ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Evaluation of *In Vitro* Utilization of Some Medicinal Plants Seeds as Source Antimicrobials

Mohamed Elkhatim Abdelmageed[1], Hyder O Abdalla[2], Omer H Arabi[3], Babiker MS[4] and Abdel Moneim E Sulieman[5]*

[1]*Department of Meat Production and Technology, Faculty of Animal Production, University of Gezira, Elmanagil, Sudan*

[2]*Department of Animal Production, Faulty of Agricultural Sciences, University of Gezira, Wad Medani, Sudan*

[3]*Department Basic Sciences, Faculty of Animal Production, University of Gezira, Elmanagil, Sudan*

[4]*Department of Poultry Production, Faculty of Animal Production, University of Gezira, Elmanagil, Sudan*

[5]*Department of Biology, Faculty of Science, University of Hail, Hail, Kingdom of Saudi Arabia*

***Corresponding Author**: Abdel Moneim E Sulieman, Department of Biology, Faculty of Science, University of Hail, Hail, Kingdom of Saudi Arabia.

Received: September 01, 2018; **Published:** December 13, 2018

Abstract

Introduction: Medicinal plants may represent an alternative treatment for non-severe cases of infectious diseases as they serve as possible source of new and cheap antibiotics.

Objective: The aim of the study is to evaluate the *in vitro* antimicrobial effects of some medicinal plants seeds including Black cumin (BS) Fenugreek (FENS), *Moringa oleifera* (MORS), *Clitoria ternatea* (CLIS), Sunflower seeds (SFS using petroleum ether, methanol and water extracts against some bacteria and yeast and mold strains.

Materials and Methods: The discs diffusion method and sensitivity test were used. Three pathogenic bacteria strains (*Salmonella typhi, E. coli* and *Staphylococcus aureus*) and yeast and mold were obtained from Microbiology Laboratory of Faculty of Engineering and Technology University of Gezira, Sudan. The mechanical extract of the tested seeds prepared at National Oilseed Processing Research Institute University of Gezira (NOPRI) by using electric presser, while the methanolic and water extracts of the tested seeds powder were prepared by socking 25 grams from each in 250 ml of methanol and water for three days, however the petroleum ether extract was prepared by using the soxhlet.

Results and Discussion: The results showed that tested seeds (BS, CLIS, FENS, MORS and SFS) had an *in vitro* antifungal and antimicrobial effect on Gram +ve and Gram -ve bacteria, *Clitoria ternatea* petroleum ether extract had the highest inhibition zone on the tested microorganisms, even more than the commonly used antibiotics.

Conclusions: The tested seeds can be used as an *in vitro* antimicrobial natural source.

Keywords: *Black Cumin; Fenugreek; Moringa oleifera; Clitoria ternatea; Sunflower; Salmonella*

Introduction

Plants have a vital source of medicine for thousands of years. Indeed today, the World Health Organization gauges that up to 80 per cent of people still depend chiefly on conventional cures such as herbs for their medicines. Plants are also the source of many modern medicines. It is assessed that around one fourth of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances. The most popular analgesic, aspirin, was originally derived from species of *Salix* and *Spiraea* and some of the most valuable anti-cancer agents such as paclitaxel and vinblastine are derived solely from plant sources [1,2].

Medicinal plants and herbs can be utilized in numerous ways. A standout amongst the most widely recognized ways is adding new herbs to sauces, stews, soups, plates of mixed greens, and as accents for as accents for finished meals. Another mainstream technique for utilization is herbal teas. Also, medicinal herbs can be found in supplements, tinctures - liquid extracts of herbs that you take orally - and salves that can be applied topically to address bites, sores, rashes, and a wide range of other skin issues.

