

Phytochemical Examination and Antibacterial Activities of Thyme Leaves and their Use with Goat's Milk as a Traditional Remedy

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Abstract

Plants are a source of a great abundance of drugs including several groups such as antimicrobials, anticancer, antispasmodics, etc. A large number of the plants are own antibiotic characteristics in conventional usage and are also utilised widely by the tribal people extensive.

Objectives: Thyme is one of the vital plants around the world because it contains various essential secondary metabolites which are considered as bioactive ingredients for the remedy of several diseases. The present study aimed to a qualitative and quantitative phytochemical screening of RE, preparation of a boiled mixture of thyme leaves and goat's milk TMGT as a traditional drug, and examination of the biological activity of each of RE and TMGT against some sorts of pathogenic bacteria.

Methodology: Preparation of Thyme-Milk Goat's Treatment (TMGT): where fresh wild thyme leaves were added to fresh goat's milk, and mixed very well, followed by added virgin olives oil with well mixing, and boiled a while, a thick, yellowish-oily liquid {Thyme-Milk Goat's Treatment (TMGT)} was obtained.

Preparation of Thyme Aqueous Raw Extract (RE): Where a coarsely fine powdered of thyme leaves was extracted with appropriated solvent by using soxhlet extractor apparatus.

Results: Percentage Yields: The percentage yields of RE were 89%, while the pH for RE and TGMT were 6.7 and 7.3, individually, and the acid value of raw goat's milk was 6.7g.

Preliminary Phytochemical Screening: The preliminary phytochemical screening of thyme aqueous crude extract revealed the presence of plentiful amounts of bioactive ingredients, which are tannins, phenols, Fats and Fixed oils, Amino acids alkaloid, flavonoids and steroids and triterpenoids.

Quantitative Phytochemical Investigation: The percentage yields obtained from the leaves regarding quantitative phytochemical investigation were 81, 72 and 90 % for Flavonoids Alkaloids and Saponins, separately.

Antibacterial: The thyme aqueous raw extract (RE) was tested against each of the bacterial; *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus*, *Streptococcus pneumonia* and *Staphylococcus aureus*, were revealed 19, 20, 21, 19 and 23 mm, correspondingly. However, the TMGT showed 23, 22, 25, 24 and 25 mm consistently.

Keywords: *Thymus vulgaris L.*; Thyme-Milk Goat's Treatment; Phytochemical Screening; Pathogenic Bacterial

Introduction

Scientific Name: *Thymus vulgaris L.*

Local Name: Zaatar

Thyme (*Thymus vulgaris L.*) widespread planted in temperate climates and the Mediterranean Nations. And is one of the great commercially valuable herbs, Besides, this plant has an adequate pungent aromatic, wherever flowers, leaves and oils extracted from the plant are widely used, special as a medicament for treating diseases or in food ingredients for a variety of cuisines, such is much appropriated to give a flavouring to soups for delightful savour. Moreover, historically, thyme is reported extended back to ancient Rome and Egypt. Where the Romans had it ere feeds and delivered it to warriors as a symbol of bravery and courage and admiration. While the Egyptians, involving the thyme, in the mummification methods. As for the ancient England communities, they used it as a spice when cooking their meals. Given that thyme contains vital and effective components of secondary metabolic constituents, which made it play a major role in prevention, treatment and other uses, the presence of compounds such as carvacrol, Linalool and others, which would resist and kill microbes. Additionally, were used as a medicine of numerous of diseases in the nowadays and in old time. Accordingly, and from these benefits makes it safe in utilising in many purposes such as a pesticide, mainly presence of thyme oil in thyme, which is the principal effective component thymol were have also demonstrated anti-microbial, anti-fungal, and maybe for other abilities. Thymol is additionally known by several names such as 2-isopropyl-5-methyl phenol (IPMP) or isopropyl-*m*-cresol, 1-methyl-3-hydroxy-4- isopropyl benzene [1], is a natural monoterpene phenol derivative of cymene, C₁₀H₁₄O, isomeric with carvacrol, found in oil of thyme, and extracted from *Thymus vulgaris L.* (common thyme), and also found in other diversities of plants. Carvacrol, or cymophenol, C₆H₃(CH₃)(OH)C₃H₇, is a monoterpene phenol, also, it has a distinctive sour. The natural occurrence of carvacrol is present in the essential oil of *Thymus vulgaris L.* while Carvacrol, or also known as cymophenol, C₆H₃(CH₃)(OH)C₃H₇, is a monoterpene phenol, has a distinctively pungent and tempered scent of oregano [2]. Carvacrol is present in the extracted essential oils of some plants such as oil from thyme, *Origanum vulgare* (oregano), and oil collected from pepperwort. Where, thyme essential oil contains subspecies between 5% and 75% of carvacrol [3]. Carvacrol has antimicrobial activity against several diverse bacterial and strains [4]. Moreover, to these incredible benefits,

