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EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF *THYMELAEA HIRSUTA* (L.) ENDL, AND THAT UTILISED AS A CONVENTIONAL TREATMENT OF INFERTILITY AND DIABETIC IN LIBYA.

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ABSTRACT

Thymelaea hirsuta (L.) Endl is used in Libya, as a folk medicine for the medication, firstly traditionally used to treat infertility, and secondly used for another disease's treatment such as diabetics. The results of qualitative phytochemical screening it is determined that the plant containing a rich amount of secondary phytoconstituents metabolites such as soluble starch, resin, balsam, flavonoids, tannins & phenols, diterpenes, triterpenes & sterols, saponins, alkaloids, polypeptides (proteins & amino acids), cardiac glycosides, carbohydrates, volatile oils, and fixed oils and fats were present in the plant extracts, while each of anthraquinones and phlobatanins were not present. Quantitive evaluation, the percentages yields of each of aqueous and ethanolic extracts were 89.3, 93.20 % respectively,while the percentages yields of each of flavonoids, saponins and alkaloids 35.23, 29.41, 39.72, 89.3, 93.20 % correspondingly. The effectiveness of aqueous and ethanolic extracts of the *Thymelaea hirsuta* (L.) Endl plants against the bacterial strains, where the crude plant extracts have good inhibitors against bacterial and it was among 8 to 12 mm. In addition, the aqueous and ethanolic extract has the highest inhibition zone 12 mm against *Escherichia Coli*, while each

of *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were 11 mm, though, 10 mm were from aqueous extract against each *of Pseudomonas aeruginosa* and *Staphylococcus Epidermis*, though 9 mm were from aqueous and ethanolic extracts against each of *Klebsiella pneumonia* and *Staphylococcus Epidermis*, and 8 mm were from aqueous and ethanolic extracts against each of *Proteus vulgaris* and *Staphylococcus Aureus*, respectively.

KEYWORDS: *Thymelaea hirsuta* (L.) Endl, Secondary Phytoconstituents Metabolites, Quantitive Evaluation, Antibacterial, Infertility.

INTRODUCTION

Thymelaea hirsuta (L.) Endl, is an endemic plant desert species in Libya is growth in the Green Mountain and also West Mountain regions and belonging to the Thymelaeaceae family. *Thymelaea hirsuta* (L.) Endl is called locally in Libya by "Al Methnan or Azaz" is a small an evergreen shrubs and its length about a meter. Likewise, have its place to the flowering plant family Thymelaeaceae, which is native to the Mediterranean region. This species of this genus were served in conventional medication because of having unusual biological activities, for instance, antimicrobial, antidiabetic, anti-inflammatory, antioxidant activity and antihypertensive.[1,3] Because of the presence of many active compounds which have a great role in the prevention and treatment of many diseases, this plant is given great importance to study. Reproductive and urinary system diseases are considered one of the difficulties of now medical sciences which their medication is significant. Thus the medicinal plants are not only a nutritious source but also are holding a potential reservoir of medicines and supplements which useful for human health. In ancient time and nowadays the important remedies values of medicinal plants has attracted considerable care in many countries including Libya where rich sources of some kinds of plants are available. Where exposed people to several agents in job and living environments that could endanger their reproductive system function. In Libya, as a folk medicine for the medication is used firstly traditionally to treat infertility, and secondly used for another disease's treatment such as diabetics, dermatitis, hair-fall, constipation, vermicide, warts, herpes, sterility, and psoriasis. In addition, has Antihyperglycemic effect, and the hyperglycemia via a partial inhibition of the glucose gut absorption.[4,9] The medicinal significance of this plant is due to the existence of secondary phytoconstituents metabolites that have a sure physiological purpose in the human body. Altered phytochemicals were originated to have an extensive variety of medicinal possessions. Therefore, the healing life-sustenance of and healthy good kinds of

nutrients are essential for the creation of healthy sperm and can increase semen parameters. Furthermore, significant nutrients in the male reproductive system for proper hormone metabolism, like motility and sperm production.

