungi.

The MIC&MLC of different plant and algae extracts against tested bacterial strains:-

In the experiment only the five plants which showed promising effect against the tested bacterial strains and the three algal strains were investigated.

It is clear from the results in **Table (3)** that all tested plants and algae showed no MIC against all tested microorganisms. *L. nobilis* only showed MLC against *S. typhimurium* at concentration of 256mg/ml. *P. guajava* showed high MLC effect against both *E. coli* and *S. typhimurium* at the level of 16mg/ml and moderate effect with *B. subtilius* and *Staph. aureus* (32mg/ml) but lower one against *L. monocytogenes* (128mg/ml). *P. agranatum*, which could be the best amongst the tested plants showed high MLC (15mg/ml) and moderate one against the other tested microorganisms (32mg/ml).

E. globulus comes in third place in MLC where it showed good activity against *B. subtilis*, *S. typhimurium* and *L. monocytogenes* (32mg/ml) where the MLC for *Staph. aureus, E. coli* was lower than that (64mg/ml). *R. officinalis* comes in fourth place to give low MLC (128mg/ml) against *E. coli*, *L. monocytogenes*, *Staph. aureus* and *S. typhimurium*, but very low one (256mg/ml) against *B. subtilius*.

Table (2): Inhibition zones (mm)* of various plants and algae and using different solvents against fungal strains.

Samples	Different extracts tested against different fungal strains											
	Ethanol : Water			Hexane			Chloroform			Methanol		
	Candida	*Asp.*	*Penic.*	*Candida*	*Asp.*	*Penic.*	*Candida*	*Asp.*	*Penic.*	*Candida*	*Asp.*	*Penic.*
Lupinus termis	-	-	-	-	-	-	-	-	-	-	-	-
Laurus nobilis	13	-	-	-	-	-	-	-	-	-	-	-
Nigella sativa	-	-	-	-	-	-	-	-	-	-	-	-
Psidium guajava	15	-	-	13	-	-	-	-	-	-	-	-
Curcuma domestica	-	-	-	-	-	-	-	-	-	-	-	-
Punica granatum	25	+	-	-	-	-	-	-	-	-	+	-
Zingiber officinale	20	-	-	-	-	-	-	-	-	-	-	-
Cinnamom umverum	15	+	-	15	-	-	-	-	-	25	-	-
Elettaria cardamomim	15	+	-	13	-	-	-	-	-	12	-	-
Matricaria chmomilla	12	-	-	-	-	-	-	-	-	12	-	-
Syzygium aromaticum	20	+	+	20	-	-	17	+	-	15	+	-
Cymbopogon proximus	-	-	-	15	-	-	-	-	-	12	-	-
Eucalyptus globulus	23	-	+	-	-	-	-	+	-	-	+	-
Rosmarinus oficinalis	16	+	+	22	-	-	-	+	-	-	+	-
Menthavaridis	13	-	-	-	-	-	-	-	-	-	-	-
Spirogyra sp	-	-	-	14	-	-	-	-	-	-	-	-
Corallina officinalis	-	+	+	-	-	-	-	-	-	-	-	-
Ulva lactuca	18	+	+	20	-	-	-	+	-	-	+	-
control	-	-	-	-	-	-	-	-	-	-	-	-
*Distance between the edge of the disk and the end of the inhibition zone (mm).												

Candida: Candida albicans *Asp: Aspergillus parasiticus* *Penic: Penicillumroqueforti*
(+) refer to stopped growth (-) refer to no effect

Regarding the MLC of the three tested algae, *Spirogyra sp*. was the best where it showed high MLC against *Staph. aureus* (32mg/ml) but lower than that against *B. subtilis*, *L.monocytogenes* and *E.coli*(64mg/ml) and no MLC against *S. typhimurium*. *C. officinalis* and *U. lactuca* only showed MLC against *S. aureus* and *B. subtilis* (64mg/ml) but no effect against the other tested bacterial strains.

The MIC&MLC of different plant and algae extracts against tested Fungal:

Regarding the MLC and MIC against the two tested fungi; *A. parasiticus* and *P. roquefortii*, data in **Table (4)** indicate that *Rosmarinus oficinalis* gave the highest effect 8.16 mg/ml for MLC and MIC, respectively against *A. parasiticus* while *L. nobilis* become second in its effect 8.64 mg/ml. *P. guajava*, *Spirogyra sp* and *U. lactuca* showed no effect while the other tested extracts gave the same effect 32, 64 mg/ml.