thyme also contains many vitamins (A, C, E, K, and B6) and minerals (calcium, iron, phosphorous, manganese and magnesium) as well as omega-3 and omega-6. On the other hand, for the therapeutic advantage of the thyme plant since ancient times, against bacteria and viruses that cause allergies, asthma, mouth and throat infections, which makes it the focus of the attention of many scientists and the practice of using it to prove its effectiveness and great advantageousness. Therefore, to shield against pathogenic microorganisms, the immune system must be strengthened by eating dietary therapy foods containing active chemical ingredients, especially if the food is focalised on herbs, and what is medicinal, and to get prevention of diseases, and eliminate them; moreover, air disinfection factor to prevent transferring of microbes; also, surface sterilize agent to provide a sanitized environment. The presence of the biochemical components differs from one plant to another and from one species to another; this depends on the genetic factors as well as on the place and conditions that the plant lives in. The functional groups, compositions and structures of chemical compounds play an important role in determining their activity against microbes, hence the distinction of each plant from another in its activity against one type of these microbes. For instance, the presence of phenolic groups exists in plant extract will cause an effect on microbes [7], while the fractions of essential oil will stimulate the cell membrane, will alter permeability and leakage of vital intracellular components, also the impairment of the bacterial enzyme system and cell respiration [8,9].

Health benefits and uses

Thyme is one of the main herbs in diverse fresh and cooked meals menus around the world. Usually, it's typically used in little quantities, because it has an efficacious and influential aroma and wild flavour; for the reason that, is indicating of consists of a variety of significant Ingredients and nutrients. In Libya since early times, *Thymus vulgaris L.* plant and Goat's milk was utilised to treat various diseases and people are still applied for health care and as natural remedies until now. Libyans used thyme in foods as a flavour, and further used plenty of aromatic herbs are added to the milk in the manner of forming remedies, essentially as a medicine to treat different ailments, particularly chests diseases, before-mentioned they used Goat's milk and thyme in treating chest diseases such as cough and chronic cough which may take more than one month or longer, and that is considering an inconvenience to patients. As this effective treatment is prepared in the following way, in a glass ves-

sel with a capacity of 3000 ml, 1000 ml of not boil fresh goat's milk, added to it, and then 1000g of fresh wild thyme leaves is added and mixed very well, following by 1000 ml of virgin olives oil is added, then with well mixing, and placed on the heater for three hours, from the beginning of the boil time, after that, the mixture is filtered using a suitable piece of cotton gauze. As a result, 500 ml of a thick, yellowish-oily liquid was obtained; this filtrate is then cooled and kept the product in the refrigerator at a temperature of 4 degrees Celsius until further use as a treatment by drinking it twice daily in the morning and at night. Thymol is used as a remedy concerning fungal infection of the skin like dermatophytosis, dermatophyte infection or tinea. As well as, is applied in infection to treat Hookworm Disease [10]. Goats' milk has less allergenic than other kinds of milk and discourages surplus mucous formation [11]. This green herb is attainable from wild, farms; the vase or growing in the garden at home may be used as dried, fresh, or in oil extracted from, and possesses many wellness advantages. Therefore, and due to the ease of obtaining it, its simple use and its effective, fast and positive effect, it was added to goat milk as a treatment and at the same time as a nutrient for the human body. Sometimes it is difficult for the human body to obtain all the nutrients that it needs during its daily lunch. Thyme is a good source of vitamin A, rich in vitamin C, which makes it a helpful aid for restoring health during colds. It is also a good source of fibre, manganese, copper and iron. Milk has biological, nutritional and biological value, making it an important part of the human diet. Thus, each type of milk of different kinds of animals has dissimilar biochemical and physicochemical composition. Nutrition and living conditions goats have a great impact on the various products of excellent quality that would give the biological value and the energy needed, in relative to other types of milk. The unbalanced and insufficient diet of the human body leads to many disorders, meaning that the imbalance in fat intake may be a major cause of obesity and the associated diseases such as high blood pressure and cardiovascular disease. The acid value is defined as the number of milligrams of required potassium hydroxide to neutralize the free fatty acids contained in one gram of fat. And it is that free fatty acids are formed naturally during the decomposition of triglycerides, meaning that they are considered a relative measure of rancidity.