METHODS AND MATERIALS

Collection and identification of plant materials

Fresh leaves of *Thymelaea hirsuta* L. Endl were collected from the West Mountain east of Libya in 2018. The plants were identified by the Department of Biology, Science College University of El-Mergeb Al-Khums, Libya. The collected samples washed by tap water then by distilled water and dried in shadow for 4 days and the drying was completing in an oven at 45 0C. The samples of dried leaves were crushed into the fine powder using a grinder in consequence packed in sterilized dark polyethene bags until further use.

Family	Scientific name	Local name
(Thymelaeaceae) Theligonaceae	*Thymelaea hirsuta* (L.) Endl.	Methnan or Azaz

EXTRACTION OF PLANT MATERIALS

The aqueous and ethanol extracts of the fine powdered of leaves of *Thymelaea hirsuta* (L). Endl were prepared, separately, by Soxhlet were 20 g of the dry powdered plant material in 40 mL with appropriate solvents (Distilled water, Ethyl Alcohol) for 6-8 hours. The extracts were filtered by using a Whatmann filter paper. The extracts were concentrated using a rotary vacuum evaporator with the water bath set at 44 - 45˚C. A dark green sticky substance was obtained from ethanolic extract, while the aqueous extract has a dark brown sticky substance was obtained. The percentage yield of extracts were 89.3 and 93.20 %w/w, respectively. Where crude extracts were stored in the refrigerator at 4˚C until further use.

PHYTOCHEMICAL SCREENING

QUALITATIVE SCREENING

Phytochemical screening: The crude extracts were qualitatively tested for the presence of various secondary metabolites using standard established methods.[10,18]

SOLUBLE STARCH TEST

The crude extract of 0.2 gm. was boiled in 1 mL of 5% KOH, cooled and acidified with H_2SO_4. Yellow colouration indicates the presence of soluble starch.

RESIN TEST

TURBIDITY TEST

The Resinous drug is insoluble in water, 1mg of extract mixed with 4-5 mL of ethyl alcohol and then about 3-4 mL of water was added in excess to form turbidity indicated the presence of resins. **$FeCl_3$ test:** 5 mL of crude extract was mixed with 10 mL of ethyl alcohol then 3-4 drops of $FeCl_3$ solution. Formation of greenish-blue colour indicated the presence of resins. **HCl test:** 0.5 gram of the crude extract was mixed with 10 mL of acetone and 5 mL of dilute HCl was added. Formation of pink colour after warming the solution in a water bath for 30 minutes indicated the presence of resins.

Balsam test: (Alcoholic $FeCl_3$)

5mL of crude extract was mixed with 2-3 drops of alcoholic ferric chloride solution. A green colour indicates the presence of balsam.

FLAVONOIDS TEST

Alkaline reagent test: 5 mL crude extract was mixed with 2mL of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on the addition of a few drops of diluted hydrochloric acid which indicated the presence of flavonoids.

Lead acetate test: 2 mL of 10% lead acetate solution was added into 2 mL of the petroleum ether crude extract then. And allowed to stand for 1 minute after which the mixture was observed for colour change usually yellowish colouration and precipitate formed.

Phlobatanins Test: 5 mL of 1% HCl was added to 2 mL of crude extract and then was boiled. Deposition of a red precipitate indicated the presence of phlobatanins.

TANNINS & PHENOLS TEST

Ferric Chloride: 10 g of the crude extract was mixed with 20 mL of distilled water in a test tube then boiled. After, that filtrate was treated with a few drops of 0.1% ferric chloride. Observed for brownish green or a blue-black colouration indicate the presence of tannins.

Ellagic acid test: 10 mL of crude extract was mixed with 5mL of 5% glacial acetic acid then 5mL of 5% sodium nitrite was added mixed well. A muddy brown colour appears which indicates the presence of phenols.