For the second tested fungi, *P. roquefortii*, the data indicate that only four extracts gave MLC and MIC effect (32, 64 mg/ml); *E. globulus, R. oficinalis, Corallina officinalis* and *U. lactuca*. The other extracts gave no effect against the same fungi.

This could be due to the resistance of *P. roquefortii* and also the possibility of using the other extracts against *A. parasiticus* which showed lower resistance to the used extracts.

Table (3): MIC&MLC of different plant and algae extracts against microorganisms.

microorganisms	***E.coli***		***L. monocytogenes***		***S. aureus***		***S.typhimurium***		***B.subtilis***	
Sample	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
Laurus nobilis	-	-	-	-	-	-	-	256	-	-
Psidium guajava	-	16	-	128	-	32	-	16	-	32
Punica granatum	-	32	-	32	-	32	-	16	-	32
Eucalyptus globulus	-	64	-	32	-	64	-	32	-	32
Rosmarinus oficinalis	-	128	-	128	-	128	-	128	-	256
Spirogyra sp	-	64	-	64	-	32	-	-	-	64
Corallina officinalis	-	-	-	-	-	64	-	-	-	64
Ulval actuca	-	-	-	-	-	64	-	-	-	64
MLC&MIC (mg/ml for algae and plant extracts)										

Bioassay of the effect of feeding rats on crude algae and plants on liver and kidney function of albino rats:

To determine the edibility and safety limits for feeding animals. Rats were fed on normal diet supplemented with three levels of four treated powder plant and algae. i.e. *C. officinalis, U. lactuca, E.globulus* and *Spirogyra sp.* three levels of treated, including 10,20 and 40% in case of *C. officinalis* (groups II,III and IV), *U. lactuca* (groups V,VI and VII), *E. globulus* (groups VIII,IX and X) and *Spirogyra sp*. (groups XI,XII and XIII) were used separately in this experiment in addition to control (groups I).

Table (5) showed the obtained results. Among different experimental groups there were no significant differences, regarding the mean value of initial body weight. While there was significant effects were observed for the final body weight and weight gain in the treated groups.

Results in **Table (6)** showed that there were significant changes in liver enzyme activities ALT or AST in the treated groups compared with control rats. Also the same **Table (6)** indicated that there were significant effects in levels of urea and creatinine as compared with control. Also, there were no significant changes in bilirubin compared with control.

All liver and kidney function were improved by replacing algae and rat feeding the most effective replace concentration were 20, 20, 40 and 20 for *C. officinalis, U. lactuca, E. globulus* and *Spirogyra sp.* respectively.

In conclusion, the addition of the four powders mentioned above didn't affect the body organs and also gave good body weight. This means that the addition of such materials could be safe for human use.

Table (4): MIC&MLC of different plant and algae extracts against tested Fungal strains.

microorganisms	***Asperagillus parasiticus***		***Penicillium roquefortii***	
sample	MIC	MLC	MIC	MLC
Laurus nobilis	8	64	-	-
Psidium guajava	-	-	-	-
Punica granatum	32	64	-	-
Eucalyptus globulus	32	64	32	64
Rosmarinus oficinalis	8	16	32	64
Spirogyra sp	-	-	-	-
Corallina officinalis	32	64	32	64
Ulva lactuca	-	-	32	64

Microbiological analyses of Ras Cheese:

Total viable counts (TVC) and fungi counts

Results in **Table (7)** show the effect of treatments and storage period on TVC and fungi counts. The control at the 90^{th} day had highest TVC and fungi of 2.9×10^3 and 3.3×10^3 cfu/g, respectively. Whereas, the results also indicated that treatment E2 and C2 had significantly lower counts than other treatments at different ripening periods.

Coliform bacteria and *E. coli*

The influences of treatments and storage period on coliform bacteria and *E. coli* (cfu/g) have been showed in **Table (8).** Ras cheese of treatment E2, C2 were free from E. Coli either when fresh or during ripening periods.

Treatment E2, C2 as main effect, significantly influence each of TVC, Fungi, coliform bacteria and *E. coli.*

Organoleptic properties:

Results presented in **Table (9)** showed the effect of the interaction between treatment and ripening period on organoleptic properties of Ras cheese. It is insignificantly affected flavor, body & texture, appearance and total score of Ras cheese; it is because cheese treatment with plant and algae extracts was only on the surface. The E_1andC_1 ripened for 30 days had higher total score of 89.50; however both the E_1 and C_2 ripened for 60and30 days gained lower total score of 81.58 and 82.33 points, respectively.