Objective

This research study was involved for the preliminary analysis in the laboratory of some chemical constituents of thyme leave

extract (RE), as well as preparation of traditional medicinal drug (TMGT), for the treatment of some chest diseases such as asthma and for other diseases. Also, the plant aqueous extracts of thyme leaves and further the therapeutic mixture were separately tested against some types of pathogenic bacteria that may cause chest and other diseases.

Methodology

Collection of the materials required for preparation

- **Raw Goat's Milk:** The raw milk sample was milked from goats which grazed and fed on thyme plants, moreover, is in the mountainous areas surrounding the city of AlKhums, It is located 120 km to the west of the capital, Tripoli, and these areas are known for the growth of the thyme plant. From where they are milked afterwards which will significantly enhance the efficiency of qualities of milk and also yield. After very good cleaning up udder and teats of the goat (It's needed, by water to do one squirt, on each teat to flesh out any blockage and bacteria, (Take a look at that milk just collected from the first squirt of each teat, to make sure there are no blood or clumps of milk which could indicate mastitis). The fresh milk was milked from the goat and then was collected in sterile plastic utensils. Selected samples were immediately transported under suitable requirements conditions and as planned to work on it, with great care to avoid any contamination.
- **Thyme:** The fresh wild thyme was collected locally from the wilds of the mountains area surrounding the city of AlKhums, The plants were used for their phytochemical analysis, moreover for the Preparation of Thyme-Milk Goat's Treatment (TMGT). The plants collected were identified botanically in the Biology Department, College of Sciences Alkhums University of Elmergib Alkhums Libya. The healthy leaves were sorted from the plant, and washed well with tap water and then with distilled water to ensure the removal of dust and all unwanted desirable, after that the leaves were dried with a clean towel and spread in the shadows for 72 hours with occasional stirring, and then transferred to a drying oven at a temperature 40 minutes for 24. The plant sample was coarsely powdered using mortar and pestle to obtain a fine powder which was stored in opaque and sealed bottles and kept in a cool and dry place until use.
- **Virgin olives oil:** the virgin olives oil was obtained from the farms around the city of Alkhums, also.

Preparation of thyme aqueous raw extract (RE)

A finely powdered sample, 20 g was extracted with 400 ml of distilled water by using Soxhlet Extractor Apparatus. The extraction was carried out for about five hours till the colour of solvent at the last siphoning time returned colourless. The solvent was completely separated to achieve a proper dry yield by evaporating under reduced pressure using Rotary Evaporator Apparatus. The yield percentage was calculated as followed:

$$\% \text{ yield} = \text{Mass of Extract} / \text{Mass of Leaves} \times 100$$

Preparation of thyme-milk goat's treatment (TMGT)

Preparation method

In a beaker with a capacity of 3000 ml, 1000 ml of fresh goat's milk was added to it, and then 1000 g of fresh wild thyme leaves were added and mixed very well, followed by 1000 ml of virgin olives oil were added with well mixing, and placed on the heater for two hours, from the beginning of the boil time, after that the mixture was filtered. As a result, 500 ml of a thick, yellowish-oily liquid {Thyme-Milk Goat's Treatment (TMGT)} was obtained. And keep the product in the refrigerator at a temperature of 4 degrees Celsius until further use.

Measuring of pH value

The pH value was measured at $24 \pm 2^\circ\text{C}$ using a pH meter with a combined electrode. The obtained result for each RE and TMGT were exhibited in table 1.

Measurement of acid value (AV)

The acid value was expressed concerning oleic acid throughout the experiment.

Acid value (AV) is defined as the mg of KOH required to neutralize the free acids existent in 1g of fat or oil. The AV is often used as a quality indicator in frying oils, where a limit of 2 mg KOH/g oil is sometimes used. = 1.3 mg.

Extraction of fatty oil

A sample of raw goat's milk was centrifuged, filtered off, dried and pulverised, from this, 20g was sifted through the No.2 sieve, after that, extracted by soxhlet extractor with 400 ml of n-hexane for about 3 hours, cooled, filtrated and evaporate the solvent to dryness at reduced pressure in a rotary evaporator, at finally, the fatty oil Collected.

Preparation of reagents

- **Phenolphthalein indicator:** 1g of phenolphthalein was dissolved in 100 mL of ethanol.
- **Sodium hydroxide titrant:** 4.0g of sodium hydroxide was dissolved with 1000 mL of distilled water in a volumetric flask.
- **Ethanol-ether solution:** The ethanol solution is prepared with dimethyl ether immediately before use. Where that, (1:1, v/v) a mixture of ethanol and diethyl ether and neutralize with sodium hydroxide titrant and add 1.0 mL of phenolphthalein indicator until pink colouration is observed.
- **Standardization of sodium hydroxide titrant:** 1.2g of potassium hydrogen phthalate, was dried to constant weight in an oven at 105°C , then in a conical flask was dissolved with 50 ml of distilled water, and shake well. 4 drops of phenolphthalein indicator was added.