DITERPENE TEST

Copper Acetate Test: 10 mL of crude plant extract is treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Triterpenes and sterols test: (Liebermann- Burchard)

About 0.1 g of the crude extract was dissolved in 1 mL acetic anhydride and then in 0.5 mL of chloroform was added. Formerly 1 mL of concentrated H_2SO_4 is added. At the contact zone of the two liquids, a brownish-red ring was formed indicating the presence of sterols and triterpenes.

Terpenoids Test (Salkowski's test)

0.5 g each of the extract was mixed with 2 mL of chloroform. 3 mL of concentrated H_2S0_4 was carefully added to form a layer. A reddish-brown colouration of the interface indicates the presence of terpenoids.

Saponins Test

Foam test: About 50 mg of the extract was dissolved in 20 mL. of distilled water. The suspension was shaken in a graduated cylinder for 15 minutes. Creation of Foam indicates the presence of Saponins.

Frothing test: 1 g of the plants powdered was placed in a test tube and 10 mL of distilled water was added and shaken vigorously for 1min. It was then allowed to stand for 30 min and observed. Formation of honeycomb froth indicates the presence of saponins.

Alkaloids Test

5 mL of extract treated with 3-4 drops of Dragendorff's reagent, appearance appear in visible light in the form of orange colour.

POLYPEPTIDES (proteins & Amino Acids) TEST

5 mL of the extract were added to 3 mL of (0.25% w/v) ninhydrin reagent and boiled for a few minutes. Formation of a blue colour indicates the presence of amino acid.

Anthraquinones Test

0.5 g of the extract was mixed with 10 mL of sulphuric acid (H_2SO_4) then boiled after that filtered while hot. The filtrate was shaken with 5 ml of chloroform and then the chloroform

layer was separated and 1 mL of dilute ammonia was added. The resulting solution was observed for colour changes.

GLYCOSIDES TEST

Sodium Nitropreside: 5 mL of the crude extract were treated with sodium nitropreside in pyridine and sodium hydroxide. Formation of pink to red colour indicates the presence of Cardiac Glycosides.

Killer Kiliani test: 10 mL of each crude extract was mixed with 8 mL of glacial acetic acid and a drop of ferric chloride solution. Carefully, concentrated sulphuric acid was added along the side of the test tube, a reddish-brown colour at the junction of two liquid and bluish-green on upper layer indicating the presence of cardiac glycosides.

Borntrager's test: 10 mL of the crude extract mixed with 3-4 drops of diluted Sulphuric acid, then boiled for 5 minutes and filtered. In the cold filtrate, an equal volume of benzene was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red colour in the ammoniacal layer indicates the presence of Anthraquinones glycoside.

CARBOHYDRATE TEST

Fehling's Test: An Equal volume of Fehling solution A and Fehling solution B are mixed and a few drops of the sample are added and boiled, a brick red precipitate indicates the presence of reducing sugar.

Molisch's Test: Treat the 2 mL of the test solution with a few drops alcoholic α-naphthol solution in a test tube and the1 ml of concentrated Sulphuric acid was added carefully along with a side of the test tube. Formation of the vitrioled ring at the junction indicates the presence of carbohydrates.

VOLATILE OILS TEST

Odour test: The ether extract was evaporated to dryness. The residue had a characteristic and distinguishing odour, thus the plant product contains volatile oils.

Petroleum ether test: 5 mL of petroleum ether crude extract was mixed with 1 mL of diluted sodium hydroxide followed by 5 mL of hydrochloric acid. This was then allowed to stand for 5 seconds and observed for a light blue colour change and precipitate formed.

Fixed Oils and Fats test: (spot test): A small quantity of plant powder was pressed between two filter papers. Oil strains on the filter paper indicated the presence of fixed oils.

