Journal of Advances in Microbiology

3(1): 1-15, 2017; Article no.JAMB.33383
ISSN: 2456-7116

Antimicrobial Efficacy of Some Medicinal Plants on Different Bacterial Isolates Associated with Semen of Infertile Men

Mohamed M. S. Farag[1*], Mamdouh S. El-Gamal[1], Aziza M. A. Mansour[2], Essam H. Abd El-Shakour[1], Mohammed H. EL-Haw[1] and Alaa M. Soliman[1]

[1]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.
[2]International Islamic Center for Population Studies and Research, Al-Azhar University, Nasr City, Cairo, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2017/33383
Editor(s):
(1) P. Rama Bhat, PG Biotechnology, Alva's College, Karnataka, India.
(2) Naim Deniz Ayaz, Department of Food Hygiene and Technology, Kirikkale University, Kirikkale, Turkey.
Reviewers:
(1) Kampon Kaeoket, Mahidol University, Thailand.
(2) Marco Aurelio Romano, Universidade Estadual do Centro Oeste, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/19072

Original Research Article

Received 12th April 2017
Accepted 11th May 2017
Published 15th May 2017

ABSTRACT

Infection of the male genitourinary tract represents a significant health care problem and account for almost 15% of cases of male infertility. The present study aims to isolate and identified different bacterial isolates collected from semen samples of infertile men attending to infertility clinic and evaluation of the effects of bacteriospermia on semen quality. Five medicinal plants extracts were used as an alternative therapeutic agent targeting these isolates. Seventy seven infertile men were evaluated by standard bacterial culture methods. Among total cases, 22 cases (28.6%) showed at least one pathogen: 10 (45.5%) *Escherichia coli*, 9 (40.9%) *Staphylococcus aureus,* and 3 (13.6%) *Pseudomonas aeruginosa*. Our results showed that samples infected with *Pseudomonas aeruginosa* recorded the highest ratio of abnormalities (96.5%). On the other hand samples infected with *Escherichia coli* recorded the second highest ratio of abnormalities (93.75%), while the samples

**Corresponding author: E-mail: Mohamed.farag@azhar.edu.eg, moh_man_s1977@yahoo.com;*

infected by *Staphylococcus aureus* showed the least ratio of abnormalities (92.88%). From these five medicinal plants that extracted by ethanol-methanol and/or chloroform-metahnol methods, the most potent plants extracts against bacterial isolates were detected for both *Syzygium aromaticum* (Clove) and *Thymus vulgaris* (Thyme). In conclusion, the present study showed that the microbiological investigation should be performed, as a routine test, to all infertile men attending to infertility clinics**.** Flavonoids of the selected plants have a good antioxidant and antibacterial activity, and can be used for medicinal and therapeutic applications.

Keywords: Semen; bacterial isolates; men infertility; medicinal plant extracts.

1. INTRODUCTION

Infection is a powerful mechanism that can lead to sperm damage, deformity and eventually, male infertility. Genital tract infection and inflammation have been associated to 8-35% of male infertility cases [1]. Infection can affect different sites of the male reproductive tract, such as the testis, the epididymis and male accessory sex glands [2,3]. Spermatozoa subsequently can be affected by infections at different points in their development and maturation. Acute and chronic infections can compromise spermatogenesis, resulting in quantitative and qualitative reductions [3,4]. Among bacterial species that interact with spermatozoa are well known causative pathogens of genitourinary tract infection such as *Escherichia coli, Ureaplasma urealyticum, Mycoplasma hominis* and *Chlamydia trachomatis* [5,6,7].

E. coli probably represents the most frequently isolated microorganism in genitourinary infections. *E. coli* rapidly adheres to human spermatozoa *in vitro*, resulting in agglutination of spermatozoa [8]. Agglutination of spermatozoa was produced only by live pathogenic *E. coli* whereas killed bacteria failed to do so [9]. While studying other uropathogenic microorganisms found significant decrease in sperm motility when spermatozoa were co-incubated with *Staphylococcus aureus*. Jiang and Lu [10] reported that *S. aureus* was the predominant flora in infertile men with a significant decrease in sperm motility. *S. aureus* was one of the predominant flora in the infertile men and commonly present in the cervix of females immobilizes the spermatozoa [11]. Attempts to improve the efficacy of available antibiotics, particularly the older and cheaper ones have been suggested [12].

Medicinal plants continue to play a central role in the healthcare systems of large proportions of the world's population, particularly in developing countries, where herbal medicine has a long and uninterrupted history of use [13]. This raises the prospects of obtaining novel chemotherapeutic compounds if this vastly untapped resource could be adequately explored. The prospect of obtaining drugs from plants has been demonstrated by some notable examples of important pharmaceuticals derived from plant precursors [14]. The rich chemical diversity in plants has also been reported to be a promising source of antibacterial compounds raising hopes of obtaining novel antibiotics that can aid the fight against resistant infection [15].

2. MATERIALS AND METHODS

2.1 Collection of Samples

Seminal fluid specimens were collected from males attending the fertility clinic at the International Islamic Center for Population Studies and Research (IICPSR), Al-Azhar University, Cairo, Egypt. The samples were collected from patients during the period of April 2013 to December 2014. Upon collection, samples were transferred to the laboratory in a temperature that is as close as possible to body by placing the container inside a plastic bag.

2.2 Examination of Specimens

All semen samples included in this study were examined for physical appearance, volume, viscosity, total motility, and sperm count.

2.2.1 Appearance

Semen samples were examined immediately after liquefaction or within one hour of ejaculation. A normal sample has homogenous gray opalescent appearance. It may appear less opaque if the sperm concentration is very low or brown when red blood cells are present.

2.2.2 Volume

The volume of the seminal fluid was measured by decanting the whole sample aseptically into a

graduated centrifuge tube and the level was recorded in ml ± 0.1. The universal bottle was preserved for cultural examination and for further experimentation.

2.2.3 Viscosity

The viscosity of the sample was determined with the aid of Pasteur pipette. A drop of semen was allowed to fall back to the sample and the length of the thread was observed. A normal sample leaves the pipette as small discrete drops while in abnormal cases, the drop forms a thread greater than 2 cm long.

2.2.4 Total motility

Total motility of the samples was done by applying a drop of the sample onto a slide, covered with cover slip. The sample was then viewed under the microscope using x40 objective lens. The microscopic field was scanned systemically and the motility of each spermatozoon encountered was graded a, b, c and d that is, (a) Rapid progressive motility, (b) Slow or sluggish motility, (c) Nonprogressive motility and (d) Immobility. The number of spermatozoa in each category was counted with the aid of a laboratory counter. Usually, four to six fields were scanned to classify 100 successive spermatozoa. All motile spermatozoa with the ones that had their heads moving were recorded.

2.2.5 Sperm count

Improved Neubauer Counting Chamber was used for the count. One twenty (1/20) dilution of semen was done with formol saline as diluents. Count = $N \times 10^6$/ml.