This solution was titrated with the sodium hydroxide titrant till the pink colouration was observed. Where, towards the completion of the titration, potassium hydrogen phthalate was completely dissolved. The concentration of the sodium hydroxide titrant was calculated according to the following equation:

$$C_{\text{NaOH}} = \frac{W_{\text{C}_8\text{H}_5\text{K}_4} \times P_{\text{C}_8\text{H}_5\text{K}_4} \times 1000}{V_{\text{NaOH}} \times \text{Mw}_{\text{C}_8\text{H}_5\text{K}_4}}$$

Where: C_{NaOH} = Molarity of sodium hydroxide titrant (mol/L), $W_{\text{C}_8\text{H}_5\text{K}_4}$ = Weight of potassium hydrogen phthalate used (g), $P_{\text{C}_8\text{H}_5\text{K}_4}$ = Purity of potassium hydrogen phthalate (%), V_{NaOH} = Volume of sodium hydroxide titrant used (mL) and $\text{Mw}_{\text{C}_8\text{H}_5\text{K}_4}$ = Molecular weight of potassium hydrogen phthalate = 204.22g.

Titration of test solution

Weighed the accurate quantity of the fatty oil being examined as shown in Table 1 and mixed with 50 mL of ethanol-ether solution was placed in a 250-mL conical flask. And then, shake the mixture well with heating until melting the mixture was put on the shaker, then heated under the reflux apparatus until dissolved completely, then, was titrated with sodium hydroxide titrant to pink colouration was seen which continues to 30 s. The volume of used sodium hydroxide titrant was measured and the acid value was calculated according to the following equation:

$$\text{Acid value} = \frac{V_{\text{NaOH}} \times 5.61}{w}$$

where: V_{NaOH} = Volume of used sodium hydroxide titrant (mL), W = Weight of the *fatty oil* which was measured (g).

Phytochemical screening

Chemical experiments were achieved on the plant's aqueous raw extract (RE) and the soft powdered samples by using standard methods to identify the components as described by Harborne (1973), Trease and Evans (1989), Sofowora (1993), [12-15].

Test for tannins and phenolic compounds

- **By magnesium and hydrochloric acid (HCl) reduction:** few scattered fragments of magnesium ribbon were added to a solution of 50 mg the extract was previously dissolved in 10 ml of alcohol, then in dropwise the concentrated of HCl were added, the developer of pink to the crimson colour indicated the presence of flavonol glycoside's [12].
- **By alcoholic ferric chloride test:** 4 drops of neutral 5%, ferric chloride solution were added to a solution of 50 mg the extract was previously dissolved in 10 ml of alcohol, the dark green colour indicates the presence of a phenolic compound [14].
- **By aqueous ferric chloride solution:** 50 mg of the extract was dissolved in 5 ml of deionized water and then treated with 3 drops of ferric chloride solution. A greenish-brown precipitate indicated the presence of tannins [12].
- **By lead acetate test:** 3 ml of 10% lead acetate solution was added to a solution of 50 mg the extract was previously dissolved in 10 ml of distilled water, a large white precipitate indicates the presence of phenolic compounds.
- **By alkaline reagent test:** a yellow fluorescence indicates the presence of flavonoids when was added to an aqueous solution of the extract was treated with 10% ammonium hydroxide solution.
- **By gelatin test:** 2 ml of 1% solution of Gelatin containing 10% NaCl were added to a solution of 50 mg the extract was previously dissolved in 10 ml of distilled water, white precipitate indicates the presence of phenolic compounds [15].

Saponification test (fats and fixed oils)

5 drops of 0.5 N alcoholic potassium hydroxide solutions were added to 5 ml of extract along with 1 drop of phenolphthalein indicator. Then the mixture was heated in a water bath for one and half hours. The partial neutralization of alkali or soap formed confirms

the excess of fats and fixed oils [16].

Test for amino acids

50 mg of the extract was dissolved in 5 ml of distilled water and filtered through Whatman No. 1 filter paper then the filtrate was investigated to check for Amino acids by a Ninhydrin test, where 2 drops of Ninhydrin solution (5 mg of Ninhydrin in 100 ml of acetone) were added to 1 ml of aqueous filtrate. The appearance of purple colour shows exists of amino acids [17].

