Original Research

Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Journal of Environmental Biology

JEB™ p-ISSN: 0254-8704
e-ISSN: 2394-0379
CODEN: JEBIDP

DOI : http://doi.org/10.22438/jeb/42/2/MRN-1663

Genotoxicity effects of medicinal plants extracts against bacterial species, *Mycoplasma hominis*

M.H. Yassin[1], Y. Alghamdi[1], E.H. Mohamed[1,2], S.A. Mostafa[1,3], A. Merghani[4], H.H. Amer[5,6], S.H. Alotaibi[5], M.M. Soliman[7,8], H. Nasr-eldeen[9] and M.M. Hassan[10]*

[1]Department of Biology, Turabah University College, Taif University, 21995, Saudi Arabia
[2]Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44519, Egypt
[3]Immunobiology and Immunopharmacology Unit, Animal Reproduction Research Institute, Giza, 11753, Egypt
[4]Department of Molecular Genetics, Aljazeera University, Al-khartoum, 34228, Sudan.
[5]Chemistry Department, University College of Turabah, Taif University, Turabah, Taif, 21995, Saudi Arabia.
[6]Animal Medicine and Infectious Diseases Department, Faculty of Veterinary Medicine, University of Sadat City, Sadat, 14174,Egypt
[7]Clinical Laboratory Sciences Department, Turabah University College, Taif University, Taif, 21944, Saudi Arabia
[8]Biochemistry Departemt, Faculty of Veterinay Medicine, Benha University, Toukh, 13736, Egypt
[9]Microbiology Laboratory, King Faisal Medical Complex, Taif, 11211, Saudi Arabia
[10]Department of Biology, College of Science, Taif University, Taif, 21944, Saudi Arabia

***Corresponding Author Email :** m.khyate@tu.edu.sa

Received: 17.08.2020 *Revised*: 26.11.2020 *Accepted*: 01.01.2021

Abstract

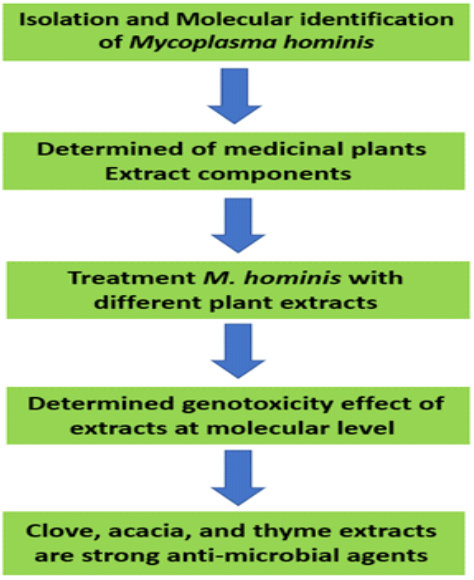
Aim: To assess the antimicrobial activity and genotoxicity of three medicinal plants used by Saudi Arabian people as traditional medicine against *Mycoplasma hominis*.

Methodology: Different concentrations of *Syzygium aromaticum* (clove), *Vachellia nilotica* (acacia), and *Thyme vulgaris* (thyme) extracts were used as antimicrobial agents against *M. hominis*, and their lethal effects on *Mycoplasma* genome DNA were analyzed using repetitive element PCR(Rep-PCR).

Results: The aqueous extracts of clove and *Acacia* at 3.125 mg ml^{-1} were found to be active antimicrobials against three tested *Mycoplasm*. Thyme extract exhibited antimicrobial activity at 12.5 mg ml^{-1}. Moreover, this extract revealed potent lethal activities as growth turbidity decreased with increasing concentration or exposure time as compared to untreated *Mycoplasma*. The results of Rep-PCR clearly indicate that changes occured in the number of genetic bands in treated *Mycoplasma* at certain concentrations as compared to untreated *Mycoplasma*.

Interpretation: These results indicate the possibility of using these extracts as a source of antibacterial compounds for treating infections caused by *Mycoplasma*.

Key words: Antimicrobial activity, Genotoxicity, *Mycoplasma hominis*, Medicinal plants, *S. aromaticum*



How to cite : Yassin, M.H., Y. Alghamdi, E.H. Mohamed, S.A. Mostafa, A. Merghani, H.H. Amer, S.H. Alotaibi, M.M. Soliman, H. Nasr-eldeen and M.M. Hassan: Genotoxicity effects of medicinal plants extracts against bacterial species, *Mycoplasma hominis*. *J. Environ. Biol.*, **42**, 220-228 (2021).

Introduction

Mycoplasma causes various respiratory and urogenital diseases in both animals and humans. These organisms are highly sensitive and depend on the host's vital biological precursors (Gaber *et al.,* 2015; Yassin *et al.,* 2018). *Mycoplasma* has minimum organelles needed for reproduction and growth. Their genome comprises circular DNA molecule. They lack cell wall, and therefore, are completely resistant to penicillin and cephalosporins (Mohamed *et al.,* 2018; Oishi *et al.,* 2019). Urogenital infection with *Mycoplasma* may adversely affect the reproductive system and increase infant mortality. Therefore, it is essential to detect and diagnose these infections at an early stage using appropriate diagnostic methods (Gaber *et al.,* 2015; Mohamed *et al.,* 2020). *Mycoplasma* infection can lead to male and female infertility. Genital infection with *M. hominis* causes fertility disorders (Ahmadi *et al.,* 2010; Maldonado-Arriaga *et al.,* 2019). Fertility in males is checked by semen quality as concentration, motility and abnormalities that can be associated with conception. Infectious processes represent plausible candidates for male infertility. *Mycoplasma hominis* has been proved to cause infertility in males and females.