2.3 Cultural Methods

Culture of seminal fluid samples were cultured in aseptic condition, within 1 h of collection the seminal fluids were cultured using Blood Agar, Chocolate Agar and MacConkey Agar media and incubated at 37°C for 24 h. The cultures were examined for bacterial growth; the isolation and identification of bacterial isolates were carried out according to Bergey's Manual of Determinative Bacteriology. All our identified strains were confirmed by 16s ribosomal RNA.

2.4 Plant Extractions

Two different methods were used for extracting the active components from the five medicinal plants used (Table 1). The source of the five medicinal plants was provided kindly by faculty of agriculture, Cairo University. Accepted names of plant species were listed according to the following link: www.theplantlist org database.

2.4.1 Extraction methods

Two different extraction methods were used for extracting the active components from the medicinal plants as the following:

2.4.1.1 Ethanol-methanol

Fifty grams of crushed plant material was packed in percolator and extracted with 100 ml of 95% ethanol [16].

2.4.1.2 Chloroform-methanol

Fifty grams of crushed plant material was homogenized with chloroform /methanol (2/1) to a final volume 100 ml [17].

2.5 Screening for the Antimicrobial Potential of the Medicinal Plant Extracts

A concentration of 0.5 McFarland standards was prepared from fresh cultures of microorganisms.

Table 1. Five medicinal plants were used in the present study

Code	Common name	Scientific Name	Family	Part used
Z	Ginger	*Zingiber officinale* Roscoe	*Zingiberaceae*	Rhizome
A	Chamomile	*Chamaemelum nobile* (L.) All	*Asteraceae*	Flower
T	Thyme	*Thymus vulgaris* L.	*Lamiaceae*	Flower
S	Clove	*Syzygium aromaticum* (L.) Merr. & L. M. Perry	*Myrtaceae*	Buds
R	Rosemary	*Rosmarinus officinalis* L.	*Lamiaceae*	Leaves

Each microorganism was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, filter paper discs (6 mm in diameter) saturated with each extract (50 µL) was placed on surface of each inoculated plate [18].

2.6 Screening for the Antimicrobial Potential of the Plant Extract Fractions

A concentration of 0.5 McFarland standards was prepared from fresh cultures of microorganisms. Each microorganism was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, filter paper discs (6 mm in diameter) saturated with plant extract fractions (50 µL) were placed on surface of each inoculated plate [18]. The plates were incubated at 37ºC for 24 h. After this period, it was possible to observe inhibition zone. Cultured bacteria with halos equal to or greater than 7 mm were considered susceptible to the tested extract. The control was the solvent used.

2.7 Thin Layer Chromatography

Thin layer chromatography technique (TLC) was used for testing purity of the more potent antibacterial fraction of the plant extract [19,20].

2.8 Identification of Pure Antibacterial Compound

Purified antibacterial compounds were identified by physical and spectral measurements using Ultraviolet Spectroscopic Analysis, ^{1}H-NMR spectroscopy, and ^{13}C-NMR Spectroscopy [21,22,23].

3. RESULTS

3.1 Frequency of Bacterial Isolates

Seventy seven samples of semen collected from men attending the fertility clinic of the International Islamic Center for Population Studies and Research (IICPSR), were examined routinely, for normal semen characteristics and bacterial count. Among total cases, 22 cases (28.6%) showed at least one pathogen. Three genera of bacterial isolates were obtained and these include *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa,* with the highest counts recorded for the genera *Escherichia* 10 (45.5%), *Staphylococcus* 9 (40.9%) and *Pseudomonas* 3 (13.6%) (Table 2).

3.2 Distribution of Isolates Relative to Sperm Characteristics

Tables 2 showed the distribution of the bacterial isolates relative to sperm characteristics including sperm count, total motility, progressive motility and abnormalities. In the present study the semen samples infected by *Staphylococcus aureus* showed higher sperm count (4.62×10^6) than *Escherichia coli* (4.53×10^6) and *Pseudomonas aeruginosa* (4.03×10^6) that showed lowest sperm count.

The highest total and progressive motility were recorded from samples infected with *Staphylococcus aureus*, the motility was 25% and 8.33%, respectively. Regarding to samples infected with *Escherichia coli,* the total and progressive motility was 15.1% and 6.5% respectively. The least motility parameters were recorded with samples infected with *Pseudomonas aeruginosa*, the total and progressive motility was 1.67% and 0.00% respectively.

The results showed that samples infected with *Pseudomonas aeruginosa* recorded the highest ratio of abnormalities (96.5%). On the other hand samples infected with *Escherichia coli* recorded the second highest ratio of abnormalities (93.75%), while the samples infected by *Staphylococcus aureus* showed the least ratio of abnormalities (92.88%) (Table 3).

3.3 Identification of Bacterial Strains by Biochemical and 16s Ribosomal RNA

The bacterial strains identification was carried out according to Bergey's Manual of Determinative Bacteriology (Buchanan, Gibbons, 1978; Gerhardt et al. 1994). All our identified strains were confirmed by 16s ribosomal RNA. Sequences were compared with bacterial strains sequences in the GenBank database using BLASTN (Table 4).

3.4 Antimicrobial Activity Caused by Different Medicinal Plant Extracts

The antimicrobial activities of five medicinal plants against our bacterial isolates were

Table 2. Frequencies of bacterial isolates in the present study

Group	Bacterial isolate	Frequency	Percentage (%)
A	*Escherichia coli*	10	45.5
B	*Staphylococcus aureus*	9	40.9
C	*Pseudomonas aeruginosa*	3	13.6

Table 3. The distribution of isolates relative to sperm characteristics

Group	Bacterial isolates	Percentage (%)	Sperm count	Total motility (%)	Progressive motility (%)	Abnormalities (%)
A	*Escherichia coli*	45.5	4.53 x 106	15.1	6.5	93.75
B	*Staphylococcus aureus*	40.9	4.62 x 106	25	8.33	92.88
C	*Pseudomonas aeruginosa*	13.6	4.03 x 106	1.67	0.00	96.5

3.3.1 Bacterial samples sequences

3.3.1.1 Samples No. 1: Escherichia coli

TTGGCGCTGC GTCAGGCCTA CACATGCAGA GAGGTAACAG GAAGAGCTTG CTTCTTTGCT
GACGAGTGGC GGACGGGTGA GTAATGTCTG GGAAACTGCC TGATGGAGGG GGATAACTAC
TGGAAACGGT AGCTAATACC GCATAACGTC GCAAGACCAA AGAGGGGGAC CTTCGGGCCT
CTTGCCATCG GATGTGCCCA GATGGGATTA GCTTGTTGGT GGGGTAACGG CTCACCAAGG
CGACGATCCC TAGCTGGTCT GAGAGGATGA CCAGCCACAC TGGAACTGAG ACACGGTCCA
GACTCCTACG GGAGGCAGCA GTGGGGAATA TTGCACAATG GGCGCAAGCC TGATGCAGCC
ATGCCGCGTG TATGAAGAAG GCCTTCGGGT TGTAAAGTAC TTTCAGCGGG GAGGAAGGGA
GTAAAGTTAA TACCTTTGCT CATTGACGTT ACCCGCAGAA GAAGCACCGG CTAACTCCGT
GCCAGCAGCC GCGGTAATAA