Test for alkaloid: (Mayer's, Wagner's reagent)

5g Thyme of leaves ground powder was well mixed with 50 ml of chloroform, where a required porridge texture was obtained. Then this was mixed with 10 ml of ammonia-chloroform solutions (0,05 N), after that, the mixture was filtrated, and the filtrate was very carefully treated with a 2 N H_2SO_4 5 ml and strongly was shaken a while, two layers were formed after 4-6 minutes which separated, the upper layer was a solution in H_2SO_4 and another one was solution solved in chloroform and was stored for the terpenoid test, later, by adding 1-2 drops of Mayer's reagent on the side of a test tube, a yellow precipitate indicated the presence of alkaloids. While, when 5 ml of the filtrate, was treated with 2 ml of Wagner's reagent on the side of the test tube. A raise of yellow or brown precipitate indicated the presence of alkaloids. Whereas, when 5 ml of the filtrate, was treated with 2 drops of Dragendorff's reagent, was added by the side of the test tube, an obvious orange or yellow precipitate indicated the presence of alkaloids [18].

Test for Flavonoid: (sianidin test, Shinoda test)

1g Thyme of leaves ground powder was well mixed with 15 ml of methanol, this mixture was filtrated off, where a required extract was obtained and then heated for about 5 minutes. After that 5 drops of concentrated HCl were added and followed by the addition of a few amounts of magnesium powder. The changing colour into red or pink indicated the presence of flavonoids [19].

Test for steroids and triterpenoids

0.5 ml of the extract solution was mixed with 1ml of chloroform and then concentrated Sulphuric acid was added carefully and slowly on the sides of the test tube. A reddish-brown colour (steroidal ring) at the interface and turning the upper layer to green indicated the presence of steroids, while the formation of deep red colour indicated the presence of triterpenoids [12,20].

Quantitative analysis:**Flavonoid's determination**

5g of plant powdered is mixed with 50 ml of aqueous methanol (80%) and was left to stand for 24 hours at room temperature. This extraction was replicated three times and each time with filtered off by using a Whatman filter paper number 42 (125 mm) and transferring into a crucible and dried on a water bath for evaporation. After cooling the crucible in a desiccator was weighed the obtained flavonoids were and percentage yield was calculated as [21]:

$$\% \text{ Flavonoid} = \text{Weight of flavonoid} / \text{Weight of sample} \times 100 \text{----- (1)}$$

Alkaloid's determination

Quantitative estimation of alkaloid was carried out by mixing 5g of thyme powder with 400 ml of 10% acetic acid in ethanol. Then, allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume then the addition of concentrated ammonium hydroxide dropwise to the extract till the precipitation was finished shortly after filtration. After 3 hours of mixture precipitation, the supernatant was discharged and the precipitates were washed with 40 ml of 0.1 M of ammonium hydroxide after that was filtered off, dried in an oven and the percentage of the alkaloid is calculated as [22,23]:

$$\% \text{ Alkaloid} = \text{Weight of alkaloid} / \text{Weight of sample} \times 100 \text{----- (2)}$$

Saponins determination

The quantitative determination of saponins was carried out by mixing up 3g of the plant powder with 70 ml of aqueous ethanol (20%), whereas this mixture was heated for 4 hours at a temperature of 55°C, with continuous stirring. After the first filtering for the mixture, the extracting process of residual was repeated twice with the same condition method of the extraction process. After the merged extracts were evaporated to 30 ml over a water bath at 90°C a 20 ml of diethyl ether was added to it in a separator funnel with vigorous agitation and then left side for. It was left aside for a while to form two phases, and this process was repeated two times, and each time the ether phase was discarded while the aqueous phase was added to it 60 ml of 1-butanol and was extracted with the addition of 10 ml of sodium chloride solution and the sodium chloride phase was disposed of, and the solution was taken and heated on a water bath for thirty minutes, and the concentrated solution was transferred to a crucible and put in an oven until a constant weight was obtained, and then the percentage of the obtained product was calculated [24].

$$\% \text{ Saponins} = \text{Weight of saponins} / \text{Weight of sample} \times 100 \text{----- (3)}$$

Antibacterial activity [25-26]**Preparation of growth media**

Nutrient agar media was constituted of Beef extract 3.0g, Agar 15.0g and Peptone 5.0g. And it was served as a growth media by dissolving 40g of nutrient agar in 700ml of distilled water. After the whole dissolution, the concluding volume of the media was raised to 1000 ml by adding further distilled water. Then media were boiled using a hot plate. The pH was adjusted to 7.0 at 26°C, using 0.1M NaOH and 0.1M HCl. The required media and altogether glassware were sterilized by autoclaving at 15psi at 121 for 20 minutes. Approximately 20ml nutrient agar was plated in Petri dishes and left to solidify for 30 minutes.