QUANTITIVE SCREENING

The quantitive phytochemical screening was carried out via the crude extracts were quantitatively evaluated for the various secondary metabolites using standard established methods.[19,21]

DETERMINATION OF FLAVONOID

Into 500 mL empty beaker a 5 g plant powder was mixed with 100 mL of 80% aqueous methanol, enclosed, and permissible to stand for 24 hours at room temperature. After that separate, the supernatant and the residue was re-extracted (three times) with a similar volume of ethanol. Whatman filter paper number 125 mm was used to filter the complete solution of each sample. All sample filtrate was far along moved into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until a constant weight was gained. The percentage yield of flavonoid was calculated as:

Flavonoid's Yield % = Weight of flavonoid / Weight of sample × 100.

DETERMINATION OF SAPONINS

Into 500 mL conical flask a 10 g plant powder was mixed with 200 mL of 20% of ethanol, the mixture was heated over a hot water bath for 4 hours with incessant stirring at 55 ^{o}C. The mixture is then filtered and the residue re-extracted with another 200 mL of 20% ethanol. The collective extracts were reduced to 80 mL over a water bath at about 90^{o}C. The concentrated is then moved into a 500 mL separating funnel and 20 mL of diethyl ether is added to the extract and vigorously shaken. The aqueous layer is recovered while the diethyl ether layer is castoff and the separation procedure is repeated. 60 mL of 1-butanol is added and the combined 1-butanol extracts were washed twice with 10 mL of 5% sodium chloride. The residual solution is then warmed in a water bath and after evaporation; the samples are dried in the oven to a constant weight. The percentage yield of flavonoid was calculated as

Saponins Yield % = Weight of Saponins / Weight of Sample × 100.

DETERMINATION OF ALKALOIDS

Into 500 mL empty beaker a 5 g plant powder was mixed with 400 mL of 10% acetic acid in ethanol, and allowed to stand for 4 hours. the extract was concentrated on a water bath to one-quarter of the original volume after that addition of concentrated ammonium hydroxide

drop wise to the extract until the precipitation was complete directly afterwards filtration. Subsequently, permissible to stand for 3 hours for suitable mixture precipitation, the supernatant was castoff and the precipitates were washed with 40 cm^3 of 0.1 M of ammonium hydroxide and formerly filtered using filter paper 12.5 cm. the residue was dried in an oven and the percentage yield of the alkaloid is calculated as:

Alkaloid's Yield % = Weight of alkaloid / Weight of sample × 100. (2) 2.5.3.

ANTIBACTERIAL ACTIVITIES

Microbial Strains

Bacterial strains were selected among (negative Gram bacteria: Klebsiella pneumonia, Escherichia coli, Proteus vulgaris and Pseudomonas aeruginosa, positive Gram bacteria: Staphylococcus aureus, Staphylococcus Epidermis. These Bacterial strains were provided from the laboratory of Al-khums Teaching Hospital, Libya, and examined against the Thymelaea hirsuta (L.) Endl, aqueous and ethanol extracts.

Preparation Inoculums

A roomful of strain was inoculated in 30 mL of nutrient broth in a conical flask and incubated on a rotary shaker at 37º C for 24 hours to activate the strain.

Antimicrobial Assay

The antimicrobial evaluated used as the standard Agar Disc Diffusion Assay adapted from.[22] Mueller Hinton Agar was prepared for the study. Mueller Hinton agar plates were swabbed with a suspension of each bacterial species, using a sterile cotton swab. Then, the sterilized filter paper discs (6mm) were wholly saturated with the test compound (40 µl). The impregnated dried discs were placed on the surface of each inoculated plate. The plates were incubated overnight at 37ºC, 24 h., plant leaves were tested against each organism in triplicate. Sterile filter paper discs (6 mm diameter) were dripped with tested extracts to load 40 µl of a given extract per disc and were located on the agar plates consistently inoculated with the test microorganisms. The paper discs with aqueous (distilled water only) and 90% ethanol were used as a negative control. Ciprofloxacin, Amoxicillin, Penicillin, Tetracycline, Augmentin and Ceftriaxone were used as a positive control (~300µg/disc). The diameter of the clear zone surrounding the disc after 24 h incubation at 37 ºC was the measure (millimeters) of antimicrobial activity of given extracts.