3.3.1.2 Samples No. 2: Staphylococcus aureus

CGTAGACCGC TGGCCGGCGT GCCTAATACA TGCAAGTCGA GCGAACGGAC GAGAAGCTTG
CTTCTCTGAT GTTAGCGGCG GACGGGTGAG TAACACGTGG ATAACCTACC TATAAGACTG
GGATAACTTC GGGAAACCGG AGCTAATACC GGATAATATT TTGAACCGCA TGGTTCAAAA
GTGAAAGACG GTCTTGCTGT CACTTATAGA TGGATCCGCG CTGCATTAGC TAGTTGGTAA
GGTAACGGCT TACCAAGGCA ACGATGCATA GCCGACCTGA GAGGGTGATC GGCCACACTG
GAACTGAGAC ACGGTCCAGA CTCCTACGGG AGGCAGCAGT AGGGAATCTT CCGCAATGGG
CGAAAGCCTG ACGGAGCAAC GCCGCGTGAG TGATGAAGGT CTTCGGATCG TAAAACTCTG
TTATTAGGGA AGAACATATG TGTAAGTAAC TGTGCACATC TTGACGGTAC CTAATCAGAA
AGCCACGGCT
AACTACGTGC CAGCAGCCGC GGTAATAA

3.3.1.3 Samples No. 3: Pseudomonas aeruginosa

TGCGCTGCGG CAGGCCTAAC ACATGCAAGT CGAGCGGATG AAGGGAGCTT GCTCCTGGAT
TCAGCGGCGG ACGGGTGAGT AATGCCTAGG AATCTGCCTG GTAGTGGGGG ATAACGTCCG
GAAACGGGCG CTAATACCGC ATACGTCCTG AGGGAGAAAG TGGGGGATCT TCGGACCTCA
CGCTATCAGA TGAGCCTAGG TCGGATTAGC TAGTTGGTGG GGTAAAGGCC TACCAAGGCG
ACGATCCGTA ACTGGTCTGA GAGGATGATC AGTCACACTG GAACTGAGAC ACGGTCCAGA
CTCCTACGGG AGGCAGCAGT GGGGAATATT GGACAATGGG CGAAAGCCTG ATCCAGCCAT
GCCGCGTGTG TGAAGAAGGT CTTCGGATTG TAAAGCACTT TAAGTTGGGA GGAAGGGCAG
TAAGTTAATA CCTTGCTGTT TTGACGTTAC CAACAGAATA AGCACCGGCT AACTTCGTGC
CAGCAGCCGC GCCAAATAA

Table 4. 16s RNA for bacterial isolates included in our study. Our result showed the bacterial strains were *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Sequences were compared with sequences in the GenBank database using BLASTN

Samples cod#	Holomogue sequences (Sequences identity %)	Accession number
S#1	• *Escherichia coli* str. K-12 substr. MG1655, complete genome (98%)	NC 000913.3
	• *Escherichia coli* O157:H7 str. Sakai chromosome, complete genome (98%)	NC 002695.1
	• *Escherichia coli* O83:H1 str. NRG 857C chromosome, complete genome (97%)	NC 017634.1
S#2	• *Staphylococcus aureus* subsp. aureus NCTC 8325 chromosome, complete genome (99%)	NC 007795.1
	• *Staphylococcus aureus* subsp. aureus N315 chromosome, complete genome (99%)	NC 002745.2
	• *Staphylococcus epidermidis* ATCC 12228 chromosome, complete genome (97%)	NC 004461.1
S#3	• *Pseudomonas aeruginosa* PAO1 chromosome, complete genome (99%)	NC 002516.2
	• *Pseudomonas denitrificans* ATCC 13867, complete genome (97%)	NC 020829.1
	• *Pseudomonas stutzeri* A1501 chromosome, complete genome (97%)	NC 009434.1

present study, two different extraction methods were used for extracting the active components from the medicinal plants as the following:

3.4.1 Ethanol- Methanol extract methods

The results showed that *Escherichia coli* was sensitive for both *Syzygium aromaticum* (Clove) and *Thymus vulgaris* (Thyme), while *Staphylococcus aurous* was sensitive to all plant extracts included our study by ethanol-methanol methods. Regarding to *Pseudomonas aeruginosa* it was sensitive to *Syzygium aromaticum* (Clove) alone. From these five medicinal plants that extracted by methanol-ethanol method, the most potent plants extracts against bacterial isolates were detected for both *Syzygium aromaticum* (Clove) and *Thymus vulgaris* (Thyme) (Table 5).

3.4.2 Chloroform – methanol extract method

The results showed that *Escherichia coli* was sensitive for both *Syzygium aromaticum* (Clove) and *Thymus vulgaris* (Thyme), while *Staphylococcus aurous* was sensitive to all plant extracts except *Zingiber officinale* (Ginger). Regarding to *Pseudomonas aeruginosa* it was sensitive to *Syzygium aromaticum* (Clove) alone. From these five medical plants that extracted by chloroform-methanol method, the most potent plants extracts against bacterial isolates were detected for both *Syzygium aromaticum* (Clove) and *Thymus vulgaris* (Thyme) (Table 6).

Table 5. Antimicrobial activity for medicinal plants extracted by ethanol-methanol methods against selected bacterial isolates (inhibition zone/mm)

Isolates/Medicinal plant	***Escherichia coli***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Zingiber officinale Roscoe	-	16	-
Chamaemelum nobile (L.) All	-	18	-
Thymus vulgaris L.	8	19	-
Syzygium aromaticum (L.) Merr. & L. M. Perry	**12**	**23**	**10**
Rosmarinus officinalis L.	-	13	-
Control	-	-	-

Table 6. Antimicrobial activity for medicinal plants extracted by chloroform-methanol methods against selected bacterial isolates (inhibition zone/mm)

Isolates/medicinal plant	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Zingiber officinale Roscoe	-	-	-
Chamaemelum nobile (L.) All	-	15	-
Thymus vulgaris L.	8	19	-
Syzygium aromaticum (L.) Merr. & L. M. Perry	**10**	**16**	**9**
Rosmarinus officinalis L.	-	13	-
Control	-	-	-

3.5 Fractionation by Column Chromatography for the Most Potent Medicinal Plant

Syzygium aromaticum was chosen for fractionated after extraction by ethanol-methanol and/or chloroform-methanol methods because it has the most potent activity against all bacterial isolates, 15 fractions for each method were collected separately. All fractions were tested for determination the most potent fraction against our bacterial isolates by agar diffusion method.

3.5.1 Antimicrobial activity of Syzygium aromaticum fractions after extraction by ethanol-methanol method

Table 7 showed that fraction number 8 was the most potent fraction against all bacterial isolates using agar diffusion method.

3.5.2 Antimicrobial activity of *Syzygium aromaticum* fractions after extraction by chloroform-methanol method

Table 8 showed that fraction number 8 was the most potent fraction against all bacterial isolates using agar diffusion method.