Mycoplasma are more resistant to many antimicrobials as they lack cell wall. Antimicrobials that are widely used to treat *Mycoplasma* include tetracyclines, macrolide-lincosamide-streptogramin-ketolide antibiotic group and fluoroquinolones. The method for mycoplasma treatment needs extra effort from researchers to treat. Therefore, searching safe alternate medication is the goal for physicians. Recently, medicinal plants have been widely used to prepare life-saving drugs. Bioactive compounds extracted from plants are used as food additives, pesticides, antimicrobials, antioxidants and anticancer agents (El-Tarras *et al.,* 2013; Gaber *et al.,* 2015; Shewamene *et al.,* 2017; Diyanat *et al.,* 2019). Herbs have been used since hundreds of years, not only as oxidative stress relievers and antioxidants agents but also for their strong antimicrobial activity. These compounds belong to a group known as secondary metabolites. Many people worldwide depend on medicinal plants for treating certain diseases (Adwan *et al.,* 2012; Djordjevic, 2019). The main advantage of medicinal plant components is that they do not enhance " resistance", which usually occurs when long-term use of synthetic antibiotics (Shewamene *et al.,* 2017). Traditional medicines have been used for numerous centuries by a major population of Saudi Arabia (El-Tarras *et al.,* 2013; El-Shabasy *et al.,* 2016).

Herbal medicines contain natural substances that promote health and alleviate illness. *Syzygium aromaticum* is a herbal medicine that can be used for the treatment of various diseases. In traditional medicine, clove is used to treat several diseases, extending from inflammation to intestinal disturbances and even cancer (El-Shabasy *et al.,* 2016). Clove contains numerous active ingredients of anti-inflammatory and antioxidant properties (Mohamed *et al.,* 2020). Clove buds have been used in folk medicine for their aromatic properties its carminative activity and as a diuretic stimulant (El-Tarras *et al.,* 2013; El-Shabasy *et al.,* 2016). Essential oil from leaves of *Thyme vulgaris* has *in-vitro* antimicrobial activity against some oral human pathogens, including *Streptococcus pyogenes, Streptococcus mutans* and *Candida albicans* (Maldonado-Arriaga *et al.,* 2019). The present study aimed to isolate and identify Human pathogenic bacteria (*M. hominis*) and study the genotoxicity of traditional herb plants (clove, Acacia, and thyme) against *M. hominis*.

Materials and Methods

Clinical specimens: Semen samples from 92 infertile men were collected from the infertility clinic of King Faisal Hospital, Taif, Saudi Arabia from May 2017 to April 2018. Patients did not intake any antibiotics in the last week before collecting the semen sample. Before sample collection, the patients had to wash their hands and genital area with water and soap. Samples were collected in sterile plastic containers. All specimens were stored at −70°C until further DNA extraction.

Isolation and identification of *Mycoplasma* species: The samples were cultured in brain heart infusion (BHI) broth. The following composition was used for preparing1l of medium: 37 g BHI broth (Hi media, India), 200 ml horse serum (VACSERA, EGYPT), 100 ml freshly prepared yeast extract, 8 ml 10% thallium acetate (Sigma-Aldrich), 1 g ampicillin (EIPICO, EGYPT), 5 g L-arginine (Sigma-Aldrich), 2 ml 1% phenol red, and 700 ml of purified water, at pH 7. The growth of *Mycoplasma* was observed by an alkaline shift from yellow to pink color. Antimicrobial susceptibility testing was performed in BHI broth without inhibitors (ampicillin and thallium acetate). BHI agar was prepared from 54 g of BHI agar (Hi media) and supplemented in the same way as BHI broth.

Antibiotic susceptibility test: Drug sensitivity tests were conducted using standard disk diffusion technique with 20 antibiotic disks (Bio-analyse, Ankara, Turkey): neomycin (N30), vancomycin (VA30), rifampin (RA5), erythromycin (E15), ciprofloxacin (CIP5), clindamycin (DA2), tetracycline (TE30), pefloxacin (PEF5), piperacillin (PRL100), cloxacillin (CX1), clarithromycin (CLR15), nalidixic acid (NA30), chloramphinicol (C30), ceftazidime (CAZ), flocloxacin (FL5), netlimicin (NET30), tobramycin (TOB10), streptomycin (S10), cephalothin (KF30), and metrondiazole (MET5) as described by AOAC (2005).

Amplification and sequencing of 16S rRNA gene in *Mycoplasma*: All *Mycoplasma* isolates were cultured in BHI broth overnight at 37°C. DNA was extracted from *Mycoplasma* isolates according to Yassin *et al.* (2018). PCR amplification of 16S rRNA gene was achieved using forward primer GPO-3 and reverse primer MGSO. PCR was performed using Go Taq Green Master Mix (Promega) following to the methods of Yassin *et al.* (2018). Approximately, 280 bp 16S rDNA fragments were sequenced using same primers and sequencer (Gene Analyzer 3121, Macrogen Co., Seoul, South Korea). The obtained 16S rDNA sequences were analyzed with known 16S rDNA sequences from GenBank by BLAST program, and a phylogenic tree was constructed using MEGA program version 7.10.

Preparation of natural extracts: Three medicinal plants, *T. vulgaris* (thyme), *S. aromaticum* (clove), and *Vacellia nilotica*

(acacia) were purchased from Taif herb store, Taif, Saudi Arabia. All three plants were shade dried at room temperature and ground to a powdered form and thereafter passed through a 2 mm diameter mesh. Cellular materials were disrupted by sonication, and suspensions were rapidly shaken on an orbital shaker for 48 hr. The material in suspension was removed *via* repeated centrifugation at 4000 rpm, followed by vacuum filtration through a 0.45 µM pore nitrocellulose membrane. The extracts were stored at 4°C, and the extracts were characterized by (GC-MS).

Antimicrobial activity of natural extracts: The antimicrobial activities of clove, acacia, and thyme extracts were assessed by the method described by Hannan (2000) with slight modifications. Each isolate was adjusted to approximately 10^6 CFU ml^{-1} and 25 µl of each strain was spread to the surface of BHI agar by running drop technique. Thereafter, 20 µl of each extract was loaded with 6 mm sterile discs, and the discs were placed on the agar plate medium. Ciprofloxacin disc (5µg ml^{-1}) was used as a positive control, and the plates were incubated at 37°C under 10% CO_2 tension for 48 hr. The results were expressed by measuring the inhibition zones. All experiments were repeated in triplicate. The minimum inhibitory concentration (MIC) of each extract was determined using a sterile 96 well plate using micro-dilution method of Al-Momani *et al.* (2007).

