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Efficiency of Essential Oil of *Mentha spicata* (L.) and Study of their Biological Activities against Some Gingivitis Bacteria

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Abstract

This experiment was carried out in the Laboratory of Postgraduate Studies / Biotechnologies / College of Science / University of Kufa / for the period from 10/1/2022 to 1/ 4/2023.This study was conducted to alcoholic extract the active ingredients and essential oils in the leaves of the mint plant (*Mentha spicata*). The essential oils were extracted from the fresh leafs of plant using the Clevenger hydro-distillation method and oils were detected using sulfuric acid reagent and crystallization method. Characterize their chemical and physical properties of these oils (the degree of acidity, Color, taste, and specific density of essential oils. Also, evaluate the cellular toxicity of essential oils and leafs extracts using the method of blood lysis of healthy human, and study their biological activities against bacteria that cause gingivitis isolated from infected male gums. Four concentrations of Oils and alcoholic leafs crude extract were tested 1:1, 2/1, 5/1, 10/1 (v/v) ml of oil /ml of distilled water, in addition to the standard substance as menthol(S1) as a control agent, and Crude Oil as standard (S2) and Kanologe as Positive control (P^*) to evaluate the antibacterial activity that causes gingivitis, using the agar diffusion method, at a rate of three replicates for each concentration and 9 replicates for each treatments. The bacterial samples that cause gingivitis were obtained from Al-Ameen Center for Research and Advanced Biotechnology in Al-Najaf City. The diagnosis of bacteria isolate was also confirmed with the Vitek2 Compact. The results showed that there was a conformity with the ideal specifications of the oils with international standards or close to them, where the acidity value was pH: 2.87. The results of blood hemolysis tests showed that there was no clear hemolysis in all concentrations used of essential oils in compare with control groups (P^*) which gave a percentage of hemolysis of blood cells. The results showed that all the concentrations used gave an area of bacterial inhibition growth rate that increased with increasing oil and leafs extract concentration. The highest inhibition rate *S.mutans* was for oils at a concentration of (1/10) v/v ml Oils: Water, with an average inhibition rate of (26), and that the highest inhibition rate was for leafs extracts at a concentration of (1/10) v/v ml extract: Water, with an average inhibition rate of (21). While the lowest inhibition rate was for the Oils and leafs extracts at a concentration of (1/1) v/v ml Oils: Water with an average inhibition of (16 and 4) mm respectively. However, the highest inhibition rate *E.coloi bacteria* was for oils at a concentration of (1/10) v/v ml Oils: water, with an average inhibition rate of (26), and that the highest inhibition rate was for leafs extracts at a concentration of (1/10) v/v ml extract: Water, with an average inhibition rate of (21). While the lowest inhibition rate was for the Oils and leafs extracts at a concentration of (1/1) v/v ml Oils: water with an average inhibition of (16 and 4) mm respectively.

Keywords: Essential Oil, *Mentha Spicata* (L.), Biological Activities, Bacteria

Introduction

Medicinal plants possess a rich source of phytochemicals that are a successful alternative to many manufactured antibiotics that are resistant to pathogenic microorganisms [4, 17] indicated that about 80% of the world's population uses traditional herbal preparations as primary treatment. Essential oils are good alternatives that are gaining more attention in the treatment of a wide range of periodontal diseases [15]. Some strategies to combat periodontal disease have been improved mainly through phytochemicals. Gum disease is one of the most common infectious diseases that destroy gum tissue and is an important public health problem. New research and studies aim to discover alternative, effective and natural materials extracted from safe and effective medicinal plants for the prevention and treatment of these diseases. The plant *Mintha spicata* (L) is one of the most important types of medicinal aromatic plants used against bacteria that cause gum disease through its biochemical content [22]. The plant contains 50% menthol oil and 25% to 28% of tannin, the chemical compound Punicallin, Granatine C, and Tanic acid, which are potent antimicrobial agents [19]. Gum disease is one of the most common diseases caused by gram-negative

anaerobic bacteria. Due to the occurrence of periodontitis, the increased resistance of oral bacteria to antibiotics and the negative effects of some antibacterial currently used in dentistry, there is a need to search for safe and effective alternative products, so the current study aims to:
Extraction of essential oils from the leaves of the mint plant and qualitative detection of the oils and testing the inhibitory ability of essential oils against the bacteria that cause gingivitis to Production of natural antibiotics that may replace chemicals antibiotic.