3.6 Photochemical Characterization and Structure Elucidation of the Most Potent fraction of *Syzygium aromaticum*

For photochemical characterization and structure elucidation of the most potent fraction of *Syzygium aromaticum* physical properties and spectra profile were carried out. Fraction no. 8 for *Syzygium aromaticum* was the most potent fraction has biological activity against all bacterial isolates extracted by both ethanol-methanol and chloroform-methanol methods. The spectroscopic analysis of these active fractions using UV spectrum and NMR spectrum were carried out.

3.6.1 Photochemical characterization and structure elucidation of the most potent fraction of *Syzygium aromaticum* using ethanol-methanol method

Physical properties and spectra profile were assessed for elucidation and identification fraction number 8 that showed the most potent activity against all bacterial isolates using ethanol-methanol method. Theses physical properties and spectra profile (Table 9), UV spectrum (Fig. 1), ^{1}HNMR spectrum (Fig. 2) and ^{13}CNMR spectrum (Fig. 3) confirmed that this compound was elucidated and identified as Apigenin-7-O- glucoside ($C_{21}H_{21}O_{11}$).

3.6.2 Photochemical characterization and structure elucidation of the most potent fraction of *Syzygium aromaticum* using chloroform-methanol method

Physical properties and spectra profile were assessed for elucidation and identification fraction number 8 too that showed the most potent activity against all bacterial isolates using chloroform-methanol method. Theses physical properties and spectra profile (Table 10), UV spectrum (Fig. 4), ^{1}HNMR spectrum (Fig. 5) and ^{13}CNMR spectrum (Fig. 6) confirmed that this compound was elucidated and identified as **Apigenin ($C_{15}H_{10}O_5$).**

4. DISCUSSION

Male urogenital tract infection is an important cause of men infertility. The etiological role of infections in male infertility has been paid attention in recent years. Asymptomatic bacteriospermia may play a major role [24,25]. Infectious processes may lead to deterioration of spermatogenesis, impairment of sperm function and obstruction of the seminal tract [26]. As a result, microbiological investigation can reveal a probable infection.

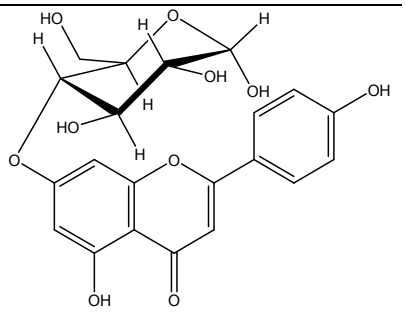
Table 7. Antimicrobial activity of *Syzygium aromaticum* fractions after extraction by ethanol-methanol method (inhibition zone/mm)

Fractions numbers	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
control	-	-	-
Fr.1	16	-	-
Fr.2	10	-	-
Fr.3	8	9	-
Fr.4	10	8	-
Fr.5	-	12	-
Fr.6	8	8	-
Fr.7	11	10	-
Fr.8	**20**	**18**	**10**
Fr.9	16	18	10
Fr.10	14	14	-
Fr.11	8	10	8
Fr.12	-	-	-
Fr.13	-	-	-
Fr.14	-	-	-
Fr.15	-	-	-

Table 8. Antimicrobial activity of *Syzygium aromaticum* fractions after extraction by chloroform-methanol method (inhibition zone /mm)

Fractions numbers	*Escherichia coli*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
control	-	-	-
Fr.1	12	14	-
Fr.2	8	10	-
Fr.3	15	20	-
Fr.4	14	22	8
Fr.5	9	18	-
Fr.6	8	18	-
Fr.7	13	18	8
Fr.8	**18**	**25**	**9**
Fr.9	16	24	9
Fr.10	-	21	-
Fr.11	-	20	-
Fr.12	-	-	-
Fr.13	-	-	-
Fr.14	-	12	-
Fr.15	-	-	-

Table 9. Photochemical characterization and structure elucidation of the most potent fraction of *Syzygium aromaticum* using ethanol-methanol method

Physical and spectra data profile	Structure
R_f: Acetic acid 15%: 0.18, BAW: 0.68, **COLOR** :U.V.: Violet, U.V. + NH_3: Yellow green, **U.V**.: MeOH: 227 sh, 273, 335, NaOMe: 275, 306 sh, ▲389, $AlCl_3$: 277, 330 sh, ▲380, $AlCl_3$+ HCl: 279, 302 sh, ▲383, NaOAc: 280, 340, ▲390, NaOAc+ H_3BO_3: 276, 342, **^{1}H NMR** : (DMSO-D6), δ: 2(s, 4H, alip-OH), 2.5(d, 1H, alip-H), 3.2(m, 4H, alip-H), 4(m, 1H, alip-H), 5(s, 2H, Ar -H), 5.8(s, 1H, Ar-H), 6.3 (d, 2H, Ar-H), 7.2 (m, 5H, J=5.7, Ar-H), **^{13}CNMR** : (DMSO-D6), δ: 66(1) , 68(1) , 70(1) , 73(1) , 82(1) , 96(1) , 98(1) , 105(2) , 116(2) , 124(1) , 129(1) , 155(1) , 159(1) , 162(1) , 166(1) , 170(1) , 183(1) , **$C_{21}H_{21}O_{11}$: Apigenin-7-O- glucoside**.	

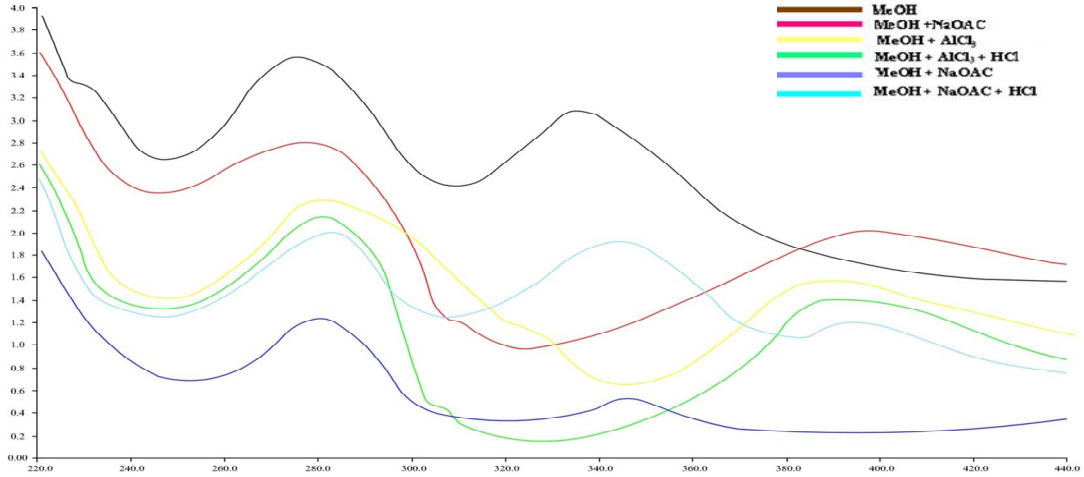


Fig. 1. UV spectrum of *Syzygium aromaticum* fraction extracted by ethanol–methanol method