RESULTS AND DISCUSSION

Qualitative screening

Quantitative Phytochemical Screening of *Thymelaea hirsuta* (L.) Endl.

Table 1: Quantitative Phytochemical Screening of *Thymelaea hirsuta* (L.) Endl.

Name of the plant	Percentage Yields (%)				
	Flavonoid	Saponins	Alkaloids	Aqueous Extract	Ethanol Extract
Thymelaea hirsuta (L.) Endl	35.23	29.41	39.72	89.3	93.20

The percentages yields of each of aqueous and ethanolic extracts were 89.3, 93.20 % respectively, while the percentages yields of each of flavonoids, saponins and alkaloids 35.23, 29.41, 39.72, 89.3, 93.20 % correspondingly. Flavonoids, saponins and alkaloids are significant secondary metabolites and are responsible values for medicinal principles of the *Thymelaea hirsuta* (L.) Endl. The Saponins reason for a decrease of blood cholesterol by stopping its reabsorption which makes it beneficial in. Furthermore, saponins own anti-mutagenic activities and antitumor and will minor the danger of human tumours, by stopping tumour cells from developing. Also, saponins are supposed to respond with the cholesterol-rich membranes of tumour cells, thus preventing their development and capability.[23] Saponins have useful properties including lowering of cholesterol Level, cytotoxic permeabilization of the intestine harmful and also active in the biological efficiency.[24] While alkaloids are vital in medication and establish a maximum of the valued medicines have an effective physiological consequence.[25]

QUALITATIVE SCREENING

Table 2: Results of the phytochemical screening of the *Thymelaea hirsuta* (L.) Endl. Leaves.

Chemical Component	Tests & Used Regents	Ethanol extract
Soluble Starch	KOH	+++
	Chloroform	+++
Resin	Turbidity test	+++
	$FeCl_3$	+++
	HCl	+++
Balsam	Alcoholic $FeCl_3$	+++
Flavonoids	Alkaline reagent test	+++
	Lead Acetate	+++
Phlobatanins	HCl	-
Tannins & Phenols	Gelatin	+++
	Ferric chloride test	+++
	Ellagic acid test	+++

Diterpenes	Copper Acetate	+++
Triterpenes &sterols	Liebermann- Burchard	+++
Saponins	Foam'stest	+++
	Fretting Test	+++
Alkaloids	Dragendorff's Test	+++
Polypeptides (proteins & Amino Acids)	Xanthoproteic Test	+++
	Ninhydrin Test	-
Anthraquinones	Ammonia solution's test	-
Cardiac Glycosides	Killer kiliani test	+++
	Sodium Nitropreside	+++
	Borntrager's test	+++
Carbohydrates	Fehling's test	+
	Molisch's test	+
Volatile Oils	Petroleum Ether Test	+++
Fixed Oils and Fats	Spot'stest	++

High presence: +++, Moderate presence: ++, Low presence: + and Absences: - of phytochemical constituents.

Well-disposed to result of qualitative phytochemical screening it is determined that the plant enclosed abundant amount of secondary bioactive constituents such as Soluble Starch, Resin, Balsam, Flavonoids, Tannins & Phenols, Diterpenes, Triterpenes & Sterols, Saponins, Alkaloids, Polypeptides (proteins & Amino Acids), Cardiac Glycosides, Carbohydrates, Volatile Oils & Fixed Oils and Fats were present in the plant extracts, while each of Anthraquinones and Phlobatanins were not present. These secondary metabolites present in the leaves which could be used to remedy different ailments traditionally, as well as used to prepare medicines by pharmaceutical industries. Consequently, this plant includes large amounts of chemical constituents which that may explain its different activities against numerous microbes. Such as phenols, phenolic acids derivatives, flavonoids, tannins etc... Saponins considered as triterpenoids glycosides or steroids which are defined by their unpleasant taste and foaming properties which causing a haemolytic effect on red blood cells.[26,28] Plants yield saponins which have potentials to contest infections against bacteria, parasites and also in human's saponins functions as immune system supporter. Correspondingly, reduced risk of cancer, cardiovascular disease and heart diseases by saponins non-sugar part.[28,29] Flavonoids are well-known as responsible for coolers of vegetable and fruits in addition to considerable health-promoting effects for instance diuretics, anti-inflammatory, anti-cancer, anti-allergic, antioxidant, anti-viral and tumour inhibitory effects.[30] As for tannin having anti-diabetic properties and is promoting wound healing.[31] While the existence of phenol in the leaves of *Thymelaea hirsuta* (L.) Endl