Monitoring *Mycoplasma* growth: To study the antibacterial effect of clove, acacia and thyme extracts, different concentrations of extracts were diluted with BHI broth media to make final concentrations (0, 3.125, 6.25, 12.5, 25, and 50 mg ml^{-1}) of extracts. Ten microliters of 10^6 tested *Mycoplasma* was used to inoculate the BHI broth media containing different concentrations of extracts and was incubated at 37°C for 48 hr at 150 rpm in a shaking incubator.

Rep-PCR analysis: Six different rep-PCR primers were used for molecular characterization of the effects of plant extracts (clove, acacia, and thyme) on DNA of *Mycoplasma* isolates grown in BHI broth media supplemented with different concentrations (0, 3.125, 6.25, 12.5, 25, and 50 mg ml^{-1}) of each extract. Six repetitive sequence primers were used to amplify the genomic DNA of *Mycoplasma* isolates. Six primer sequences are as follows: Rep 12 (5'- AGAGAGAGAGAGAGAGC-3'), Rep 18 (5'-ACACACACACACACACG-3'), Rep 19 (5'-AGAGAGAGAGAGAGAGTT-3'), BOX A1 (5'-CTACGGCAAGGCGACGCTGACG-3'), and (GTG)5 (5'-GTGGTGGTGGTGGTG-3'). PCR amplification was performed according to Mazrou *et al.* (2020).

Statistical analysis: The differences between the means of studied treatments were tested by ANOVA at 5% probability.

Table 1: Inhibitory zones (mm) of tested antibiotics against *Mycoplasma* isolates (n = 3)

Antibiotic	Inhibitory zone	Antibiotic	Inhibitory zone
Neomycin (N30)	R (0.0)	Clarithromycin (CLR15)	R (0.0)
Vancomycin (VA30)	R (0.0)	Nalidixic acid (NA30)	R (0.0)
Rifampin (RA5)	R (0.0)	Ceftazidime (CAZ)	R (0.0)
Erythromycin (E15)	R (0.0)	Flocloxacin (FL5)	S (25)
Ciprofloxacin (CIP5)	S (33)	Netlimicin (NET30)	R (0.0)
Chloramphinicol (C30)	S (45)	Tobramycin (TOB10)	R (0.0)
Clindamycin (DA2)	S (44)	Cloxacillin (CX1)	R (0.0)
Tetracycline (TE30)	S (45)	Streptomycin (S10)	R (0.0)
Pefloxacin (PEF5)	S (27)	Cephalothin (KF30)	R (0.0)
Piperacillin (PRL100)	R (0.0)	Metrondiazole (MET5)	R (0.0)

Table 2: Minimum inhibitory concentration (MIC) and importance of Clove, Acacia and Thyme extracts as alternate medication against *Mycoplasmas hominis*

Mycoplasma Isolates	Extracts	MIC data (mg ml^{-1})		
		Range	MIC_{50}	CIP_{50}
M. hominis No. 8	Clove	3.1-6.4	3.13	
	Acacia	2.9-5.8	2.92	3.125
	Thyme	12.5-16.8	12.53	
M. hominis No. 9	Clove	3.2-6.4	3.2	
	Acacia	3-6	3.0	3.125
	Thyme	12.5-16.8	12.53	
M. hominis No. 22	Clove	3.1-6.4	3.13	
	Acacia	2.9-5.8	2.92	3.125
	Thyme	12.5-16.8	12.53	

CIP = Ciprofloxacin (5µg ml^{-1})

Table 3: Potential inhibitory zone for examined extracts (Clove, Acacia and Thyme extracts) against *Mycoplasmas hominis*

Mycoplasma isolates	Extracts	Inhibition zone (mm) at different concentrations			
		3.125 (mg ml^{-1})	6.25 (mg ml^{-1})	12.5 (mg ml^{-1})	CIP (5µg ml^{-1})
M. hominis No. 8	Clove	28^{a}	44^{a}	ND	
	Acacia	22^{c}	36^{d}	ND	33^{b}
	Thyme	ND	25^{i}	39^{c}	
M. hominis No. 9	Clove	26^{b}	40^{b}	ND	
	Acacia	20^{e}	31^{e}	ND	34^{a}
	Thyme	ND	27^{h}	43^{a}	
M. hominis No. 22	Clove	18^{f}	39^{c}	ND	
	Acacia	21^{d}	30^{f}	ND	34^{a}
	Thyme	ND	28^{g}	41^{b}	

CIP = Ciprofloxacin (5µg ml^{-1}); ND = Not detectable, P< 0.05

When the means were significantly different, Duncan's Multiple Range Tests were applied using CoStat software program (Version 6.400).

Results and Discussion

In the present study, 7 of the 92 semen samples (7.6%) were positive for *Mycoplasma* and were subjected to biochemical assays. Biochemical characterization revealed that all isolates were sensitive to digitonin and tested positive for arginine deamination and negative for glucose fermentation. Three isolates with same antibiotic susceptibility profile were selected for further study. These isolates were resistant to neomycin (N30), vancomycin (VA30), rifampin (RA5), erythromycin (E15), piperacillin (PRL100) and ampicillin (AM); however, they were susceptible to ciprofloxacin (CIP), clindamycin (DA), clarithromycin (CLR15), nalidixic acid (NA30), ceftazidime (CAZ), netlimicin (NET30), tobramycin (TOB10), cloxacillin (CX1), streptomycin (S10), cephalothin (Kf30) and metrondiazole (MET5). They were highly sensitive to tetracycline (TE30) and chloramphinicol (C30) with inhibition zone of 45 mm, moderately resistant to ciprofloxacin (CIP5) with inhibition zone of 33 mm, and less resistant to flocloxacin (FL5) with inhibition zone of 25 mm (Table 1).

These results are in accordance to those obtained by Yassin *et al.* (2018). Moreover, these results were inconclusive because *Mycoplasma* has the potential to develop antibiotic resistance which may be related to some changes in both genome and protein. These include changes in the gene expression of several genes and cellular proteins related to resistance and virulence (Mihai *et al.,* 2011). Effective treatment with a specific antibiotic reduces the risk of *Mycoplasma* in humans (Stanley *et al.,* 2001; Mihai *et al.,* 2011). PCR was simultaneously performed for all samples tested in this study using universal *Mycoplasma* primers. Seven semen samples detected one specific band for *Mycoplasma* at approximately 280 bp; however, in other semen samples the band was absent. The 16S rDNA nucleotide sequences revealed 99% similarity and the partial sequences were deposited in the GenBank database (Fig. 1). When these sequences were aligned with the database sequences, seven *Mycoplasma* isolates were identified as *M. hominis*, which infected the reproductive channels in males and females; *M. hominis* Tu7 presented 98% sequence similarity with *M. hominis* NR025971 and *M. hominis* Aj002265. The phylogenetic relationships of *Mycoplasma* strains were inferred using neighbor-joining method.