Herbal medicine is described by the utilization of restorative plants or plant parts containing substances or classes of substances in charge of their remedial activity after accumulation forms, stabilization when appropriate, and drying, and may exist in full shape, grodd, grodd and chopped [3]. Co-administration of medicinal plants alongside prescription medications can cause unexpected interactions [4] through carelessness in relation to their utilization, regularly determined, by philosophies of life, personal and cultural customs. Some of these factors derive from patients, but may also arise through health professionals without expertise in this area [5].

The need for new antimicrobial agents is closely linked with the problem of emergence of strains that are resistant to most synthetic antibiotics. This has arisen due to extensive use of antibiotics, which renders most of the current antimicrobial agents inefficient in controlling some bacterial diseases [6]. There is an increased evidence to proof that medicinal plants may represent an alternative treatment for non-severe cases of infectious diseases. They could also serve as possible source of new and cheap antibiotics to which pathogenic strains are not resistant and several works provide scientific bases for the popular use of plants against infectious diseases [7]. Herbs, spices and their extracts were already used thousands of years ago in Mesopotamia, Egypt, India China and old Greece, where they were appreciated for their specific aroma and various medicinal properties [8]. In the present study, the in vitro antimicrobial effects of some medicinal plants seeds were assessed.

Material and Methods

Culture media

The cultured media contains the required nutrients in the correct amount, suitable osmotic pressure and pH. Microorganism were incubated in an atmosphere and temperature most suited to their metabolism [9].

In vitro Antimicrobial activity test

Three pathogenic bacteria strains (*Salmonella typhi, E. coli* and *Staphylococcus aureus*) and yeast and mold were obtained from Microbiology Laboratory of Faculty of Engineering and Technology University of Gezira, Sudan. The mechanical extract of the tested seeds prepared at National Oilseed Processing Research Institute University of Gezira the (NOPRI) by using electric presser, while the methanolic and water extracts of the tested seeds powder were prepared by socking 25 grams from each in 250 ml of methanol and water for three days, the mixture was placed at room temperature, then the extraction was filtrated using filter paper, then the extract was kept in plastic tubs and stored at 37°C pending using. On the other hand, the petroleum ether extract was prepared by using the soxhlet. The extracts were used as crude materials without any dilutions (100%).

The antimicrobial activity test of petroleum ether, mechanical, methanolic and water extracts of the tested seeds powder, were performed according to the procedure of Bauer Kirby, as described by Prescot., et al [10]. In the procedure of Bauer Kirby, 0.1 ml of bacterial and fungal inocula were transferred to sterile Petri-dishes containing nutrient agar for bacteria growth and Potato dextrose agar (PDA) for yeast and molds growth. The inocula were spreaded on the surface of the agar with aid of sterile glass spreaders. Sterile antibiotic assay discs 5 mm in diameter) were immersed for 10 minutes in petroleum ether, mechanical, methanolic and water extracts of the tested seeds powder (300 mg/ml). The impregnated discs were precisely placed on the surface of inoculated nutrient agar. The plates were incubated for 24 hour at 30°C. The inhibition zones were measured in (mm).

Sensitivity test

The test done to evaluate the sensitivity of *E. coil, Salmonella typhi* and *Staphylococcus* to antibiotics were performed according to the procedure of Bauer Kirby, as described by Prescot., *et al* [10]. In the procedure of Bauer Kirby, 0.1 ml of bacterial and fungnal inocula were transferred to sterile Petri-dishes containing nutrient agar for bacteria growth and Potato dextrose agar (PDA) for yeast and molds growth. The inocula were spreaded on the surface of the agar with aid of sterile glass spreaders then airing of antibiotics precisely placed on the surface of inoculated nutrient agar. The plates were incubated for 24 hour at 30°C. Then the inhibition zones of antibiotics in the growth compared with the highest inhibition zones of different extracts from tested seeds on the *E. coil, Salmonella typhi* and *Staphylococcus.*

Statistical analysis

The data was analyzed using MSTAT program.