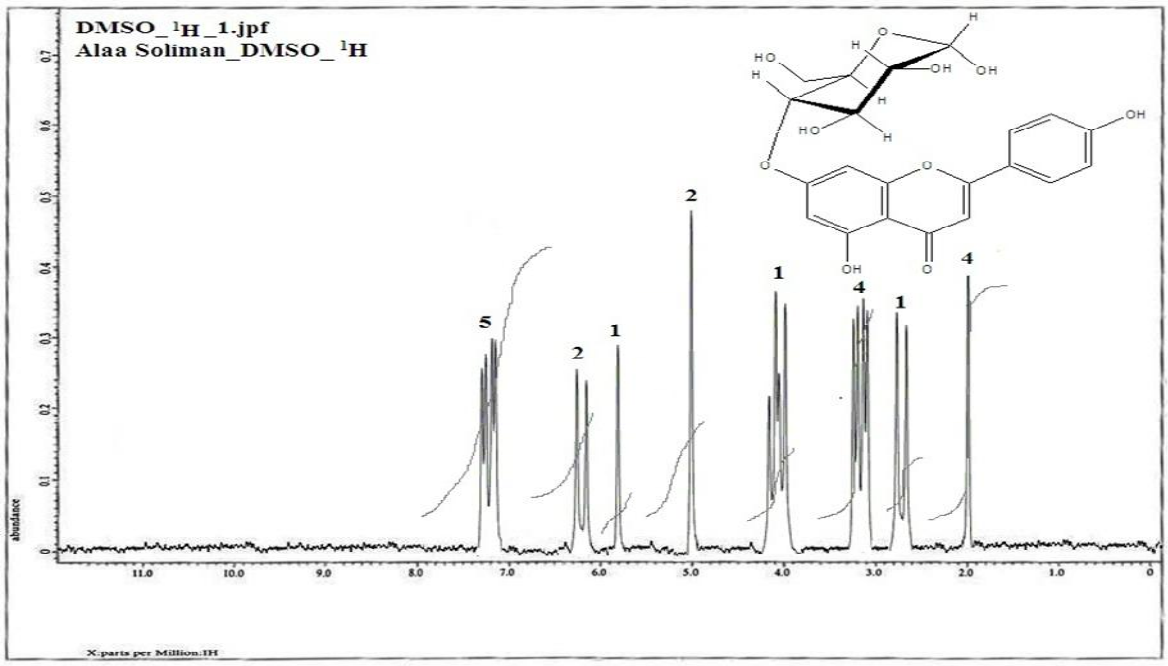
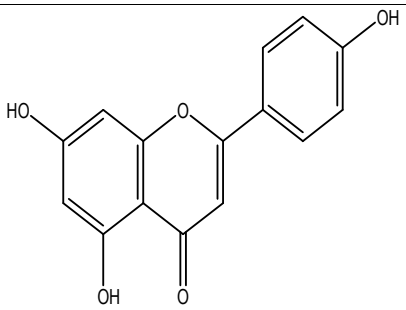


Fig. 2. ^{1}HNMR spectrum of *Syzygium aromaticum* fraction extracted by ethanol–methanol methods

The present study aims to isolate and identified different bacterial isolates collected from semen of infertile males attending infertility center at International Islamic center for population studies and Research, Al-Azhar University. Five medicinal plants extracts were used as an alternative therapeutic agent targeting these isolates. Fractionation of the most antimicrobial active extracts against the bacterial isolates was done. Phytochemical screening on the most antimicrobial active fractions and its identification was carried out using spectroscopic analysis. Seventy seven samples of semen were collected and examined for normal semen characteristics and bacterial count. Among total cases, 22 cases (28.6%) showed at least one pathogen. Three genera of bacterial isolates were obtained and these include *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa,* with the highest counts recorded for the genera

Escherichia 10 (45.5%), *Staphylococcus* 9 (40.9%) and *Pseudomonas* 3 (13.6%). Our study was in agreement with previous studies examined the impact of genital tract infections and bacterial semen contamination in male fertility and should that microorganisms can affect the male reproductive function directly, causing the agglutination of motile sperm, reducing the ability of acrosome reaction and causing alterations in cell morphology-and indirectly, through the production of reactive oxygen species generated by the inflammatory response to the infection [27]. Our study was in agreement with Emokpae et al. [28] reported that presence of *S. aureus* should not be ignored as it can lead to decrease in the number of spermatozoa, the suppression of their motility, changes in their morphology and fertilizing capacity, *S. aureus* could to be the dominant flora in infertile men with a significant decrease in sperm motility.

Table 10. Photochemical characterization and structure elucidation of the most potent fraction of *Syzygium aromaticum* using chloroform-methanol method

Physical and spectra data profile	Structure
R_f: Acetic acid 15%: 0.09, BAW: 0.85, **COLOR** :U.V.: Violet, U.V. + NH_3: Faint Yellow, **U.V**.: MeOH: 271, 340, NaOMe: ▲ 279, ▲ 400, $AlCl_3$: 280, 301 sh, 391, $AlCl_3$+ HCl: 275, ▲ 394, NaOAc: 271, ▼ 382, NaOAc+ H_3BO_3: 270, 342, **^{1}H NMR** (DMSO-D6), δ: 5(s, 3H, Ar-OH), 6.3(d, 2H, Ar-H), 7.1(m, 3H, Ar-OH), 7.8(s, 2H, Ar-H), **^{13}C NMR** (DMSO-D6), δ: 97(1), 105(1), 108(1), 116(2), 122(1), 130(1), 156(1), 160(1), 165(1), 168(1), 182(1), $C_{15}H_{10}O_5$: **Apigenin.**	

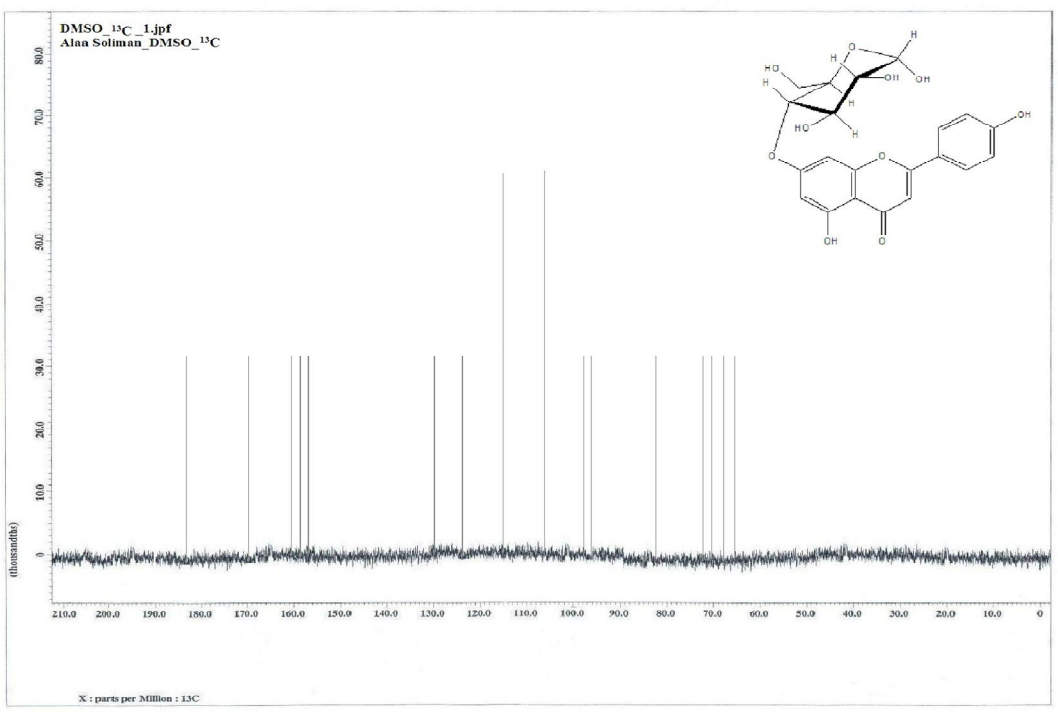


Fig. 3. ^{13}CNMR spectrum of *Syzygium aromaticum* fraction extracted by ethanol–methanol methods