Preparation of bacterial concentration

The dry crude extract which separated from the plant's aqueous raw extract (RE) by rotary vacuum evaporate was dissolved in Dimethyl Sulfoxide (DMSO) at a concentration of 100mg/1ml and filtered through 0.45 µm sterile filter membranes.

Cultivation of bacteria (Disc diffusion method)

By the disc diffusion method, this research study intended at evaluating the *in vitro* antibacterial activity of the thyme leaves aqueous extract and a thick, yellowish-oily liquid product (TMGT) against each of bacterial sorts; a one Gram-negative bacteria *Escherichia coli* and four Gram-positive bacterial sorts; *Staphylococcus aureus*, *Lactobacillus*, *Streptococcus pneumonia* and *Staphylococcus aureus*, in presence of four antibiotics types Azithromycin (Azi), Tetracycline (Tet), Gentamicin (Gen), Norfloxacin (Nor), respectively, against to each sort of pathogenic bacteria as a positive control, while DMSO and raw milk's goat as a negative control. After the media in the dishes hardened, were cultivated in each plate with a type of bacteria selected for the experiment. 50 µL of bacterial inoculum containing 10⁸ CFU/mL were spread over plates containing prepared agar, and discs 6 mm in diameter impregnated with 40µL of the raw extracts solutions (RE) 1 mg/disc and also, 40µL of the TMGT were placed on the surface of the media, separately (Meaning that in each Petri dish previously cultured with a type of bacteria, separately, where the discs were placed in it as follows: a saturated disc containing 40µL of the aqueous extract of thyme leaves "RE", the disc saturated with 40µL a thick, yellowish-oily liquid product "TMGT", the discs of appropriate antibiotics that affect

the bacteria already cultivated on the Petri dish, a saturated disc containing 40µL of DMSO and a saturated disc containing 40µL of distilled water used in the extraction of thyme leaves, separately). Too, the two control discs were used containing DMSO and raw milk's goat while 40 µg/ disc of each type of antibiotics were used as negative and positive controls, correspondingly. And then all the dishes were incubated in the incubator at a temperature of 37 °C for 24 hours. The diameter of the zone of inhibition revealed by the extract was measured and correlated with the standard. Each sample was used in triplicate for the assaying of antibacterial activity.

Note: (Currently, the evaluation of TMGT without wild thyme leaves extract is abandoned in this research work, which is now under progress for prospective studies to be conducted meanwhile to obtain a comprehensive comparison of its effect and efficacy against antimicrobial applications and as diseases treatments.)

Results and Discussion

Prepared solution	Shape	Colour	Taste	Odour
RE	Dry-Resinous	Brown	Thyme	Thyme
TMGT	Thickly-Oily	Yellowish-oily	Thyme with a little bit of brackish	Thyme

Table 1: Results of physical state parameters of RE and TMGT.

Table 1 showed the results of physical state parameters of RE and TMGT, as the shape was Dry-Resinous, colour was Brown, taste and odour were thyme, while for TMGT the shape was Thickly-Oily, colour was Yellowish-oily, while the taste was thyme, with a little bit brackish and with the usual thyme scent.

In the human diet, fats and oils are of utmost importance due to the high percentage of essential and essential fatty acids needed by the human body tissues. Due to the ease of digestion of goat milk for its nutritional value, it was recommended for children, the elderly and sick people, with caution due to its fat content. As well, measuring the acidic value of raw goat's milk to know the percentage of polyunsaturated fatty acids it contains. Also, the high content of fatty acids in goat milk may be the cause of rancidity during its storage period. That affects the quality of the milk and leads to a specific odour due to the decomposed components such as peroxides, free fatty acids, fatty oils, the production of low molecular weight ketones low molecular weight aldehydes. As shown in

Table 2 the results of yield and pH of RE was 89, 6.7 %, separately, while the pH and Acid Value for TGMT were 7.3 and 7, individually. And the acid value of raw goat's milk was 6.7g. As well many of the monounsaturated like oleic acid and polyunsaturated fatty acids such as ruminic acid that exists in the milk have advantageous influences on human wellness. Excessive consumption of foods containing high in fats has a major role in causing diseases such as vascular disease, cancer, heart disease, diabetes, obesity, and others. And because milk fat is considered one of the complex natural fats, it contains about 500 types of these acids, it is well known that monounsaturated fatty acids do not cause cholesterol aggregation and convert into rancid, as is the case with polyunsaturated fatty acids.