Results and Discussion

In vitro antimicrobial effects of different seeds extracts:

Table 1 shows that Petroleum ether extract of CLlS shows the highest inhibition zone (31.5 mm) followed by BS (20.125 mm), MORS (10 mm) and the lowest inhibition zone shows by SFS without any effect on *E. coil.* The CLlS shows a highest inhibition zone (17 mm) on *Salmonella typhi* followed by BS (14.5 mm). MORS (7 mm) however *Salmonella typhi* was resistant to (SFS and FENS) extracts. CLlS extract appear as a strong inhibitor with (20.8 mm) zone on *Staphylococcus aureus*, while BS shows (9 mm). On contrast (SFS, MORS and FENS) had no effects on *Staphylococcus aureus*. SFS shows positive effect on *Candida* (10 mm) while (BS and MORS) shows same inhibition zone (7 mm). Nevertheless CLlS and FENS shows no effect on *Candida*.

Mechanical extract effects on *E. coli* as in table 1 shows that the highest zone (15 mm) was recorded by BS and MORS which exhibited (14 mm) zone however (SFS, CLlS and FENS) were resisted by the *E. coli*. There was no effect of any mechanical extract on *Salmonella typhi* and *Staphylococcus aureu*s except BS which recorded (9 mm) on *Salmonella typhi* and (17.6 mm) on *Staphylococcus aureus*. *Candida* shows sensitivity for BS (10 mm) and MORS (7 mm) and it was resistant to SFS, CLlS and FENS.

Methanolic extracts of the tested seeds had no effects on *Salmonella typhi, Staphylococcus aureus, Candida* and *E. coli* except FENS (15 mm) on *E. coli*. Water extraction of BS shows the highest inhibition zone (13.5 mm), followed by MORS (13 mm), CLlS (12.8 mm) and (9 mm). FENS had no effect on *E. coil*. SFS extraction appeared to have (14.6 mm) as the highest inhibition zone on *Salmonella typhi* followed by CLlS (13 mm), MORS (12.5 mm) and BS (12.3 mm), while FENS had no effect on *Salmonella typhi*. *Staphylococcus* was sensitive to CLlS

(11.3 mm) and MORS (7 mm), while BS, SFS and FENS had no effects on *Staphylococcus aureus*. The same water extract of CLlS and MORS shows 12.8 mm, 13 mm inhibition zone on *Candida*, while BS, SFS and FENS had no effects.

BS extracts had antimicrobial effects on Gram positive and Gram negative bacteria specially the petroleum extraction and the mechanical extract (oil), the results were in agreement with many authors (Salman., *et al*. 2008). Also the results shows that BS had antifungal activity, this result agreed with Akhtar., *et al* [11]. The antimicrobial activity of this oil may be attributed to the presence of thymoquinone The results shows that BS methanolic extract had no antimicrobial effect, which agreed with Rakhshandeh., *et al.* [14], who reported that BS methanolic extract had antimicrobial effect on *E. coli* and disagreed with the same author who found that the methanolic extract had no antimicrobial effect against *Staphylococcus aureus,* and with Islam., *et al.* [15] who reported that methanol extracts of BS shows high inhibitory effect against Gram-positive and Gram-negative clinical bacterial strains during germination phases as compared to BS seed extract, the extracts shows highest activity from 5th day to 11th day of germination. The result was in agreement with Karrouchi., *et al.* [16] who reported that methanolic extract had no antifungal effect.

The MORS Petroleum (oil) and aqueous extracts appeared as effective antimicrobial on Gram positive and Gram negative bacteria the results were in agreement with Chuang., *et al* [17]. The water extracts appeared to be more effective as antimicrobial against gram positive and Gram negative bacteria, the results were in agreement with Saadabi [18]. The observations on both positive and Gram negative effects in the same plant extracts may be explained by the presence of a wide spectrum of bactericidal substances, or by the action of toxins produced by the plant. The present study demonstrated that MORS oil was effective as antifungal, which agreed with Chuang., *et al.* [17] and the aqueous extract was active as antifungal which agreed with Chuang., *et al* [17]. These results were disagreed with Abu Sayeed., *et al.* [19] who reported that methanol extract possessed moderate antibacterial activity against bacterial strains, *Staphylococcus aureus, Salmonella typhi,* and *Proteus* species and antifungal activity against pathogenic fungi; *Alternaria* sp, *Colletotrichum* sp, *Curvularia* sp and *Fusarium* sp.