benefits as reduces inflammation antiseptic. From these bioactive agents act as an irritant influence while treated to the skin.[32] As for the Cardiac glycosides possesses a strong effect on the heart, improves in supporting its influence and rate of contraction when the heart is failing.[32] Similarly, Steroidal composites are of significance in pharmacy due to their association with composites such as sex hormones.[33] And this encourages the use of this plant in traditional medicine by the Libyans, where they used it in the treatment of many diseases, especially in the treatment of infertility. Likewise, *Thymelaea hirsuta* (L.) Endl consists of saponins and phenolics. And according to the saponins as the manner concerning for the anti-bacterial characteristics possibly will involve owing to membranolytic action of the saponins accompanied by decreasing of the surface part tension of the extracellular medium,[34] while phenolics which they function through chelating metal ions similar manganese, cobalt where are essential as co-factors for microbial enzymes.[33,34]

ANTIMICROBIAL ASSAYING

Table 3: Results of the antibiotics and *Thymelaea hirsuta* (L.) Endl. Crude extracts against pathogenic microbes.

Bacterial Strains / Extracts & Antibiotics		*Klebsiella pneumonia* (mm)-	*Escherichia Coli* (mm)-	*Pseudomonas aeruginosa* (mm)-
Thymelaea hirsuta (L.) Endl.	Aqus. Extr.	9	12	10
	EtOH Extr.	11	12	11
Ciprofloxacin		S	S	S
Amoxicillin		S	S	S
Penicillin		S	S	S
Tetracycline		S	S	S
Augmentin		R	R	R
Ceftriaxone		R	S	R
Cefotaxime		R	S	R

R = Resistant, S = Sensitive, (mm) = millimeters

Table 4: Results of the antibiotics and Thymelaea hirsuta (L.) Endl. Crude extracts against pathogenic microbes.

Bacterial Strains / Extracts & Antibiotics		Proteus vulgaris (mm)-	Staphylococcus Epidermis (mm)+	Staphylococcus Aureus (mm)+
Thymelaea hirsuta (L.) Endl.	Aqus. Extr.	8	10	9
	EtOH Extr.	8	9	8
Ciprofloxacin		S	S	S
Amoxicillin		S	R	S
Penicillin		S	S	S

Tetracycline	R	S	S
Augmentin	S	S	S
Ceftriaxone	S	S	R
Cefotaxime	R	S	S

R = Resistant, S = Sensitive, (mm) = millimeters.