Material and Methods

Plant Collection and Oils Extracts Preparation

The fresh leafs of *Mintha spicata*, was collected from the growing plants in the home garden in the Najaf city in October 2022 and kept in the laboratory at room temperature even use. Leafs crude extracts were done by maceration methods for 24 hours over night. The essential oil was extracted or separated from the leaves according to the method used by [16], using the method of hydro distillation using the Apparatus Clevenger by immersing 80g of fresh leaves with distilled water in a 1-liter glass flask.

Physical and Chemical Properties

Color and taste of Essential oils

The Essential oils color was determine on the naked eye, and a sensory evaluation was conducted to determine the taste of the aromatic oils. The volatile oils differ in the degree of their natural colors after extraction [2].

Specific density

Is the ratio between the weight of a certain volume of volatile oil and the weight of an equal volume of water at a temperature of 11 ° C [14].

Acidity

Is the number of milligrams of potassium hydroxide (KOH) needed to neutralize the free fatty acids present in one gram of oil or fat. The acidity was calculated as follows:

$$IA = \frac{VxNx\ 56.1}{m}$$

Where,
IA= Acidity
V= Volume of KOH
N= molarity of KOH solution
m= The mass of the oil sample. [8]

Qualitative Detection of Oils

H2SO4 Reagent

Take 21 drops of oil and mixed with 1-3 ml of sulfuric acid to be colorless with peppermint oil.

Crystallization

Oil samples were placed in glass tubes at a low temperature of 4 °C, which leads to crystallization of oil particles or their transformation into colorless crystals. [20].

Cytotoxicity test

The cytotoxicity of essential oils was tested according to the method by [22] was followed in the toxicological tests of the studied Bloods sample mixed with 30 µL of the mint plant extract at different concentrations (10:1,5:1,2:1 and 1:1 v/v, O:W.with (control treatment S1) representing crude oil and (control treatment S 2) representing menthol oil, and Positive control (P*) representing Kanologe drug.The blood suspension was incubated at room temperature for 1 hours, After the incubation period, the suspension was centrifuged at 10,000 r/min within 5 min. The supernatant was read in a 96-hole plate using a Mark X microplate scanning spectrophotometer (Beurad, USA) at 550 nm The hemolysis ratio was calculated as follows:

$$H = \frac{ODM - ODOil\ X - 100}{OD\ Oil\ x\ 100}$$

Where,
H = Hemolysis rate
OD = optical density

Samples Preparation

To prepare the concentrations used in this study, the volumetric dilution method used by [1] was followed by dissolving a specific volume of oil or crude leafs extracts into a specific volume of water and achieving complete dissolution using 10% DMSO, where concentrations of 1:1, 1/2. 1/5, 1/10 (v/v) ml of distilled water/ ml of oil or leafs extracts were prepared, in addition to the standard substance menthol (S1), standard of crude oil or leafs extracts (S2) were placed in tightly closed glass tubes, labeled, and kept in the refrigerator at a temperature of 10 °C until subsequent experiments were carried out.

Bacteriological Tests

The test bacteria included two types of bacteria, one of which were Gram-positive, namely *Streptococcus. Mutans*, and the same were Gram-negative, namely *Escherichia coli*. The cultures were activated on broth nutrient at a temperature of 37˚C for 24 hours, then compared with McFarland's standard solution prepared as mentioned by the diffusion well agar method was used.

Results and Discussions

Physical and Chemical Properties of Oils

Color and Taste of Essential Oils

The color of the essential oils was measured based on the naked eye, and a sensory evaluation was performed to determine the taste of the essential oils [13]. Table 1, reveled that the color and taste of the essential oils under study, which was light yellow and a pungent taste, which is agreements with to what was found by [27]. Essential oils have a distinctive fragrant smell because they contain some compounds with small partial weights that volatilize at room temperatures, such as aldehydes, alcohols, ketones, esters, and other oxygen compounds [3]. It was found from studies that the oils are rarely colored, (with some exceptions) and take a gradient to a light blue color due to the presence of the chamazulene compound. The essential oils differ in the degree of their natural colors after extraction, which are either colorless or pale yellow, light yellow, greenish yellow, yellowish brown or greenish blue, which indicates the high quality and purity of the oils and their closeness to international standards for volatile oils [12].