Aqueous extraction of SFS appeared as antibacterial against both Gram negative and Gram positive, also SFS petroleum ether extract (oil) exhibited antifungal activity. These results disagreed with Subashini and Rakshitha (2012) [20] who evaluated the antimicrobial activity of methanolic extract of SFS. On the basis of results of antibacterial activity analysis, the SFS extract shows high sensitivity to *Salmonella typhi*, moderate sensitivity to *Staphylococcus aureus* and less sensitivity to for fungus, the extract of SFS. Shows high sensitivity to *Rhizopus stolonifer* and *Aspergillus fumigates*, moderate sensitivity to *Candida albicans.*

CLIS petroleum ether extract (oil) appeared as antibacterial for both Gram negative and Gram positive, also CLIS aqueous extract appeared as antibacterial for both gram negative and gram positive, which was in agreement with Mhaskar., *et al.* [21], who reported that the aqueous seed extract of CLIS shows maximum zone of inhibition against *E. coli.* CLIS aqueous extract appeared as antifungal. The activity may be due to the protein finotin as reported by Kelemu., *et al.* [22], who isolated antimicrobial and insecticidal protein finotin from CLIS. The present study was disagreed with Madhavarao., *et al. [23]*, who reported that methanol extracts of CLIS in vitro had antimicrobial activity against the microorganisms, hence methanol extract assessed had shown good antibacterial activity in *E. coli* and *Staphylococcus aureus.* The present study was disagreed with Kamilla., *et al.* [24] who reported that methanol extracts of CLIS exhibited antimicrobial activity against *Staphylococcus aureus* (Gram positive bacteria) and *Escherichia coli* and *Salmonella typhi* (Gram negative bacteria).

A protein designated as 'finotin' has been isolated from CLIS and reported to have antifungal, antibacterial and insecticidal properties [22]. It is possible that this compound was mainly responsible for the observed antimicrobial effects in this study. Mhaskar., *et al.* [21] reported that the aqueous seed extracts of CLIS shows strong antifungal activity on all the tested fungi.

As the results shows, the FENS petroleum ether and methanolic extract (oil) appeared as antibacterial for *E. coil.* The results were agreed with Abdalah [25]. The different concentrations of methanolic extract of Fenugreek seeds produced inhibition zone against bacterial isolates; *Escherichia coli* was sensitive to concentrations ranging between 125 - 1000 mg/ml, and disagreed with the same author who reported that Staphylococcus aureus was sensitive to methanolic fenugreek seeds extract at various concentrations, 125, 250, 500, and 1000 mg/ml, The results were agreed with Zaen Al-abdeen., *et al.* [26] who reported that fenugreek seeds petroleum ether had effect on *E. coli* and the FENS water extracts had no effect on *E. coli* and *S. aureus.* The results were agreed with Marzougui., et al [27]. Petroleum ether and methanolic extract had no effect on *E. coli* and at *S. aureus* and *S. typhimurium* and disagree with the same author who concluded that aqueous extract of FENS had an antibacterial activity.

This study also agreed with Kroum [28] who reported that the methanolic and water extracts of fenugreek seeds are very poor antibacterial agents against standard organisms (*Escherichia coli, Salmonella typhi* ATCC 0650), Taylor., *et al*. [29] had shown that chemical composition can vary among different varieties of fenugreek originating from different areas of the world. The results were disagreed with that reported by Haoula., *et al.* [30] who studied the antifungal potential of petroleum ether, ethyl acetate and methanolic fractions of the aerial fenugreek parts and shows that the antifungal activity resided mainly in the methanol fraction. In contrast to our findings numerous authors have reported various degrees of bacteriostatic activity of Fenugreek water extract against gram positive and gram negative bacteria [31]. The cause of variation in antibacterial influence maybe due to variation in plant components that collected from different areas.