As shown in table 3 and 4 the potency of aqueous and ethanolic extracts of the *Thymelaea hirsuta* (L.) Endl plants against the bacterial strains, where the crude plant extracts have important inhibitors against bacterial and it was among 8 to 12 mm. In addition, aqueous and ethanolic extracts has the highest inhibition zone 12 mm against Escherichia Coli, while each of Klebsiella pneumonia and Pseudomonas aeruginosa were 11 mm, though, 10 mm were from aqueous extract against each of Pseudomonas aeruginosa and Staphylococcus Epidermis, although 9 mm were from aqueous and ethanolic extracts against each of Klebsiella pneumonia and Staphylococcus Epidermis, and 8 mm were from aqueous and ethanolic extracts against each of Proteus vulgaris and Staphylococcus Aureus, respectively. As well as in comparison to the antibiotics was decent susceptible to the pathogens bacteria. In accordance with results could be the *Thymelaea hirsuta* (L.) Endl which known as a traditional medicinal plant and which utilized in the treatment of human urogenital tract, infertility and in the diabetic diseases in the earliest and current time and might remain as well for that purpose played a vital role at the present time as important raw substantial for the pharmaceutical manufacturing. Numerous medicinal plants throughout the world are utilised to healing infertility and testicular disorders. In addition, Plantago belongs to Liliaceae family are caused significant increase in testosterone concentration, can help to boost infertility through improving spermatogenesis in diabetic mice and sperm number,[35, 36] Cinnamomum Darchin, Berberis integerrima belongs to family Berberidaceae caused increase in testosterone level,[37] Aloe Vera caused increase in the number of spermatogonia, Sertoli cells, and Leydig cells in the testis in the diabetic rats,[39] Salvia officinalis belongs to family Labiatae caused significant increase in serum testosterone level, seminiferous tubules diameters, and sperm number in the tubes tunnels in male rats and Foeniculum vulgare Mill, which Estrogenically activity where existence of Anatole compound will effect on reducing the menstrual pain and increasing milk secretion, primary dysmenorrhea, and infertility.[39] There are no precision suggests of how such plants function to increase the fertility inhumane body. Nevertheless, these plants often produce phenolic composites particularly flavonoid composites which together possess anti-inflammatory and antimicrobial activities. Moreover, because of flavonoids which have antioxidant activities, the great oxidative tension is more

correlated with an increase in the probability of infertility, plenty of other plants which produce flavonoid composites with antioxidant activity. The composites with antioxidant activity remain capable to regain free radicals, inhibiting organ destruction, which senses to people, believed via the experience with the application of these plants as a treatment for diseases that by the natural methods of treatment is acceptable for them. Amongst these species which utilized in this study, there are pathogens which cause genito-urinary diseases in men and women, such as Staphylococcus Aureus, Escherichia Coli and Candida spp. These organisms are existent in the human urogenital tract and effective in causing harmful.[40] Also, Escherichia Coli exhibits a sperm-agglutinating activity in vivo. Species of the genus Candida are capable to interact with sperms making decreased motility and intense alteration to their ultrastructure.[41] May also cause infertility by hampering the sperm motility, damaging sperm, harmfully affecting sperm function, changing the chemical composition of the seminal liquid, the bacteria are recognized as a reason for urethritis which is regularly complicated via infections of other organs of the genital tract, as well as the testes. Staphylococcus aureus often exists in the semen which reason infertility in men, as well as Proteus vulgaris and Pseudomonas aeruginosa cause the contamination either. Escherichia coli is one of the extreme predominant bacteria isolated from the semen samples of male's presence infertility health center.[42] Various micronutrients like Vitamins C, B12, E, Arginine, Carnitine, Selenium and Zinc played very important roles in enhancing sperm quantity and improving its potential function.[43,46] When the diabetic patients exposure to infections from some types of bacteria such as Staphylococcus aureus, Escherichia coli, Klebsiella and Pseudomonas (pneumonia) or maybe due to aerobic gram-negative bacteria and some groups of Streptococcus which may possibly this infection will increase then leads to more health complicated problems which may likewise some of these types cause diseases as a result of esophageal disorders, impaired bronchiolar reactivity. Moreover, the risk of developing complications for the diabetics is from bacteremia in Staphylococcus aureus pneumonia, with get together increasing in death possibility.[47]

CONCLUSION

It can be concluded that the selected medicinal plant (*Thymelaea hirsuta* (L.) Endl) is the source of secondary metabolites and contains considerable constituents of secondary bioactive constituents such as flavonoids, tannins & phenols, diterpenes, triterpenes & sterols, saponins, alkaloids, cardiac glycosides, carbohydrates, volatile oils and fixed oils and fats were take place in the plant extracts. These secondary metabolites present in the leaves which

could be used to remedy different ailments traditionally, as well as used to prepare medicines by pharmaceutical industries. Due to the presence of these secondary metabolites the selected medicinal plants have high healing potential which confirmed by the obtained results of the antibacterial activity.

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