Specific density

According to the results in the same table, the specific density of essential oils was 1.0876 compared to the specific

density of menthol oil, which was 0.8898. It is less than one, and this confirms that the plant extract is naturally oily resin. [26]. When the specific density of the essential oil is less than 0.9, it means that the oil carries ordinal and aliphatic compounds, and if they are more than the correct one, then the oil contains compounds with many aromatic rings that are chemically different. From knowing the value of the specific density of the oils, can be known the degree of oil purity. This result agrees with the international standards for vegetable oils according to [4] which amounted to 0.990.

Acidity

Results in the same table show that, the pH of the oil extracted from the leafs of the *M. specata* was 2.87. This value indicates that the components of the oil did not partially decompose during distillation. The pH of an essential oil is an important criterion for estimating its quality. [11] indicated that the acid number indicates the percentage of free fatty acids in the oil, and whenever the percentage is low in the extracted oil, it is less than pH: (3)indicates that the essential oils are stable and not oxidized.

Table 1: Some physical and chemical properties of essential oils

Properties	Results
The color	light yellow
Taste	stinging
The scent	aromatic
Specific density	1.0876
Menthol specific density	0.8898
Acidity	2.87

Fig 1: Sulfuric acid reagent

Qualitative Oil Test

H2SO4 reagent

A few drops of the aromatic oil obtained by the distillation in a test tube mixed with 3 ml of concentrated sulfuric acid. It is noted that the light-yellow oil turns into a completely colorless transparent oil (Fig. 1). This indicates the high purity of the oils [23].

Crystallization

When the studied essential oil was exposed to the low temperature of 4 ° C for 72 hours, a crystallization state appeared for the oil [21]. Found that oil crystallization processes require less time, approximately 48 hours at -45 °C.

Human Erythrocyte Lysis (%)

The optical absorption spectrum of leafs extract and essential oils suspended with RBC blood cells was measured at concentrations (1:1, 2:1, 5/1, 10/1 (v/v) O: W using Triton X-100% and (tyrode (v/v), respectively as a positive control and a negative control, wavelength 550 nm was adopted for the assay. The value of the optical absorbance throughout the experiment period in 60 minutes. Determination of hemolysis is based on the degree of absorption of the hemoglobin spectrum at 550 nm using a spectrophotometer, compared to the control treatment.

The results shown in Fig (2A) indicated that the percentage of human erythrocyte lysis (%) caused by the effect of essential oils extracted from the peppermint plant in cubated after 1 hour at concentrations of (1:1, 2/1,5/1,10/1 (v / v), O:W notes that all concentrations of the *M. spicata* plant Oils recorded different low hemolysis rate (0.0006, 0.0008,0.0002 and 0.0041) respectively and also the control treatment S1,S2 and P* recorded the high percentage of the hemolysis rate was (0.0089, 0.0163 and 0.0005) respectively. A significant hemolytic effect was recorded. In addition, an increase in the percentage of hemolysis was observed reached (0.0163) in the control treatment S2 (Crude extracts) after 1 hour of incubation. Similarly, for the control treatment P* (Drugs), noticed that the percentage of red blood dissolution reached (0.0005).

Also, the hemolytic effect of the crude leafs extract showed that represented a very weak toxic effect on isolated red bloods erythrocytes, with a hemolysis rate not exceeding at two concentrations of 5:1, 10:1 (v/v), O: W (0.0022, 0.0032) compared to hemolysis in the control treatment. Fig 2B)

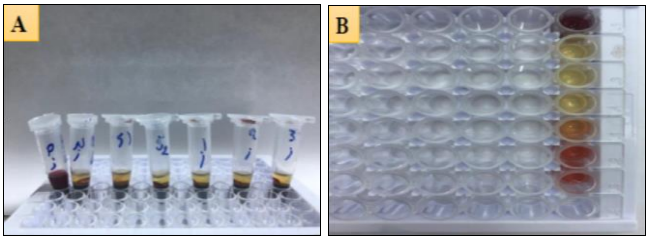

Fig 2: Hemolysis percentage of tubes containing red blood cells in the presence of various concentrations of oil, leafs for mint plant at 37 ° C (**A:** Oils **B:** leafs)

This result shows that there are no clear indications of hemolysis with increasing the concentration of the studied oils, except for the concentration of 1:10 v/v after 1 hour of incubation, which was 0.0041% compared to the control treatments. The presence of a small percentage of hemolysis in blood cells. This may be because in whole blood plasma adsorption occurs, it may affect hemolytic properties. Also, the results obtained through hemolysis tests conducted in the laboratory on red blood cells isolated from human blood, indicated that the extracts of Oils of the mint plant showed very low toxicity, so they can be important natural sources in treatment and in the pharmacological fields to alleviate various diseases. The hemolysis percentage for essential oils showed that they had almost no

Hemolysis in low concentrations of the tested oils. It is known that the interactions of the chemical components of essential oils with the membranes of RBCs depend mainly on the presence of silanol groups on the surface of the particles [18].