Phylogenetic analysis revealed 77% similarity between *M. hominis* TU7 and *M. fermentans* NR113683 that infected humans. PCR technique is a specific, simple, sensitive, and effective molecular technique for identifying *Mycoplasma* infection (Stanley *et al.,* 2001; Mihai *et al.,* 2011; Mohamed *et al.,* 2018). Medicinal plants comprise various compounds that are used to treat chronic and infectious diseases in humans and/or animals. Hence, these plants can be used as alternative treatments to the present antimicrobial therapy (Gaber *et al.,* 2015; Yu *et al.,* 2019). Clove is highly effective and is widely used in folk medicine (Cortés-Rojas *et al.,* 2014). Gas chromatography mass spectrometry (GC–MS) was used to identify the molecular ion peaks for the crude extract of clove, that is, 1a, 1b, 1c, 1d, 1e, 1f, 1g, and 1h at 331 [M^+], 581 [M^+], 344 [M^+], 300 [M^+], 207 [M^+], 416 [M^+], 430 [M^+], and 342 [M^+], respectively. The functional groups in the compounds, such as acetyl ($COCH_3$), carbonyl (CO), hydroxyl (OH), and amino (NH_2) groups contribute to the biological activity of *S. aromaticum* and clove extracts (Ali *et al.,* 2012). GC–MS was used to identify the molecular ion peaks for the crude extract of acacia, that is, 2a, 2b, 2c, 2d, and 2e at 502 [M^+], 420 [M^+], 332 [M^+], 528 [M^+], and 433 [M^+], respectively. The biological activity of *V. nilotica* extract was due to the presence of various functional groups.

Thyme contains 40% of essential oil thymol, which prevents the growth of pathogenic bacterial strains. The oil was successfully used as an antibacterial and antifungal agent in the bread and cheese industry (Maksimov, 2017). The biological activity of *T. vulgaris* (thyme) extract is due to the presence of various functional groups in the compounds, such as acetyl, carbonyl, hydroxyl, and nitro groups. The results of the present study revealed that all *Mycoplasma* species examined were

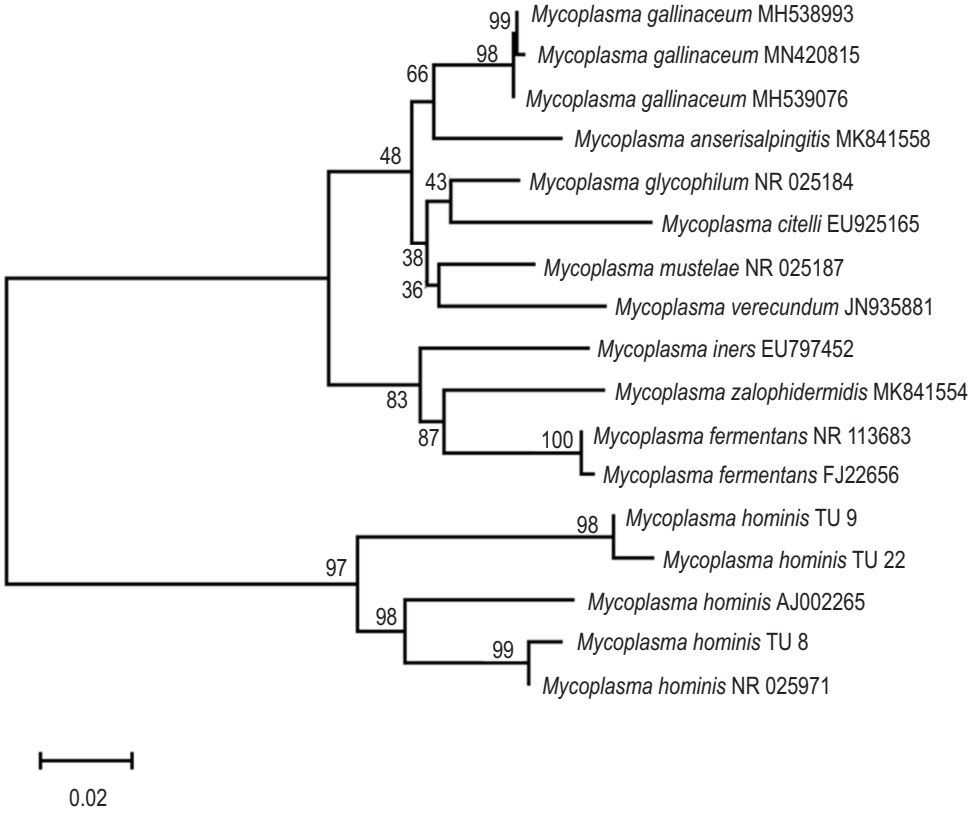


Fig. 1: Neighbor-joining phylogeny based on 16S rRNA gene sequences of three *Mycoplasma* isolates and related *Mycoplasma* species obtained from a BLAST search of NCBI database.

susceptible to *T. vulgaris, S. aromaticum* and *V. nilotica* extracts. To compare the antimicrobial activity of three-medical plant extracts, the agar well diffusion method was used. This method allows better diffusion of extracts into the medium, thus, enhancing contact with the organisms (Gaber *et al.,* 2015).