Highest inhibition zones

CLlS Petroleum extract exhibited the highest inhibition zone on *E. coli* (31.5 mm) which was higher than the inhibition zones recorded by the several antibiotics. Ampicillin/Sulbactam (16 mm), Cefotaxime (18 mm), Levofloxacin (12 mm), Gentamicin (15 mm) and Tazobactam/piperacillin (20 mm), CLlS Petroleum extract inhibition zone higher than or equal to Co-trimoxazole, Tetracycline, Ciprofloxacin, Chloramphenicol, Ceftriaxone. Ofloxacin and Amikacin which had 20 mm or more inhibition zones. The CLlS Petroleum extract shows an inhibition zone on *Salmonella typhi* (17 mm) which was higher than the inhibition zones recorded by the following antibiotics in table 2. Tetracycline 12 mm, Cefotaxime 10 mm and Ciprofloxacin 10 mm, and equal to Co-trimoxazole and lower than Tazobactam/piperacillin, Ampicillin/Sulbactam, Amikacin, Chloramphenicol (CH), Levofloxacin, Gentamicin, Ceftriaxone and Ofloxacin which recorded 20 mm or more, also CLlS inhibition zone on *Staphylococcus aureus* was (20.8 mm), which was higher than the inhibition zones recorded by several antibiotics in table 2. Co-trimoxazole, Tetracycline, Linezolid which had no inhibition zones, Ciprofloxacin (18 mm), Cephalexin (20 mm), Cefotaxime (20 mm) and lower than Ampicillin/Sulbactam, Levofloxacin, Cloxacillin, Roxithromycin, Lincomycin and Gentamicin which recorded more than 20 mm. On the other hand table 2 shows that MORS water extraction shows the highest inhibition zone on *Candida* (13 mm).

Antimicrobial Agent	*E. coli*	*S. typhi*	*Staphylococcus*
Ampicillin/Sulbactam (AS)	16	20	More than 20
Co- trimoxazole (BA)	More than 20	17	Reset
Cephalexin (PR)	-	-	20
Tetracycline (TE)	More than 20	12	Reset
Cefotaxime (CF)	18	10	20
Ciprofloxacin (CP)	More than 20	10	18
Levofloxacin (LE)	12	More than 20	More than 20
Linezolid (LZ)	-	-	Reset
Cloxacillin (CX)	-	-	More than 20
Roxithromycin (RO)	-	-	More than 20
Lincomycin (LM)	-	-	More than 20
Gentamicin (GM)	15	More than 20	More than 20
Tazobactam/piperacillin (TZP)	20	17.8	-
Chloramphenicol (CH)	More than 20	18	-
Ceftriaxone (CR)	More than 20	More than 20	-
Ofloxacin (OF)	More than 20	More than 20	-
Amikacin (AK)	More than 20	20	-

Table 2: *Inhibition zone (mm) of different microorganisms (E. coli, S. typhi and Staphylococcus) as affected by different antibiotics.*

-: Not tested

Traditional medicine use primarily water as solvent but in this study the plant extract, extracted in organic solvent shows distinct antibacterial activity than aqueous extract. This observation can be rationalized in terms of the polarity of the compound being extracted by solvent and, in addition to intrinsic bioactivity, and by the ability to dissolve or diffuse in the media used in the method.

This variation may be due to the aspects of the study, the method of extraction of medicinal plants, the method of antibacterial study, culture medium, pH, temperature, incubation period, and the genetic variation of plant, age of the plant or the environmental factors that make the comparison of published data unreliable [32]. This class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity [33]. Moreover, numerous research groups have sought to elucidate the antibacterial mechanisms of action of selected flavonoids. The activity of quercetin, for example, has been at least partially attributed to inhibition of DNA gyrase [33]. This result agreed with Taha., *et al.* [34] and Estevinho., *et al.* [35], who reported that the susceptibility of bacteria to phenolic compound and gram reaction appeared to have an influence on growth inhibition. Phenolic compounds may affect the growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration.