Table 2: hemolysis percentage of red blood cells in the presence of various concentrations of oil, leaves for mint plant at 37 ° C

Conc.	Extracts		Average
	leafs	Oils	
P*	0.0005	0.0005	0.0005
S1	0.0089	0.0089	0.0089
S2	0.0163	0.0001	0.0082
T1	0.0008	0.0006	0.0007
T2	0.0021	0.0008	0.0014
T3	0.0022	0.0002	0.0012
T4	0.0032	0.0041	0.0036

P*: Kanaloge, S2: Crude, S1:Menthol; T1(1:1), T2(1:2), T3 (1:5), T4(1:10) W:O

The results of studies by [23] have shown that lipophilic substances can often induce hemolysis because they are capable of destabilizing the lipid bilayers present in cell membranes, causing lysis of erythrocytes and increasing plasma hemoglobin levels. However, many natural compounds have properties that cause the reduction in the membrane fluidity of erythrocytes, reducing hemolytic processes that lead to lower blood viscosity. This property has a potential for pharmaceutical use.

Effectiveness of Essential Oils and Crude Leafs Extracts against *S. Mutans* and *E.Coli* Bacteria

The results in Fig 3 indicate that all concentrations of oils and crude alcoholic extracts of *M. spicata* leafs have antibacterial activity against *S.mutans* isolated from infected gums with different inhibition rates, which increases with increasing concentration. It was found that all the concentrations of *M.spicata* leaf extracts as shown in Fig (3A1, A2)gave differences in the inhibition zone between all concentrations, compared with the control group (S1) and (S2), as well as the antibiotic group used kenaloge (P*) against pathogenic bacteria, which recorded an average of (2, 16, 17) mm, respectively, (Fig. 3), and the highest average inhibition zone at concentration 10/1 (v/ v) extract / distilled water was (21) mm, compared with the studied concentrations 1/1, 1/2, and 1/5 (v/v) with an average of (10, 18, 20) mm respectively.

[10] mentioned that phenolic compounds are among the largest phytochemical compounds in the mint plant that have clear activity against bacteria that cause gingivitis in humans, and the phenolic compounds cause destruction and damage at the level of the wall of bacteria, which shows an increase in the membrane permeability of potassium protons and ions. Decrease in ATP stock within the cell and cause damage to its cellular proteins.

Also, the results from the same table show that all concentrations of essential oils of *M. spicata* plant have antibacterial activity against *S.mutans* isolated from infected gums with different inhibition zone rates, which also increases with increasing concentration. Where it was found that all concentrations gave clear differences in inhibition zone rate between all concentrations, compared with the control group S1 and S2, as well as the antibiotic group used kenalog (P*) against pathogenic bacteria, which reached an average of (2, 18, 17) mm, respectively, and was higher the average of inhibition zone at concentration 10/1 (v / v) Oil / distilled water is (26) mm compared to the other concentrations studied 1/1, 2/1 and 5/1 (v /v) O/D.W with an average of (16, 19, 22) mm respectively and as shown in Fig (3B1, B2).

Scientific studies have shown that volatile oils with MICs ranging from 19 to 100 µg/mL are potent antibacterial agents [8]. Essential oils (EOs) are composed of volatile compounds, such as terpenes, terpenoids, phenol-derived aromatic components, and aliphatic components. With strong aromatic molecules, EOs possess several biological properties, including antibacterial activities [6].