The antimicrobial activities as clear inhibition zones (in mm diameter) of water extracts of *T. vulgaris*, *S. aromaticum*, and *V. nilotica* at 3.125, 6.25, and 12.5 mg ml^{-1}, were investigated. Three extracts were used against three *M. hominis* isolates. The results presented in Table 2 indicate that the MIC_{50} of clove, acacia, and thyme ranged from 3.13 to 3.2 mg ml^{-1}, 2.92 to 3 mg ml^{-1}, and 12.5, respectively, with three treated *Mycoplasma* isolates. CIP_{50} was 3.125 in all treated *Mycoplasma* samples. Moreover, the clove and acacia extract at final concentrations of 3.125 mg ml^{-1} were active against the three *Mycoplasma* isolates and inhibition zone ranged from 20 to 28 mm, whereas, at the same concentration, thyme extract was inefficient to kill most of the treated *Mycoplasma* isolates and no inhibition zone was detected (Table. 3). In contrast, the final concentration of 6.25 mg ml^{-1} of clove and acacia extracts was more effective in increasing the inhibition zone from 31 to 44 mm. The inhibition zone of thyme extract at same concentration ranged from 25 to 28 mm. At 12.5 mg ml^{-1}, most treated *Mycoplasma* were killed and no inhibition zone was observed for clove and acacia extracts.

The inhibition zone of thyme extract ranged from 39 to 43 mm with three treated *Mycoplasma* isolates compared with ciprofloxacin (5µg ml^{-1}) treatment, which ranged from 33 to 43 mm. It was found that the inhibitory activity was dependent on the dose and duration of exposure to the extract. Moreover, due to high cost of synthetic antibiotics, their side effects and development of resistance of microorganisms to commonly used drugs, the search for natural alternatives to antibiotics is important (Yu *et al.,* 2019; Diyanat *et al.,* 2019). Finally, the optical density of *Mycoplasma* isolates treated with clove, acacia, and thyme extracts at different concentrations were measured. The optical density decreased with an increase in the concentration from 0 to 50 mg ml^{-1} of different extracts of clove, acacia, and thyme. The optical densities of *M. hominis* No. 8 isolates treated with clove, acacia, and thyme ranged from 3.565 to 0.213, 3.646 to 0.115, and 3.675 to 0.878, respectively (Fig. 2). The second *Mycoplasma* isolate (*M. hominis* No. 9) was also treated with different concentrations of clove, acacia, and thyme extracts, wherein the optical densities of clove, acacia and thyme ranged from 3.643 to 0.326, 3.667 to 0.110 and 3.342 to 0.414, respectively. In contrast, the third *Mycoplasma* isolate (*M. hominis* No. 22) was also treated with different concentrations of clove, acacia, and thyme extracts,

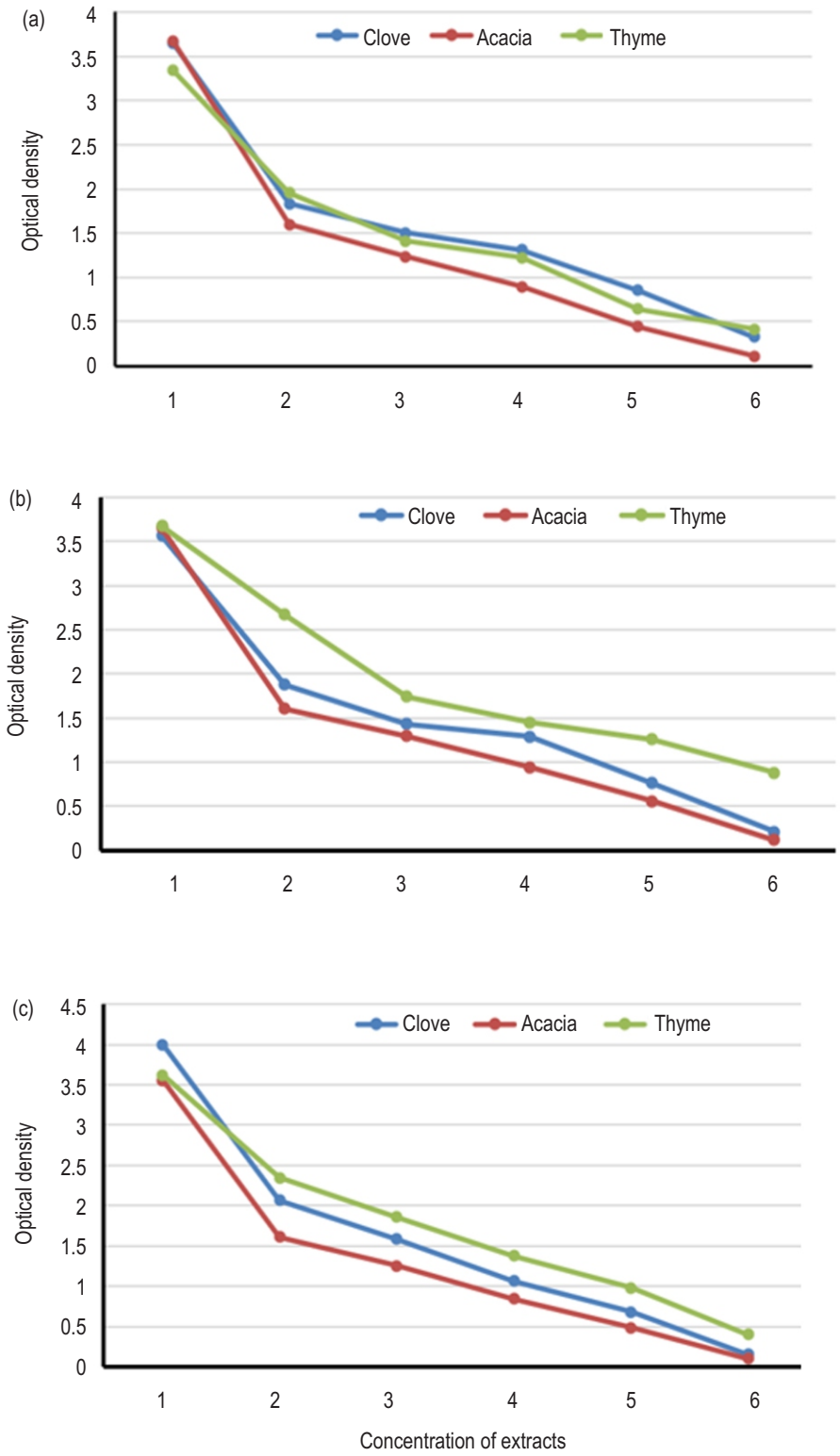


Fig. 2: Antimicrobial activity of different concentrations of clove, acacia and thyme extracts as indicated by the changes in optical density of three different human mycoplasma strains: (a) Human mycoplasma strain 8; (b) Human mycoplasma strain 9 and (c) Human mycoplasma strain 22.