There were insignificant differences found among all treatments regarding total score for organoleptic properties of Ras cheese that ranged between 89.50 and 81.58 points as shown in **Table (9)**.

Table (5): Influence of different levels of some algae and plant on body weights of male albino rats.

Group No.	Treatment	Initial body weight (g)	Final body weight (g)	Weight gain (g)
Significant		**NS**	***	***
Group I	Control	55.12±1.48	150.40±2.07^{a}	95.28±2.80^{a}
Group II	Co 10%	56.36±1.31	110.00±2.54^{b}	53.64±1.50^{b}
Group III	Co 20%	55.58±1.98	114.10±1.59^{b}	58.52±2.56^{b}
Group IV	Co 40%	56.75±1.32	104.00±1.41^{c}	47.25±1.04^{c}
Group V	Ul 10%	54.54±.853	119.18±2.46^{b}	64.64±2.55^{b}
Group VI	Ul 20%	54.86±1.27	121.46±5.78^{b}	66.60±4.60^{b}
Group VII	Ul 40%	54.82±.960	107.58±1.10^{c}	52.76±1.18^{c}
Group VIII	EU 10%	55.80±1.35	123.00±3.53^{b}	67.20±3.32^{b}
Group IX	EU 20%	54.90±2.79	116.80±2.79^{b}	61.90±2.96^{b}
Group X	EU 40%	57.75±1.84	118.37±1.79^{c}	60.62±.629^{c}
Group XI	SP 10%	55.80±2.28	136.50±17.7^{b}	80.70±19.2^{b}
Group XII	SP 20%	55.40±2.30	127.60±2.94^{b}	72.20±2.13^{b}
Group XIII	SP 40%	57.00±1.45	116.60±2.07^{c}	59.60±2.04^{c}

Values are expressed as mean ± S.D. (n=5).

Means having different superscripts within each item in the same column are significantly different.

NS: insignificant and *******: refers to significant decrease at P ≤ 0.001relative to control.

Co:*Corallina officinalis*.**Ul**: *Ulva lactuca.* **EU**:*Euca lyptus globulus*. **SP**:*Spirogyra* sp.

Table (6): Influence of different levels of some algae and plant on liver and kidney functions of male albino rats.

Group No.	Treatment	Urea (mg/dl)	%	ALT(GPT) (U/L)	%	AST(GOT) (U/L)	%	Creatinine	%	Bilirubin	%
Significant		***		*		*		*		NS	
Group I	Control	45.0±1.0^{a}	**100**	78.0±2.0^{a}	**100**	308.0±7.0^{a}	**100**	0.483±.005^{a}	**100**	0.173±.03	**100**
Group II	Co 10%	29.0±1.0^{bc}	**35.6**	46.5±2.5^{b}	**40.4**	196.0±16.0^{b}	**36.4**	0.303±.032^{b}	**37.3**	0.160±.00	**7.6**
Group III	Co 20%	26.5±.50^{c}	**41.2**	38.5±1.5^{b}	**50.7**	155.0±39.0^{b}	**49.7**	0.350±.010^{b}	**27.6**	0.150±.05	**13.3**
Group IV	Co 40%	30.5±.50^{b}	**32.3**	48.0±1.0^{b}	**38.5**	230.0±18.0^{b}	**25.4**	0.290±.000^{b}	**40**	0.150±.05	**13.3**
Group V	Ul 10%	24.0±3.0^{bc}	**46.7**	65.0±5.0^{b}	**16.7**	238.5±47.5^{b}	**22.6**	0.420±.040^{b}	**13.1**	0.183±.02	**5.7**
Group VI	Ul 20%	20.5±.50^{c}	**54.5**	44.0±3.0^{b}	**43.6**	220.5±27.5^{b}	**28.5**	0.310±.025^{b}	**35.9**	0.100±0.0	**42.2**
Group VII	Ul 40%	34.5±.50^{b}	**23.4**	56.0±3.0^{b}	**28.3**	189.0±13.0^{b}	**38.7**	0.400±.050^{b}	**17.2**	0.123±.03	**29**
Group VIII	EU 10%	24.5±1.5^{bc}	**54.4**	49.0±5.0^{b}	**37.2**	204.5±3.50^{b}	**33.7**	0.400±.050^{b}	**17.2**	0.166±.02	**4.1**
Group IX	EU 20%	34.5±1.5^{c}	**23.4**	49.0±2.0^{b}	**37.2**	246.5±21.5^{b}	**20**	0.493±.015^{b}	**2**	0.170±.01	**1.8**
Group X	EU 40%	32.0±0.0^{b}	**28.9**	45.0±3.0^{b}	**42.4**	150.5±15.5^{b}	**51.3**	0.390±0.00^{b}	**19.3**	0.130±.02	**24.9**
Group XI	SP 10%	34.0±2.0^{bc}	**24.5**	53.0±7.0^{b}	**32.1**	215.5±40.5^{b}	**30.1**	0.42±0.02^{b}	**13.1**	0.15±0.05	**13.3**
Group XII	SP 20%	26.0±-5.0^{c}	**42.3**	49.0±1.0^{b}	**37.2**	205.5±21.5^{b}	**33.3**	0.36±0.07^{b}	**25.5**	0.15±0.04	**13.3**
Group XIII	SP 40%	32.5±1.5^{b}	**27.8**	92.0±42^{b}	**17.9**	293.0±87.0^{b}	**4.9**	0.38±0.06^{b}	**21.4**	0.22±0.02	**27.1**