Prepared solution	Yields (%)	pH Value	Acid Value (g)
RE	89	6.7	-
Raw Goat's Milk	-	-	6.7
TMGT	-	7.3	-

Table 2: Results of yield, pH and Acid Value of RE and TMGT.

Phytochemical analysis

Distinct researches revealed that the involved terpenes in the milk show the involved terpenes of the ingested feed. And this means that antioxidant composites such as terpenes are transmitted to the milk able to increase the oxidative stabilizing of milk.

Qualitative phytochemical composition

Phytochemicals	Existence of Phytochemicals
Tannins and Phenolics	+++
Fats and Fixed oils	+++
Amino acids	+++
Alkaloids	+++
Flavonoid	+++
Steroids and Triterpenoids	+++

Table 3: Qualitative screening of phytochemicals from aqueous extract of Thyme leaves.

Note: Strong positive test = +++, Moderate Positive test = ++, Weak positive test = +, Negative tests = -

The result in table 3 indicated the presence of the phytochemical secondary metabolites in abundance amounts, which are tannins, phenols, Fats and Fixed oils, Amino acids alkaloids, flavonoids

and steroids and triterpenoids. Accordingly, the preliminary phytochemical examination revealed that the aqueous extract of thyme leaves contains bioactive ingredients and are responsible also for antioxidant activities. Traditionally thyme was used for healing several respiratory ailments such as bronchitis and asthma, also were used for the medication of other illnesses like antispasmodic, antiviral, antimicrobial and antiseptic. This is promoting, by it owing bioactive chemical ingredients activity existent in it. Thyme is combined with other herbs to treat ailments like sore throat, bronchitis and also such as gastritis disorders. Because the Thyme is benefited in health-promoting properties and disease-preventing related to its rich nutritional value is vital for great health, and these nutrients of this aromatic herb such as vitamins, minerals and that considered as phytonutrients. Thyme is likewise a great source of Vitamin C which provides resistance against infectious illnesses and attacks harmful pro-inflammatory free radicals, also principally Vitamin A is an antioxidant, essential for maintaining healthful mucus membranes, for good vision and skin [27]. The risk of genetic diseases such as diabetes, heart disease and cancer can be reduced by consuming foods that contain herbs that contain biologically active compounds.

Plant's Name	Flavonoids (%)	Alkaloids (%)	Saponins (%)
Thyme	81	72	90

Table 4: Results of the Quantitative Phytochemical Composition.

The percentage yields obtained from the leaves concerning quantitative phytochemical analysis were for Flavonoids 81%, Alkaloids 72% and Saponins 90%. The usefulness benefits gained from healthy foods, especially those that plants and herbs share in their components nutrients, even if they are flavoring's or natural additives to improve the quality and more acceptance for food, resulting from the fact that they contain components that have benefits for the human body, whether for treatment or even for disease resistance and prevention, such as the effectiveness of antioxidants through a potential source. Additionally, fresh thyme contains many vital compounds such as flavonoids, tannins and phenolic antioxidants. Moreover, it is rich in vitamins, selenium, magnesium, manganese, iron, calcium and potassium. While the main phenolic antioxidant components are thymol which is for antioxidant activities. Thyme contains saponins, which interact with cholesterol in the human body to form insoluble complexes, as well as prevent the intestines from absorbing endogenous and exogenous cholesterol [28].

Fatty oil

Supposed acid value ml	Weight of fatty oil being examined/g
10	4
50	2
100	1
200	0.5

Table 5: Resulted of Weight of fatty oil being examined.

For more illustration of that, an acid value which is described as the number of mg of potassium hydroxide needed to neutralize the free acid in 1g of fat, fatty oil or other related materials is examined to evaluate the rancidity of the Raw Goat's Milk sample. And according to table 5 the expected acid value of 10, 50, 100 and 200 ml were 4, 2, 1 and 0.5 for the weight of fatty oil existence investigated/g. Milk fat is the all mostly are complex natural fats that include about 400-500 fatty acids [29].

Antibacterial activities

The human body is infected with many diseases, they may be caused by microorganisms such as bacteria, viruses, fungi, etc., Where bacteria can live inside the human body or on his skin and some of them are good for human health, and it is referred to as healthy bacteria, some of which are damaging to the human body and are referred to as harmful or pathogenic bacteria. A proposal that structurally likes thymol and carvacrol already found in thyme enhances the permeability of the bacterial cell wall, therefore describing the antimicrobial effect of the thyme [30]. The presence of bacteria in raw goat milk may be due to the cultivation system and thus the lunch provided to the goats, is it organic or traditional and the year, the milking, the place, and the farms of the goats were living in. Also, the existence of pathogens bacteria depends on the wellness condition of the goats and varies greatly between milk produced farms. That is, based on the quality system used in each farm.