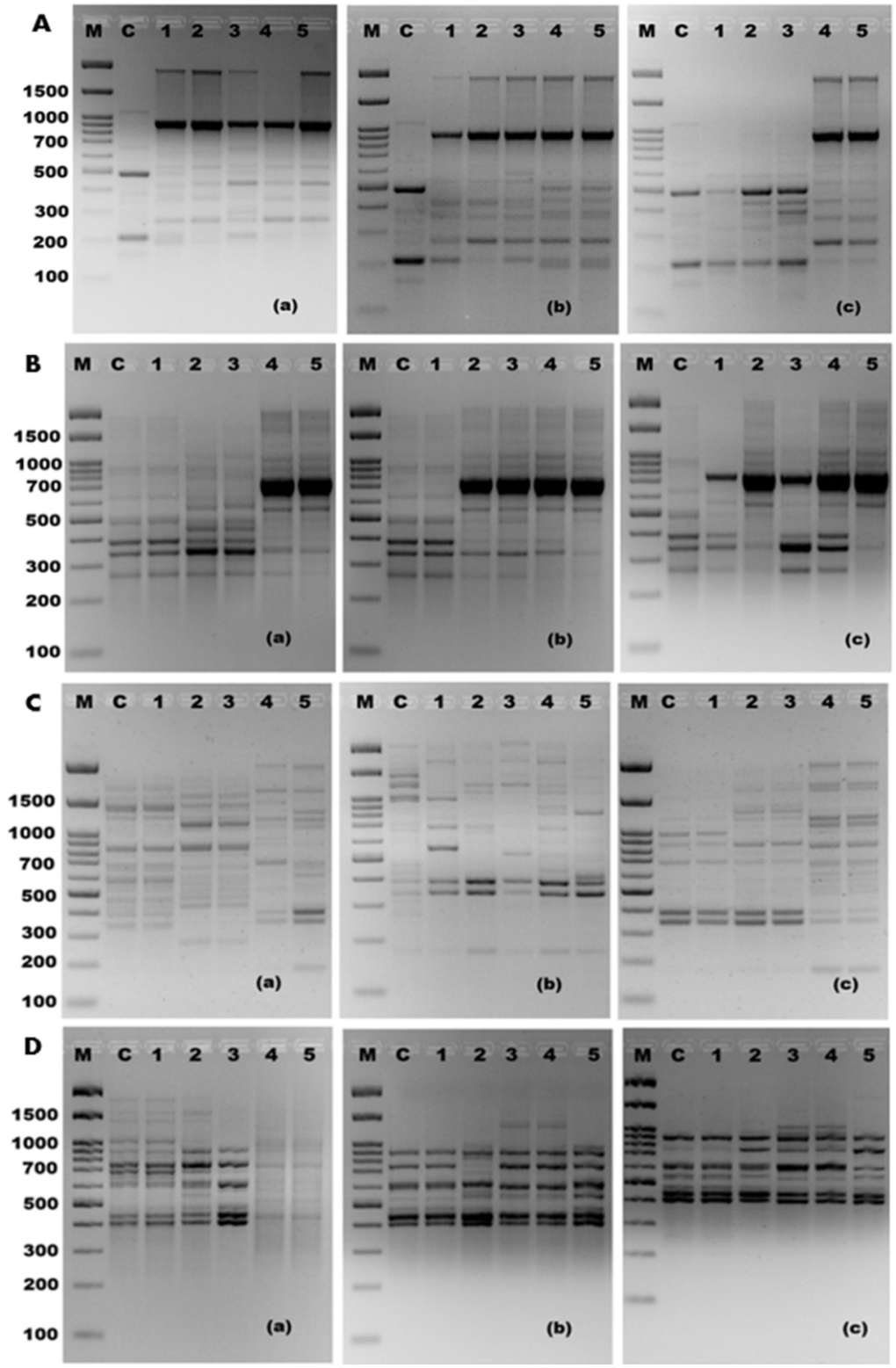


Fig. 3: Rep-PCR profile of different treated *Mycoplasma* strains generated with (A) Box A1 primer, (B) GTG5 primer, (C) rep 12 primer and (D) rep-18 primer using different concentrations of thyme extracts: (a) *Mycoplasma* strain No. 8; (b) *Mycoplasma* strain No. 9 and (c) *Mycoplasma* strain No. 22. M is 100 bp DNA ladder; C = untreated *Mycoplasma*. The concentrations of thyme extracts were 1 = 3.125, 2 = 6.25, 3 = 12.5, 4 = 25, and 5 = 50 mg ml^{-1}, respectively.

wherein their optical densities ranged from 3.996 to 0.154, 3.557 to 0.099 and 3.621 to 0.397, respectively.

Therefore, these plants can be considered readily available alternatives to drugs such as fluoroquinolones, tetracyclines, macrolides and chloramphenicols, which are presently used to treat *Mycoplasma* infection (Mohamed *et al.,* 2018; Djordjevic, 2019). In order to explore the stability of the genetic material, at molecular level, in the treated *Mycoplasma* as a result of antimicrobial activities of different concentrations of clove, acacia and thyme extracts, the changes in the *Mycoplasma* genetic material due to the treatment of plant extracts was evaluated using rep-PCR analysis for genomic DNA. The rep-PCR results indicated that numerous polymorphic numbers of genetic bands were from the electrophoretic products of PCR for treated *Mycoplasma* strain compared with those of untreated *Mycoplasma* (Fig. 3A-D). Fig. 3-A illustrates the rep-PCR profile of treated *Mycoplasma* strain No.8 generated with Box A1 primer using different concentrations of clove, acacia, and thyme extracts. In Fig. 3 A-a, in untreated *Mycoplasma* (control), two bands with molecular weights of 180 and 1100 pb were observed that were absent in treated *Mycoplasma* with different concentrations of thyme. The band with a molecular weight of 1900 was absent in the control and concentration 4 (25 mg ml^{-1}) of thyme extract. Similar results were observed in Fig. 3 A-b; the band with molecular weight 1900 was found in all treated *Mycoplasma* with different concentrations of acacia and was absent in control. Moreover, the band at 190 pb was observed at high concentrations (25 and 50 mg ml^{-1}). For thyme treatment Fig. 3 A-c, three bands with molecular weights of 1800, 900 and 230 pb were found at high concentrations (25 and 50 mg ml^{-1}).