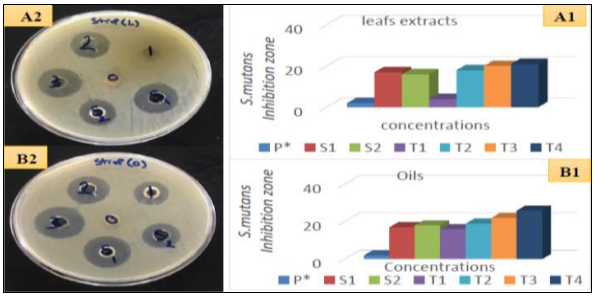

Fig 3: Inhibition zone of the growth rate of *S.mutans* bacteria using crude alcoholic extract of leaves (A1, A2) and essential oils (B1, B2)

Meanwhile, the results showed in Fig (4A1, A2) that all concentrations of crude alcoholic extracts of mint leafs have antibacterial activity against *E.Coli* bacteria isolated from infected gums with different rates of inhibition. The antibacterial inhibitory also increases with increasing the concentration of the crude extract of *M.spicata*. It was found that all the studied concentrations gave significant effects compared with the control group S1 and S2, which amounted to (17 and 18) mm, respectively, as well as the antibiotic group used kenalog ((P* against bacteria and amounted to (00) mm and the highest average zone of inhibition rate was at concentration 1 /10 (v / v) distilled water / extract is (20) mm compared with the studied concentrations 1/1, 1/2 and 1/5 (v/v) with an average of (00.14 16) mm, respectively. The results from the same table also showed that there were significant differences between the raw concentrations of the oils and the control treatments S1 and S2, which gave the highest rate of inhibition and reached (18 and 17) mm, respectively. The results showed in the study of [9] that the alcoholic extracts of the mint plant *M spicata* is less biologically effective against *E. coli* than the effectiveness of essential oils. Fig (4 B1, B2).

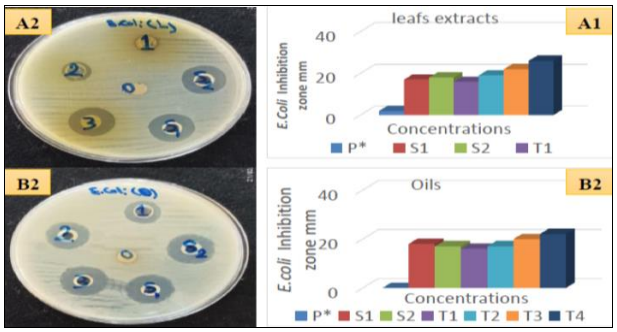

Fig 4: Inhibition zone of the growth rate of *E.Coli* bacteria using crude alcoholic extract of leaves (A) and essential oils (B)

The oils extracted from *M.spicata* plant no induced for hemolysis with all concentrations in present study (10:1, 5:1, 2:1, 1:1) v/v, O:W. Hemolysis is a process of destruction of red blood cells (erythrocytes) in which the

rupture of the plasma membrane occurs. Hemolysis is caused not only by some types of antibiotics and anti-inflammatory agents, but also by natural compounds. Several plant extracts with hemolytic activity have been described, some of which are cytotoxic or genotoxic, making it necessary to perform pharmacological and toxicological analyses of essential oils and plant extracts [5]