Values are expressed as mean ± S.D. (n=5).

NS: insignificant and *******: refers to significant decrease at P ≤ 0.001relative to control.

%: refers to decreasing.

Co:*Corallina officinalis*. **Ul**: *Ulvalactuca.* **EU**:*Eucalyptus globulus*. **SP**:*Spirogyra* sp.

Table (7): Total viable counts and fungi (cfu/g) of Ras cheeses as affected by treatment and ripening period.

Treatments*	Ripening period (days)			
	Fresh	30	60	90
TVC $\times 10^3$ cfu/g				
Control	0.21	2.7	2.8	2.9
E_1	0.22	0.26	0.24	0.25
E_2	0.23	0.24	0.23	0.21
C_1	0.23	2.2	2.3	2.5
C_2	0.24	2.4	2.2	2.1
Fungi $\times 10^3$ cfu/g				
Control	2.7	2.9	3.1	3.3
E_1	2.9	2.2	2.1	2.4
E_2	2.8	2.1	2.0	1.9
C_1	3.0	2.5	2.3	2.3
C_2	3.2	2.5	2.2	2.1

Treatments*

E1: ***Eucalyptus globulus(****covered with* plant extract 64mg)

E2*: *Eucalyptus globulus(*covered with plant extract 128mg*)

C_1:*Corallina officinali(**covered with plant extract 64mg)*

C_1:*Corallina officinali(**covered with plant extract 128mg)*

Table (8):Coliform bacteria and *E. coli* (cfu/g) of Ras cheeses as affected by treatment and ripening period.

Treatments*	Ripening period (days)			
	Fresh	30	60	90
***E.coli*$\times 10^3$ cfu/g**				
Control	0.23	1.3	4.3	5.4
E_1	0.30	0.33	1.1	2.4
E_2	0.32	0.46	1.1	2.1
C_1	3.2	0.70	1.4	3.5
C_2	3.1	1.3	2.4	3.5
Coliform$\times 10^4$ cfu/g				
Control	2.5	3.5	5.4	9.2
E_1	2.3	1.1	2.1	2.5
E_2	2.4	1.1	1.3	2.4
C_1	3.2	1.2	2.4	5.4
C_2	3.4	2.2	4.3	5.4

Treatments*

E1: ***Eucalyptus globulus (***covered with plant extract 64mg) ***E2*: *Eucalyptus globulus (***covered with *plant extract 128mg*)

C_1: ***Corallina officinali (****covered with algae extract 64mg)* **C_1:*Corallina officinali (****covered with algae extract 128mg)*

Table (9): Changes in the scores of organoleptic properties of Ras cheeses as affected by treatment and ripening period.