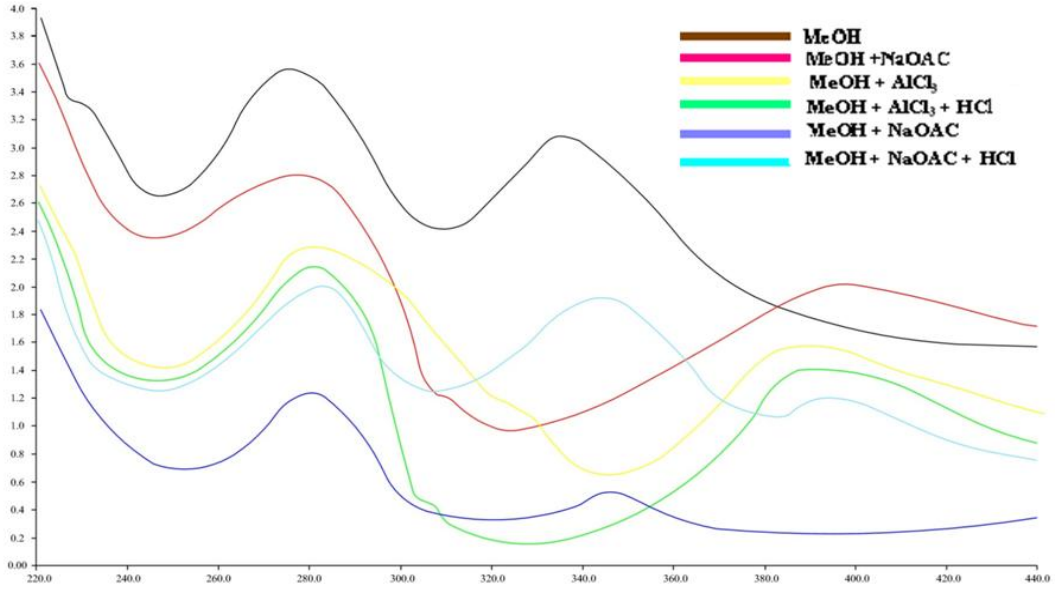


Fig. 4. UV spectrum of *Syzygium aromaticum* fraction extracted by chloroform–methanol method

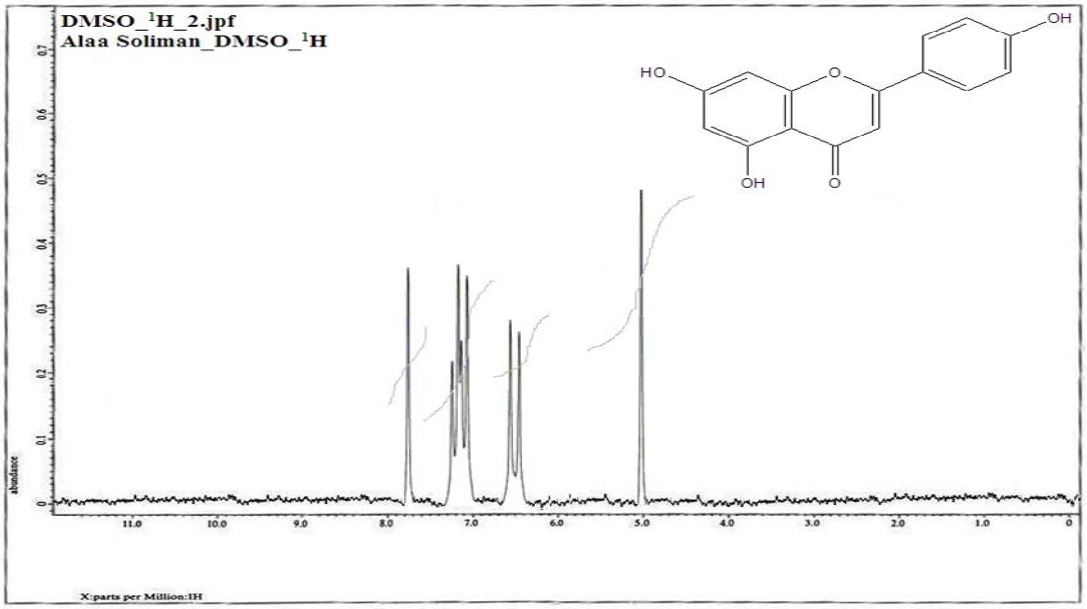


Fig. 5. ^{1}HNMR spectrum of *Syzygium aromaticum* fraction extracted by chloroform–methanol methods

Bacterial growth may be one cause of contradictory conclusions concerning the effective sperm/bacteria ratio. The absence of sperm agglutination was also observed after heat treatment of bacteria. In one of the earlier reports on effect of heat killed bacteria on spermatozoa characteristics had studied that the live pathogenic *S. aureus* obtained from cervical cultures decreases in motility and viability of human spermatozoa which was absent in the sets in which the bacteria had been killed by boiling for 30 min before mixing the ejaculate [29].