References

1. Ahmadi F, *et al*. Chemical composition, *in vitro* anti-microbial, antifungal and antioxidant activities of the essential oil and methanolic extract of Hymenocrater longiflorus Benth of Iran. Food and Chemical Toxicology. 2010; 48(5):1137-1144.
2. Abir BO, Hajjaj H. Chemical composition, biological activity and physicochemical properties of *buckthorn oil*, MSc, University of Ouargla, 2019, p67.
3. Al-Shahat NZ. Physiology and chemistry of volatile oils (of aromatic plants), Mars Publishing House, Riyadh, Saudi Arabia. MSc thesis, 1995.
4. AFNOR (Association Française de 12 (ormalisation). Norme Française NF ISO 3063 huile Essentielle d'ylang-ylang [Cananga odorata (Lamarck) J.D. Hooker et Thomson forma genuina] Paris: AFNOR, 2005.
5. Ajdzanovic V, Spasojevic I, Pantelic J, Sosic-Jurjevic B, Filipovic B, Milosevic V, *et al*. Vitex agnus-castus L. Essential Oil Increases Human Erythrocyte Membrane Fluidity. Journal of Medical Biochemistry. 2012; 31:222-227.
6. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils. Food chemical toxicology. 2008; 46:446-475.
7. Bouqawada M. Phytochemical study of pesticides and phenols in some types of local dates, MSc. thesis, University of Ouargla, 2008, p87.
8. Bourhia M, Messaoudi M, Bakrim H, Mothana, RA, Sddiqui NA, Almarfadi OM, *et al*. Citrullus colocynthis (L). Schrad: Chemical Characterization, Scavenging and Cytotoxic Activities. Open Chem. 2020; 18:986-994.
9. Chang L, Zhang Z, Ke D, Jian-Ping Y, Xiao-Kui G. Antibiotic resistance of probiotic strains of lactic acid bacteria isolated from marketed foods and drugs. Biomedical and Environmental Sciences. 2009; 22(5):401-412.
10. Calo JR, Crandall PG, O'Bryan CA, Ricke SC. Essential Oils as Antimicrobials in Food Systems-A Review. Food Control. 2015; 54:111-119.
11. De Cliff S. et Harerimana PC. Extraction de l'huile-131 Essentielle complète des fleurs de Cananga odorata de la plaine de l'Imbo: Vers la vulgarisation d'une nouvelle filière de plantes industrielles au Burundi. Revue de l'université de Burundi, Série Sciences Exactes. 2013; 28:1-17.
12. Faria TDJ, Ferreira RS, Yassumoto L, Souza JPD, Ishikawa WK, Bardosa AM. Antifungal activity of essential oil isolated from Ocimum gratissimum L. (Eugenol chemo type). An international Journal. 2006; 6:867-871.
13. Fatiha, Darraji, Karama Raja. A theoretical study on the effect of vegetable oil extraction methods on their composition and effectiveness. PHD thesis. Kasdi Merbah University - Algeria.13-Garvy *et al*, 1977.
14. Gonzalez-Tejero MR, Casares-Porcel M, Sanchez-Rojas CP, Ramiro-Gutierrez JM, Molero-Mesa J, Pieroni-Giusti ME. Medicinal plants in the Mediterranean area. J Ethn Pharmacol. 2008; 116(2):341-357.
15. Hussain AI, Anwar F, Nigam PS, Ashraf M, Gilani AH. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four Mentha species. Journal of the Science of Food and Agriculture. 2010; 90(11):1827-1836.
16. Lardry JM, Haberkorn VL'aromathérapie *et al*. Les Huiles Essentielles. Kinésithérapie La Rev. 2007; 7:14-17.
17. Lhuillier A. Contribution a l'étude phytochimique de quatre plantes malgaches: Agauria salicifolia Hook. f ex Oliver, Agauria polyphylla Baker (Ericaceae), Tambourissa trichophylla Baker (Monimiaceae) et Embelia concinna Baker (Myrsinaceae). Thèse de doctorat. Toulouse, 2007.
18. Lin YS, Haynes CL. Journal of the American Chemical Society. 2010; 132:4834-4842.
19. Moreno S, Scheyer T, Romano CS, Vojnov AA. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Readical Res. 2006; 40(2):223-231.
20. Mohamed N, boukhatem, mohamd SH, Fairoua S. et Yahia H. Extraction, composition et et propriétés physico-chimiques de l'huile essentielle du Géranium Rosat (Pelargonium graveolens L) cultivé dans la plaine de Mitidja (Algérie)»; université de saad Dahleb de Blida; Algérie, 2010.
21. Nadeem MA, Dubey R, Singh A, Pandey R, Bundelas. Processing and Quality Evaluation of Menthol Mint Oil. International Journal of Mathematics and Statistics Invention. 2017; 5(2):68-70.
22. Julie L, Valentine M, Lutfiye A, François M. Impact of Silver Nanoparticles on Haemolysis, Platelet Function and Coagulation. Nanobiomedicine. 2014; 1:4.
23. Bui-Phuc T; Nhu-Trang T, Cong-Hau N. Comparison of chemical composition of essential oils obtained by hydro-distillation and microwave-assisted extraction of Japanese mint (*Mentha arvensis* L.) grown in Vietnam. Materials Science and Engineering, 2020, p991.
24. Kini RM. Excitement Ahead: Structure, Function and Mechanism of Snake Venom Phospholipase A2 Enzymes. Toxicon. 2003; 42:827-840.
25. Ktari N, Trabelsi I, Bardaa S, Triki M, Bkhairia I, Salem RBSB, *et al*. Antioxidant and hemolytic activities, and effects in rat cutaneous wound healing of a novel polysaccharide from Fenugreek (Trigonella foenum-graecum) Seeds. Int. J. Biol. Macromol. 2017; 95:625-634.
26. Salima Hebaz. Extraction and study of the antioxidant activity of the essential oil of Ammodaucus leucotrichus. MSc. study. College of Technology. Algeria, 2019.
27. Yadav K. Dental Caries: Bacterial profile of Dental caries. Lambert Academic Publishing, Omni Scriptum GmbH and Co. KG, Germany, 2016, p120.