The rep-PCR profile of treated *Mycoplasma* strain No. 8 generated with (GTG) 5 primer using different concentrations of clove, acacia, and thyme extracts are illustrated in Fig. 3B-b. Two bands with molecular weights of 700 and 550 bp were observed in treated *Mycoplasma* with high concentrations of acacia 2, 3, 4, and 5 (6.25, 12.5, 25 and 50 mg ml^{-1}), whereas, they were absent in *Mycoplasma* treated with 3.125 mg ml^{-1} of acacia and in control, and similar results were observed in Fig. 3B-c. The band with 280 bp was observed in all *Mycoplasma* treatments and was absent in treatments 2 and 5 (6.25 and 25 mg ml^{-1}). In contrast, Fig. 3C and 3D illustrate the rep-PCR profile of different treated *Mycoplasma* strains (No. 8, No. 9 and No. 22, respectively) generated with rep-12 and rep-18 primers using different concentrations of thyme extracts. Fig. 5 illustrates the rep-12 profile of different *Mycoplasma* strains treated with thyme. Fig. 3C-a presents a band with a molecular weight of 850 bp observed in control and low concentrations and absent athigh concentrations 4 and 5 (25 and 50 mg ml^{-1}) of thyme. Moreover, a band with a molecular weight of 2000 was observed at high concentrations of 4 and 5 (25 and 50 mg ml^{-1}). Fig. 3C-b illustrates the band with molecular weight 550 bp found in treatment 1 (3.125 mg ml^{-1}) only. Fig. 3C-c depicts bands with molecular weights of 600 and 2000 pb observed at high concentrations of 4 and 5 (25 and 50 mg ml^{-1}) and absent in other treatments and control.

The band with 1350 bp was observed in treatments 2 and 3 (6.25 and 25 mg ml^{-1}) only and absent in other treatments and control. Fig. 3D-a depicts three bands found in control and low concentration and absent in high concentrations 3, 4 and 5 (12.5, 25, and 50 mg ml^{-1}) in *Mycoplasma* strain no. 8. Fig. 3D-b for *Mycoplasma* strain no. 9 presents two bands with molecular weight 1300 and 1350 bp found in *Mycoplasma* treatments 3 and 4 (12.5 and 25 mg ml^{-1}) of thyme. Fig. 3D-c presents similar results as Fig. 3 D-b, with band 1000 pb found in *Mycoplasma* treatments 3 and 4 (12.5 and 25 mg ml^{-1}) of thyme. In the present study, the genetic effects of aforementioned three medicinal plants extracts as mutagenic agents were confirmed by rep-PCR. The results of this analysis revealed a polymorphic banding pattern when compared untreated bacteria and those treated with different concentrations of clove, acacia and thyme extracts (Fig. 3A-D). This indicates the ability of these medical plant extracts to induce point mutations as a result of deletion compromising at least one nucleotide, as revealed by the disappearance of several genetic bands and change in primer matching sites as compared with those of untreated bacteria. These results suggest molecular changes as deletion or frame shift mutations in one or more loci that affect gene expression and interruption in biochemical pathways of DNA and protein synthesis, as alkaloids in clove, acacia, and thyme extracts often do. Moreover, these results are in accordance with those obtained by Al-Momani *et al.* (2007) and El-Tarras *et al.* (2013).

Some of the components of these medical plant extracts may act as intercalating agents or generate free radicals that interact with genomic DNA to account for the observed deletions as suggested by similar results obtained by Baeshin *et al.* (2008) and Mazrou *et al.* (2020). *Aspergillus terreus* in their study with *Cryptolepis sangvinolehta* and Furthermore, the damage seen in the SEM images indicates the lethal effect of high concentrations of *Rhazya stricta* leaf extract on treated bacteria (El-Tarras *et al.,* 2013; Gaber *et al.,* 2015). Similarly, oregano and thyme essential oil exhibited strong antimicrobial properties against human mycoplasmas and ureaplasmas, and the observed cells were damaged when treated with essential oils; these results are in accordance with the reports of Oishi *et al.* (2019), and revealed the mechanism of action for essential oil components in *Mycoplasma* cells. It was thought to be the degradation of cell wall damage to cytoplasmic membrane (El-Tarras *et al.,* 2013).

T. vulgaris, S. aromaticum and *V. nilotica* extracts, proved to have antimicrobial activities against *Mycoplasma hominis* and can be used as a source of antimycoplasma safe compounds. The antimicrobial activities of these extracts can be explained at molecular level by rep-PCR in concentration dependent manner.

Acknowledgment

The authors extend their appreciation to the Deanship of Scientific Research at Taif University for funding this research through the Program of Research Groups under project number 1/439/6064.

Add-on Information

Authors' contribution: M.H. Yassin, Y. Alghamdi: Microbiological work; **E.H. Mohamed, H. Nasr-eldeen:** Collected the Mycoplasma samples**, S.A. Mostafa, A. Merghani, H.H. Amer:** Biochemical analysis; **S.H. Alotaibi, M.M. Soliman, M.M. Hassan:** Genetical analysis.

Research content: The research content is original and has not been published elsewhere

Ethical approval: Not Applicable

Conflict of interest: The authors declare that there is no conflict of interest.

Data from other sources: Not Applicable

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*..

References

Adwan, G., Y. Salameh, K. Adwan and A. Barkat: Assessment of antifungal activity of herbal and conventional toothpastes against clinical isolates of *Candida albicans*. *Asian Pac. J. Trop. Biomed.,* **2**, 375–379 (2012).

Ahmadi, M., A.N. Mozaffari, B. Kazemi, M. Sedighigilani and F. Masjedianjazi: Detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* in semen of infertile men referred to Royan Institute by PCR method. *Cell J. Yakhteh.,* **12**, 371-380 (2010).

Ali, A., N. Akhtar, B.A. Khan, M.S. Khan, N. Khalid, K. Waseem, T. Mahmood and L. Ali: *Acacia nilotica*: A plant of multipurpose medicinal uses. *J. Med. Plants Res.,* **6**, 1492-1496 (2012).