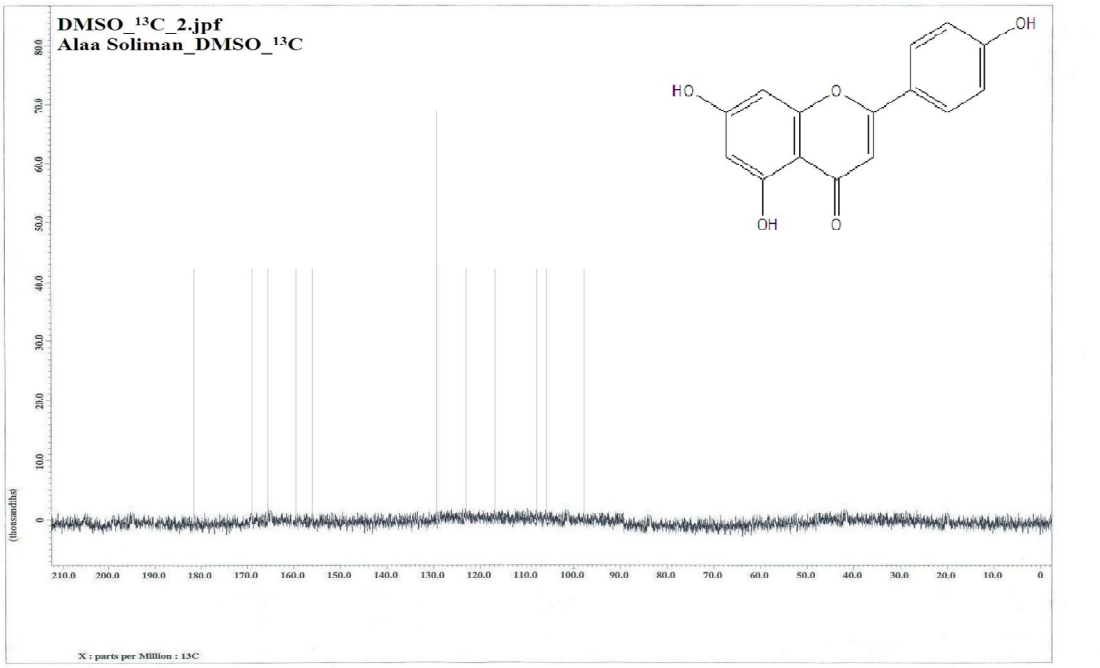


Fig. 6. ^{13}CNMR spectrum of *Syzygium aromaticum* fraction extracted by chloroform–methanol methods

Alternatively, effect of antimicrobial agents on bacteria was also observed. Treatment of cultured bacteria with different groups of antibiotics abolished the effect. This indicates that must be some adhesion sites on spermatozoa to which *E. coli* binds. The mechanisms by which *E. coli* affect sperm functions have not yet been identified [30]. Interference of *E. coli* with these receptors may influence the motility and viability. Other authors reported similar observations concerning sperm motility and agglutination after incubation with *E. coli*. We suggest in agreement with previous study that *E.* coli/spermatozoa-interaction may be a two-step process: i.e. adhesion to and subsequent destruction of the sperm membrane. This mechanism may account for any inhibitory effects of *E. coli* infection on male fertility. It is speculative whether the same mechanisms are also responsible for impaired male fertility in cases of genitourinary infections [30].

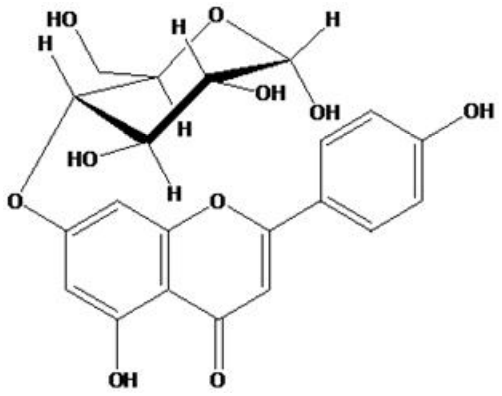
According to World Health Organization reported that approximately 75-80% of the world's population uses plant medicines either in part or entirely. Growing numbers of American health care consumers are turning to plant medicines for many reasons – low cost and seeking natural alternatives with fewer side effects are commonly cited [31].

In the present study five medicinal plants were collected from different locations in Egypt. Each medicinal plant coded and determined its family. Common and scientific name was recorded. Flower of Chamomile and Thyme, Rhizome of Ginger, buds of Clove and leaves of Rosemary were included. These five medicinal plants were extracted by ethanol-methanol and/or chloroform-methanol methods and examined as antimicrobial activity against all bacterial isolates included our study.

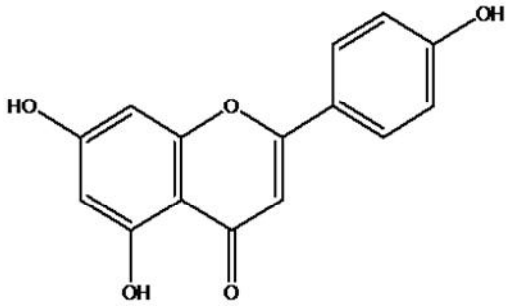
Our results showed that *Escherichia coli* was sensitive for both *Syzygium aromaticum* and *Thymus vulgaris*, while *Staphylococcus aurous* was sensitive to most plant extracts included our study by ethanol-methanol and/or chloroform-metahnol methods. Regarding to *Pseudomonas aeruginosa* it was sensitive to *Syzygium aromaticum* alone. From these five medicinal plants that extracted by ethanol-methanol and/or chloroform-metahnol methods, the most potent plants extracts against bacterial isolates were detected for both *Syzygium aromaticum* and *Thymus vulgaris*.

Our results was in agreement with Rasool results revealed that the degree of antimicrobial properties of four spices tested alone against *E. coli* growth can be put in the following order: (clove >rosemary > ginger and cumin), clove and rosemary essential oils alone were exhibited a strong antibacterial action. These spices may be selected as potentially useful anti *E. coli* agents in meat products and other foods which easily contaminated by *E. coli* through fecal oral route during bad handlings, a possible way to use these two spices in combination with other food preservatives such as salt, acid, sugar and others to select pathogenic and spoilage microorganisms and may provide better alternatives in the conventional antimicrobial additives in food [32].

In the present study *Syzygium aromaticum* (Clove) was chosen for fractionated after extraction by ethanol-methanol and/or chloroform-methanol methods because it has the most potent activity against all bacterial isolates, 15 fractions for each method were collected separately. All fractions were tested for determination the most potent fraction against our bacterial isolates by agar diffusion method. According to our data one fraction from each extract has the most potent activity against all bacterial isolates included our study. Photochemical characterization including UV spectrum, NMR spectrum and structure elucidation of the most potent fraction of *Syzygium aromaticum* was carried out. This Fraction of *Syzygium aromaticum* that extracted by ethanol-methanol and/or chloroform-methanol methods was elucidated and identified as **Apigenin-7-O- glucoside ($C_{21}H_{21}O_{11}$) and Apigenin ($C_{15}H_{10}O_5$),** respectively.



Apigenin-7-O- glucoside



Apigenin

Our results was in agreements with Hoque et al. [33] who reported that ethanol and aqueous extracts and the EOs from Clove and cinnamon exhibited antibacterial activity against food borne pathogens *in vitro*. However, there are some limitations in using spices like Clove or cinnamon, such as the antibacterial activity is decreased when spices are added to food materials containing protein, carbohydrate, and fat, and the strong flavor. The flavor of the food products may not be acceptable by some consumer groups if large amounts of spices are added to the products to inhibit the food borne pathogens. Therefore, the use of spices along with preservatives such as acid, salt, sugar and with processing and storage conditions can help in controlling microorganisms in food products.

The spectroscopic analysis currently one of the most versatile and sensitive instrumental methods applied to structural characterization of plant secondary metabolite mixtures isolated from biological material including flavonoid glycoconjugates. Resolution of the applied mass spectrometers plays an important role in structural studies of mixtures of the target compounds isolated from biological material. High-resolution analyzers allow obtaining information about elemental composition of the analyzed compounds. Application of various mass spectrometric techniques, including different systems of ionization, analysis of both positive and negative ions of flavonoids, fragmentation of the protonated/deprotonated molecules and in some cases addition of metal ions to the studied compounds before ionization and fragmentation, may improve structural characterization of natural products. The presented data indicated that the flavonoids isolated act differently on the bacterial species tested.