Treatments*	Ripening period (days)	Flavor (60)	Body and texture (30)	Appearance (10)	Total score (100)
Control	0	49.08±1.67^{b}	24.66±1.26^{a}	8.58±0.19^{a}	82.33±2.44^{b}
	30	51.25±1.00^{ab}	26.25±0.56^{a}	8.58±0.14^{a}	86.08±1.43^{ab}
	60	54.00±0.87^{a}	26.91±0.73^{a}	8.50±0.15^{a}	89.41±0.75^{a}
	90	54.00±0.87^{a}	26.25±0.56^{a}	8.58±0.14^{a}	86.08±1.43^{ab}
E1	0	48.33±1.63^{b}	24.41±1.23^{a}	8.58±0.19^{a}	81.33±2.32^{b}
	30	54.00±0.87^{a}	26.91±0.73^{a}	8.58±0.19^{a}	89.50±0.64^{a}
	60	54.00±0.87^{a}	24.50±1.25^{a}	8.66±0.18^{a}	81.58±2.39^{b}
	90	54.00±0.87^{a}	26.91±0.73^{a}	8.50±0.15^{a}	89.41±0.75^{a}
E_2	0	48.41±1.66^{a}	24.50±1.25^{a}	8.66±0.18^{a}	81.58±2.39^{a}
	30	51.25±1.00^{a}	24.50±1.25^{a}	8.58±0.19^{a}	84.33±1.79^{a}
	60	54.00±0.87^{a}	26.16±0.54^{a}	8.50±0.15^{a}	85.50±1.20^{a}
	90	54.00±0.87^{a}	24.50±1.25^{a}	8.58±0.19^{a}	84.33±1.79^{a}
C_1	0	48.50±1.68^{b}	24.25±1.17^{a}	8.50±0.15^{a}	81.25±2.28^{b}
	30	51.25±1.00^{a}	26.91±0.73^{a}	8.58±0.19^{a}	89.50±0.64a
	60	54.00±0.87^{a}	26.25±0.26^{a}	8.58±0.14^{a}	86.08±1.43^{ab}
	90	54.00±0.87^{a}	26.91±0.73^{a}	8.50±0.15^{a}	89.41±0.75^{a}
C_2	0	49.08±1.67^{a}	24.66±1.26^{a}	8.58±0.19^{a}	82.33±2.44^{a}
	30	51.25±1.00^{a}	24.66±1.26^{a}	8.58±0.19^{a}	82.33±2.44^{a}
	60	51.25±1.00^{a}	26.25±0.56^{a}	8.58±0.14^{a}	86.08±1.43^{a}
	90	54.00±0.87^{a}	26.25±0.56^{a}	8.58±0.14^{a}	86.08±1.43^{a}

Treatments*

E1: ***Eucalyptus globulus(****covered with* plant extract 64mg) **C_1:*Corallina officinali(****covered with algae extract 64mg)*

E*2: *Eucalyptus globulus(*covered with plant extract 128mg***)* **C_1:*Corallina officinali(****covered with algae extract 128mg)***

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الملخص العربى

تأثير مستخلصات بعض النباتات والطحالب كمضادات ميكروبية ومواد حافظة للأغذية.

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أجريت هذه التجربة لدراسة التأثير المضاد للميكروبات لبعض النباتات والطحالب. فقد تم استخدام خمسة عشر نباتا وثلاثة طحالب، وقد تم الحصول على المستخلصات بإستخدام الإيثانول: الماء، الهكسان، الكلوروفورم والميثانول. كما تم تحديد كل من MIC و أقل تركيز مثبط وكذلك أقل تركيز مميت. وأظهرت النتائج أن مستخلص الرمان P. granatum مع الإيثانول: المياه، أعطت أعلى منطقة تثبيط ضد الكائنات الدقيقة المختبرة ، وأعطت الطحلب Ulva أفضل النتائج عند الإستخلاص بالكلوروفورم يليها الهكسان . أعطى أيضا المستخلصات الإيثانول : المياه أعلى تثبيط ضد الفطريات المختبرة يليها الهكسان والميثانول. وكانت MLC أقل تركيز مميت لبكتريا القولون *E. coli* عند ١٦مجم/مل مع مسخلص الجوافة، ولكن وصلت إلى ٢٥٦مجم/مل مع مستخلصات الروزمارى. كما أظهرت نتائج الـــ MIC أقل تركيز مثبط ضد فطر الأسبرجلس *Asperagillus parasiticus* مع ٨ مجم/ مل لمستخلصات ورق اللورا والكافور *L. mobilis* ، *E. globulus* فقط.

ولم يكن لتغذية الفئران على المستخلصات النباتية والطحالب تأثير كبير على وزن الجسم، ولكن كانت هناك تغيرات وتأثير كبير في انزيمات الكبد، ولم يكن هناك أي تأثيرعلى الخصائص الحسية للمعاملات الخمسة التي أجريت على الجبن مما يعني أن هذه المستخلصات يمكن استخدامها بأمان للاستهلاك الآدمى.