In table 6 the results of the bioactivity of Thyme aqueous raw extract (RE) alongside each of bacterial sorts; *Escherichia coli*, *Staphylococcus aureus*, *Lactobacillus*, *Streptococcus pneumonia* and *Staphylococcus aureus*, were revealed 19, 20, 21, 19 and 23 mm, respectively. While the liquid product TMGT showed 23, 22, 25, 24 and 25 mm correspondingly, And in presence of the antibiot-

ics types Azithromycin (Azi.), Tetracycline (Tet.), Gentamicin(Gen.), Norfloxacin (Nor.) were 26, 25, 28, 26 and 27, individually, which used against to each sort of pathogenic bacteria as a positive control. Whereas the results showed that the effectiveness of the TMGT was higher than using RE and that the inhibition zone was against *Staphylococcus aureus* 25 mm compared to the antibiotic against this type of bacteria, which had an inhibition zone of 27 mm. Also, when comparing the results obtained, there is no considerable difference in the resistance of bacteria by TMGT, besides, the results of the RE against bacteria were very good when compared to the used antibiotics. Furthermore, from a comparison between RE and TMGT, we find that the results obtained are very good, which may encourage its use as a treatment against some diseases arising from those bacteria tested in this previous test. The activity of thyme is assumed to be correlated with the great presence of secondary metabolite constituents. Linalool and Carvacrol, β -pinene, α -terpineol, camphor, borneol, and thymol compounds are played a very important role as bioactive compounds against bacteria [31-33]. Additionally, Thyme consists of essential oils which are used in foods and beverages as flavour enhancements. Consequently, the existence of vital several constituents such as carvacrol, cymene and thymol are known to own antimicrobial properties. As previous studies confirmed that the presence of essential oils in thyme has an effective effect on stopping the increase in the number of cells of pathogenic microorganisms, especially when used as a milk sterilizer. Accordingly, may also, that inhibitory effects to the microorganisms revealed in this research study were by the occurrence of the thyme secondary metabolites ingredients including essential oils and which possess the potential to inhibit and inactivate five tested pathogenic microorganisms which appear more clearly in comparison between the results of RE and TMGT.

Name of Bacteria	Inhibition diameters zones size (mm)					
	RE	TMGT	Antibiotic			
			Azi.	Tet.	Gen.	Nor.
<i>Escherichia coli</i>	19	23	26	-	-	-
<i>Staphylococcus aureus</i>	20	22	-	25	-	-
<i>Lactobacillus</i>	21	25	-	-	28	-
<i>Streptococcus pneumonia</i>	19	24	-	-	-	26
<i>Staphylococcus aureus</i>	23	25	-	27	-	-

Table 6: Results of antibacterial Activities.

Antibiotics types: Azithromycin (Azi), Tetracycline (Tet), Gentamicin (Gen) and Norfloxacin (Nor).

Milk and dairy products are rich in essential nutrients, vitamins and proteins which are considered one in every of the vital nutritional components for human health. On the opposite hand, it's worth noting that if the milk contains a high percentage of steroids or antibiotics, this milk must be discarded, due to its harmfulness and also the concern it causes on the endocrine glands within the physical body, which results in inequality within the balance. Hence, the evaluation of hormones in milk as likely endocrine-disrupting composites is critical. Where normally steroid hormones exist in milk as combined forms which don't seem to be biologically effective, merely within the human system digestorium can be converted toward their free forms, therefore, it's necessary for healthy milk to contain steroid hormones at low levels which are during a safe and normal range.

Conclusion

This research was preliminarily investigated some active compounds contained in the aqueous extract of thyme leaves, and also the use of the leaves in preparing the therapeutic mixture according to the traditional methods used in preparing the prescribed herbal medicine and testing it against some types of pathogenic bacteria. Furthermore, were needed to the great inhibitory activity against pathogenic microorganism which could be achieved by using different concentrations of milk and plants crude extracts, which were needed to accomplish more bactericidal effects and to exhibit strong antibacterial activity, further researches are needed to determine their antibacterial effects on pathogens both in different milk types and the human body too. The medicinal applications of thyme related to main Biological Compounds such as phenols, carvacrol, thymol, which are acts as antimicrobial, expectorant, antioxidant, spasmolytic, mucolytic and antitussive. Positively, these components in the thyme make it is more active against different sorts of bacteria and can inhibit its growth bacteria.

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Competing Interest

The authors declare that they have no competing interests.

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