Al-Momani, W., E. Abu-Basha, S. Janakat, R.A.J. Nicholas and R.D. Ayling: *In vitro antimycoplasmal* activity of six Jordanian medicinal plants against three *Mycoplasma* species. *Trop. Anim. Hlth. Prod.,* **39**, 515-519 (2007).

Association of Official Analytical Chemists (AOAC) Official Methods of Analysis of AOAC International.18th Edn. Maryland, USA: AOAC International (2005).

Baeshin, N.A., S.H. Qari, J.S.M. Sabir and A.M. ALhejin: Biochemical and molecular evaluation of genetic effects of *Rhazya stricta* (Decne) leafs extract on *Aspergillus terreus*. *Saudi J. Biol. Sci.,* **15**, 25-33 (2008).

Cortés-Rojas, D.F., C.F. de-Souza and W.P. Oliveira: Clove (*Syzygium aromaticum*): A precious spice. *Asian Pac. J. Trop. Biomed.,* **4**, 90-96 (2014).

Diyanat, S., M. Cheniany and M. Lahouti: Investigating the use of pine needle extract as biological herbicide for weed control in bean crop. *J. Envir. Biol.,* **40**, 1164-1172 (2019).

Djordjevic, S.M.: From medicinal plant raw material to herbal remedies. In: Aromatic and Medicinal Plants: Back to Nature (Ed.: H.A. El-Shemy), pp. 269-288 (2019).

El-Shabasy. A.: Survey on medicinal plants in the flora of Jizan region, Saudi Arabia. *Int. J. Bot. Stud.,* **2**, 38–59 (2016).

El-Tarras, A.E., M.M. Hassan and M.A. El-Awady: Evaluation of the genetic effects of the *in vitro* antimicrobial activities of *Rhazya stricta* leaf extract using molecular techniques and scanning electron microscope. *Afr. J. Biotech.,* **12**, 3171-3180 (2013).

Gaber, A., M.M. Hassan, E.S. Dessoky and O.A. Attia: *In vitro* antimicrobial comparison of Taif and Egyptian pomegranate peels and seeds extracts, *J. Appl. Biol. Biotechnol.,* **3**, 012-017 (2015).

Hannan, P.C.: Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary mycoplasma species. International Research Programme on Comparative Mycoplasmology. *Vet. Res.,* **31**, 373-395 (2000).

Hassan, M.M. and E.S.B. Belal: Antibiotic resistance and virulence genes in *Enterococcus* strains isolated from different hospitals in Saudi Arabia. *Biotechnol. Biotechnol. Equip.,* **30**, 726-732 (2016).

Maksimov, O.: Thyme *Thymus vulgaris* L. Thymol CT essential oil as natural preservative. *Amer. J. Essent. Oils Nat. Prod.,* **5**, 19-22 (2017).

Maldonado-Arriaga, B., N. Escobar-Escamilla, J. C. Pérez-Razo, S. L. Alcaráz-Estrada, I. Flores-Sánchez, D. Moreno-García, R. Pérez-Cabeza, P. Mondragón-Terán, J. Shaw, C. Hernandez-Cortez, G. Castro-Escarpulli and J.A. Suárez-Cuenca: Mollicutes antibiotic resistance profile and presence of genital abnormalities in couples attending an infertility clinic. *J. Inter. Med. Res.,* **48**, e3000605 (2019).

Mazrou, Y.S., A.H. Makhlouf, E.R. Elbealy, M.A. Salem, M.F. Awad, M.M. Hassan and M. Ismail: Molecular characterization of phosphate solubilizing fungi *Aspergillus niger* and its correlation to sustainable agriculture. *J. Environ. Biol.,* **41**, 592-599 (2020).

Mihai, M., N. Valentin, D. Bogdan, C.M. Carmen, B. Coralia and S. Demetra: Antibiotic susceptibility profiles of *Mycoplasma hominis* and *Ureaplasma urealyticum* isolated during a population-based study concerning women infertility in Northeast Romania. Brazil. *J. Microbiol.,* **42**, 256-260 (2011).

Mohamed, A.A., M.H. Yassin, M.M. Hassan, A.M. Sabry and A.M. Ibrahim: Molecular and bacteriological diagnosis of *Mycoplasma* species infection in camels at Taif Governorate, Saudi Arabia. *Ann. Res. Rev. Biol.,* **23**, 1-6 (2018).

Mohamed, E.H., Y.S. Alghamdi, S.M. Abdel-Hafez, M.M. Soliman, S.H. Alotaibi, M.Y. Hassan, H. Nasr-AlDeen and H.H. Amer: Susceptibility assessment of multidrug resistant bacteria to natural products. *Dose Res.,* **20**, 1-11 (2020).

Oishi, T., K. Takahashi, S. Wakabayashi, Y. Nakamura, S. Ono, M.Kono, A. Kato, A. Saito, E. Kondo, Y. Tanaka, H. Teranishi, H. Akaike, T. Tanaka, I. Miyata, S. Ogita, N. Ohno, T. Nakano and K. Ouchi: Comparing antimicrobial susceptibilities among *Mycoplasma pneumoniae* isolates from pediatric patients in Japan between two recent epidemic periods. *Antimicrob. Agents Chemother.,* **63**, 2517-2518 (2019).

Shewamene, Z., T. Dune and C.A. Smith: The use of traditional medicine in maternity care among African women in Africa and the diaspora: A systematic review. *BMC Comp. Alter. Med.,* **17**, 382 (2017).

Stanley, W.A., C.H. Hofacre, G. Speksnijder, S.H. Kleven and S.E. Aggrey: Monitoring *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in breeder chickens after treatment with enrofloxicin. *Avian Dis.,* **45**, 534-539 (2001).

Yassin, M.H., A.A. Mohamed, M.M. Hassan, A.A. Baiomy and A.M. Ibrahim: Molecular characterization of two new *Mycoplasma* species isolated from chickens in Saudi Arabia. *Biotechnology,* **17**, 142-150 (2018).

Yu, D., I.H. Yun, N.T.D. Pham, Y.M. Jang, S.K. Park, S.H. Eom and Y.M. Kim: Antibacterial activity of *Eriobotrya japonica* leaf extracts against methicillin-resistant *Staphylococcus aureus*. *J. Envir. Biol.,* **40**, 1129-1136 (2019).