Conclusion

The different seeds extractions (petroleum ether, mechanic and aqueous) had an antifungal and antimicrobial effects on gram +ve and gram - ve bacteria. *In vitro* application of petroleum ether extraction of *Clitoria ternatea* seeds had antimicrobial effects against *E. coli, Salmonella* and *Staphylococcus*, while petroleum ether extraction of Sunflower had antimicrobial effects against *Candida*. Petroleum ether extraction of tested seeds is more effective than mechanic extract. It is recommended to identify the active ingredients and therapeutic agents of medicinal plants that can be used as bacteriostatic and bactericides.

Bibliography

1. Katzung BG. "Basic and Clinical Pharmacology". 6th Edition, London: Prentice Hall International (UK) Limited (1995).
2. Pezzuto J. "Taxol® production in plant cell culture comes of age". *Nature Biotechnology* 14.9 (1996): 1083.
3. Governo do Brasil. "Agência Nacional de Vigilância Sanitária (ANVISA). Ministério da Saúde. Resolução no 14, de 31 de março de 2010". Dispões sobre o Registro de medicamentos fitoterápicos, Brasília (2001).
4. Colalto C. "Herbal interactions on absorption of drugs: Mechanisms of action and clinical risk assessment". *Pharmacological Research* 62.3 (2010): 207-227.
5. Dias MG and Salgueiro L. "Interações entre preparações à base de plantas medicinais e medicamentos". *Revista de Fitoterapia* 1 (2009): 5-22.
6. Gustavo HF., *et al*. "Antibacterial effect (in vitro) of moringa oleifera and Annona muricata against gram positive and gram negative bacteria". *Revista Institudo de Medicalo Tropicale de Sao Paulo* 52.3 (2010): 129-132.
7. Ajibesin KK., *et al*. "Ethanobotanical Survey of Akwa lbom State of Nigeria". *Journal of Ethanopharmacology* 115.3 (2008): 387-408.
8. Greathead H. "Plants and plants extracts for improving animal productivity". *Proceedings of the Nutrition Society* 62.2 (2003): 279-290.
9. Cheesbrough M. "District laboratory Practice in tropical countries" (2000).
10. Prescott LM., *et al*. "Antimicrobial Chemotherapy". Microbiology 2th edition .W.M.C. Brown publishers (1993): 324-330.
11. Akhtar N., *et al*. "Comparison of antifungal activity of thymoquinone and amphotericin B against Fusarium solani in vitro". *Scientific Journal of King Faisal University* 8.2 (2007): 137-145.
12. Kahsai AW. "Isolation and Characterization of Active Ingredients from Nigella sativa for Antibacterial Screening". Master's Thesis, Department of Chemistry, East Tennessee State University (2002).

13. Randhawa MA and Al-Ghamdi MJ. "A review of pharmacotherapeutic effects of Nigella sativa". *Pakistan Journal of Medical Sciences* 41.2 (2002): 77-83.

14. Rakhshandeh H., *et al*. "In vitro and in vivo study of the antibacterial effects of Nigella sativ methanol extract in dairy cow mastitis Avicenna". *Journal of Phytomedicine* 1.1 (2011): 29-35.

15. Islam MH., *et al*. "Antibacterial activity of Nigella sativa seed in various germination phases on clinical bacterial strains isolated from human patients". *E3 Journal of Biotechnology and Pharmaceutical Research* 4.1 (2013): 8-13.

16. Karrouchi K., *et al*. "Antimicrobial activity of aqueous, ethanolic, methanolic, cyclohexanic extracts and essential oil of Nigella sativa seeds". *Journal of Chemical and Pharmaceutical Research* 6.8 (2014): 9-11.