5. CONCLUSION

In conclusion, the present study showed that the microbiological investigation should be performed, as a routine test, to all infertile men attending to infertility clinics**.** It should be noticed that presence of urogenital tract infection and inflammation must be eradicated by antibiotic and anti-inflammation treatment, especially before using Assisted Reproductive Techniques (ART). Flavonoids of the selected plants have a good antioxidant and antibacterial activity, and can be used for medicinal and therapeutic applications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sushila K, Arvind S, Vijay P, Rambir S, Poonam S. *Escherichia coli* attaches to human spermatozoa: Affecting sperm parameters. Arch Appl Sci Res. 2001;3: 618-623.
2. Diemer T, Huwe P, Michelmann HW, Mayer F, Schiefer HG, Wiedner W, et al. *Escherichia coli*-induced alterations of human spermatozoa. An electron microscopy analysis. Int J Androl. 2000; 23:178-186.
3. Golshani M, Taheri S, Eslami G, Suleimani Rahbar AA, Fallah F, Gourdazi H. Genital tract infection in asymptomatic infertile men and its effect on semen quality. Ir J Publ Health. 2006;35:81-84.
4. Khalili MB, Sharifi-Yazdi MK. The effect of bacterial infection on the quality of human's spermatozoa. Ir J Publ Health. 2001;30:119-122.
5. Kohn FM, Erdmann I, Oeda T, Mulla KF, Schiefer HG, Schill WB. Influence of urogenital infections on sperm functions. Andrologia. 1998;30:73–80.
6. Hosseinzadeh S, Brewis IA, Eley A, Pacey AA. Co-incubation of human spermatozoa with *Chlamydia trachomatis*serovar E causes premature sperm death. Human Reprod. 2001;16:293-299.
7. Gdoura R, Kchaou W, Chaari C, Znazen A, Keskes L, Rebai T, Hammami A. *Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis and Mycoplasma genitalium* infections and semen quality of infertile men. BMC Infect Dis. 2007;7:129-137.
8. Diemer T, Huwe P, Ludwig M, Schroeder-Printzen I, Michelmann HW, Schiefer HG, Wiedner W, et al. Influence of autogenous leucocytes and *Escherichia coli* on sperm motility parameters *in vitro*. Andrologia. 2003;35:100-105.
9. Liu JH, Li HY, Cao ZG, Duan YF, Li Y, Ye ZQ. Influence of several uropathogenic microorganisms on human sperm motility parameters *in vitro*. Asian J Androl. 2002;4:179-182.
10. Jiang J, Lu DY. Detection of bacteria from semens of infertile males and their seminal parameters. Chin J Andro. 1996;l10:196-198.
11. Ohri M, Prabha V. Isolation of sperm-agglutinating factor from *Staphylococcus aureus* isolated from a woman with unexplained infertility. FertilSteril. 2005;84: 1539-1541.
12. Lomovskaya O, Bostian KA. Practical applications and feasibility of eux pump inhibitors in the clinic–a vision for applied use. Biochem. Pharmacol. 2006;71:910–918.
13. Koduru S, Grierson DS, Afolayan, AJ. Ethnobotanical information of medicinal plants used for treatment of cancer in the Eastern Cape Province, South Africa. Curr Sci. 2007;92:906–8.
14. Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N, O'Neal JM, Cornwell T, Pastor I, Fridlender B. Plants and human health in the twenty-first century. Trends Biotech. 2002;20:522-531.
15. Smith ECJ, Williamson EM, Wareham N, Kaatz GW, Gibbons S. Antibacterials and modulators of bacterial resistance from the immature cones of *Chamaecyparis lawsoniana*. Phytochem. 2007;68:210-217.
16. Boakye-Yiadom K, Konning GH. Incidence of antibacterial activity in the connaraceae. Planta Medica. 1975;28:397-400.
17. Folch J, Lees M, Stanley GH. A simple method forthe isolation and purification of total lipides from animal tissues. J. Biol. Chem. 1957;226:497-509.
18. Bauer AW, Kirby E, Sherris EM, Turk M, et al. Antibiotic by standardized single disk method. Am. J. Clin. Path. 1966;45:493-496.
19. Bettelheim F, Landesberg J. Column and paper chromatography separation of plant pigments PP.265-271. In Saunders, W.B.

Ed. Laboratory experiments for general organic &biochemistry. Harcourt Brace Co. Publisher, Florida 32887. America; 1995.
20. Evens WC. Trease and evens Pharmacognosy. W. B. Saunders Co. London NW1 7DX. UK. 1996;119-130.
21. Chari VM, Wagner H, Neszmelyi A, et al. In "Farkas, L.; Gabor, M. and Kally, F. (Eds.). Flavonoids and Bioflavonoids". Elsevier, Ireland. 1977;293–301.
22. Markham KR, Ternai B, Stanley R, Geiger H, Mabry TJ. Carbon–^{13}NMR studies of flavonoids. Ш: Naturally occurring flavonoid glycosides and their acylated derivatives. Tetrahedron. 1978;34:1389–1397.
23. Shen CC, Chang YS, Ho LK. Nuclear magnetic resonance studies of 5,7-dihydroxy flavonoids. Phytochem. 1993;34: 843–845.
24. Askienazy-Elbhar. Male genital tract infection: The point of view of the bacteriologist. Gynecol Obstet Fertil. 2005;33:691-697.
25. HY Li, JH Liu. Influence of male genital bacterial infection on sperm function. Zhonghoa Nan KeXue. 2002;8:442-444.
26. Keck C, Gerber-Schafer C, Clad A, Wilhelm C, Breckwoldf M. Seminal tract infections: Impact on male fertility and treatment options. Hum Reprod Update. 1998;4:891-903.
27. Moretti E, Serena C, Natale F, Andrea Pammolli, Maria GF, Valentina GG. The presence of bacteria species in semen and sperm quality. J Assist Reprod Genet. 2009;26:47–56.
28. Emokpae MA, Uadia PO, Sadiq NM. Contribution of bacterial infection to male infertility in Nigerians. J. Health and Allied Sci. 2009;8:1-5.
29. Teague NS, Boyarsky JF, Glenn. Fertility and Sterility. 1971;22:281-85.
30. Kala S, Arvind S, Vijay P, Rambir S, Poonam S. *Escherichia coli* attaches to human spermatozoa: Affecting sperm parameters. Arch. Appl Sci Res. 2011;3:618-623.
31. Babb DA, Pemba L, Seatlanyane P, Charalambous S, Curchyard G, et al. Use of traditional medicine in the era of antiretroviral therapy: Experience from South Africa. E J LAS. Med. Gen. Med. 2004;6:10640.
32. Rasool MH. Antimicrobial activity of plant essential oils against the growth of *Escherichia coli*. J. Pharmacy. 2013;3:1-6.
33. Hoque MM, Bari MM, Vijay K, Juneja K, Kawamoto S. Antibacterial activity of clove and cinnamon against food borne pathogens. Rep. Natl. Food Res. Inst. 2008;72;9-21.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/19072