17. Chuang PH., *et al*. "Anti-fungal activity of crude extracts and essential oil of Moringa oleifera Lam". *Bioresource Technology* 98.1 (2007): 232-236.

18. Saadabi AM and Abu ZAI. "An in vitro antimicrobial activity of Moringa oleifera L. seed extracts against different groups of microorganisms". *Asian Journal of Advances in Basic and Applied Science* 5.5 (2011): 129-134.

19. Abu Sayeed M., *et al*. "In vitro Antimicrobial Activity of Methanolic Extract of Moringa oleifera Lam. Fruits". *Journal of Pharmacognosy and Phytochemistry* 1.4 (2012).

20. Subashini R and Rakshitha SU. "Phytochemical Screening, Antimicrobial Activity and In Vitro Antioxidant Investigation of Methanolic Extract of Seeds from Helianthus annuus L". *Chemical Science Review and Letters* 1.1 (2012): 30-34.

21. Mhaskar AV., *et al*. "Callus induction and antimicrobial activity of seed and callus extracts of Clitoria ternatea L". *Current Trends in Biotechnology and Pharmacy* 4.1 (2010): 561-567.

22. Kelemu S., *et al*. "Antimicrobial and insecticidal protein isolated from seeds of Clitoria ternatea, a tropical forage legume". *Plant Physiology and Biochemistry* 42.11 (2004): 867-873.

23. Madhavarao B and Sabithadevi Vinnakoti K. "In-vitro antimicrobial and free radicals cavenger assay of two medicinal plants Clitoria ternatea and Cardiospermum halicacabum". *International Journal of Chemical and Analytical Science* 2.11 (2011): 1253-1255.

24. Kamilla L., *et al*. "Antimicrobial Activity of Clitoria ternatea (L.) Extracts". *Pharmacology Online* 1 (2009): 731-738.

25. Abdalah ME. "The study of Antibacterial activity of fenugreek (Trigonella foenum-graecum)" (2011).

26. Zaen Al-abdeen SS., *et al*. "Antibacterial Effects of Fenugreek (Trigonella foenum-graecum)". *Basrah Journal of Veterinary Research* 10.2 (2010): 133-140.

27. Marzougui N., *et al*. "Antibacterial activity of extracts of diploid and induced autotetraploid Tunisian populations of Trigonella foenum-graecum L". *Journal of Medicinal Plants Research* 6.38 (2012): 5166-5172.

28. Kroum NEH. "Antibacterial Activity of Trigonella foenumgrea-cum linn (Fenugreek) Seed-Extracts Against Some Species of Enterobacter". M.Sc. Thesis University of Khartoum, Faculty of Public and Environmental Health (2009).

29. Taylor WG., *et al*. "Variation indiosgenin levels among 10 accessions of fenugreek seeds produced in Western Canada". *Journal of Agricultural and Food Chemistry* 50.21 (2002): 5994-5997.

30. Haoula R., *et al*. "Aqueous and organic extracts of Trigonella foenum-graecum L. Inhibit them mycelia growth of fungi". *Journal of Environmental Sciences* 20.12 (2008): 1453-1457.

31. Bhatti MA., *et al*. "Antibacterial activity of Trigonella foenum-graecum seeds". *Phytotherapy* 67 (1996): 372-374.

32. Burt S. "Essential oils: their anti bacterial properties and potential applications in food: a review". *International Journal of Food Microbiology* 94.3 (2004): 223-253.

33. Tim Cushnie TP and Lamb AJ. "Antimicrobial activity of flavonoids". *International Journal of Antimicrobial Agents* 26.5 (2005): 343-356.

34. Taha FS., *et al*. "Evaluation of the biological activity of sunflower hull extracts". *Grasas y Aceites* 63.63 (2012): 184-192.

35. Estevinho L., *et al*. "Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey". *Food and Chemical Toxicology* 46.12 (2008): 3774-3779.

Volume 15 Issue 1 January 2019