DETERMINATION OF ANTIMICROBIAL ACTIVITY OF FRUITS OF *CITRULLUS COLOCYNTHIS*

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ABSTRACT: Objective: The current study was designed to examine the antibacterial activity of various extracts of *Citrullus colocynthis* fruits against *Helicobacter pylori*, *Salmonella enterica*, *Shigella flexneri*, *Campylobacter jejuni*, *Micoplasma pneumonia*, and *Shigella dysenteriae.*

Methodology: Antimicrobial activity was evaluated by agar disc diffusion method. The available genome of *C. colocynthis* was utilized to retrieve sequence of highly conserved genes matK, rpl20, rpl32, and rps7 and to identify their relevant genes in *C. rehmii*.

Results and Conclusion: The maximum antimicrobial activity was shown by ethanol extract (*S. f.* 32mm, *C. j.* 27mm, *H. p.* 26mm, *S. d.* 22mm, *M. p.* 21mm, *S. e.* 19mm) while least activity was given by n-hexane extract (*S. f.* 13mm, *C. j.* 11mm, *H. p.* 18mm, *S. d.* 8mm, *M. p.* 10mm, *S. e.* 8mm) when compared with standard antibiotic levofloxacin (*S. f.* 30mm, *C. j.* 30mm, *H. p.* 30mm, *S. d.* 28mm, *M. p.* 25mm, *S. e*. 20mm). All extracts had given maximum response against *H. pylori* and *S. flexneri* and least response against *M. pneumoniae* and *S. enterica.* The in-silico genome-wide investigation studies showed that all four genes of *C. rehmii* were identified with high conservation based on motif distribution and phylogenetic relations.

Key words: *Citrullus colocynthis*, antimicrobial activity, *C. rehmii*, MIC.

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INTRODUCTION

Background: The bacterial infections have been increasing day by day due to their unique ability to grow exponentially. Frequent and over dosage of antibiotics makes bacteria resistant to most of drugs which results in increase of morbidity and mortality rate. Therefore it is the need of time to find an alternate method to combat bacterial diseases with minimum or no side effects (Nora *et al.*, 2015). Herbal plants had been extensively used in the cure to numerous diseases since ancient times and approximately one lac plant species have been reported to be used against various infectious diseases (Gupta *et al.*, 2012).The crude extracts of various medicinal plants using different compounds such as methanol, ethanol, water, ethyl acetate, acetone, butyl acetate and chloroform has proven to be beneficial against various pathogens in various studies (Elkamaliand Mahjoob, 2015).

Citrullus colocynthis belongs to cucurbitaceae family extremely bitter in taste and well known for its medical and nutritional benefits as well as anti-cancerous, antidiabetic, analgesic, purgative and laxative properties (Al-Snafi, 2016). It is commonly known as bitter gourd, egusi, bitter cucumber, wild gourd, desert gourd and bitter apple (Idan *et al.*, 2015). It contains different kinds of compounds such as resins, alkaloids, flavonoids, steroids, gums, citrullol, glycosides and cucurbitacin E-2 glucoside (Sharma *et al.*, 2010). It has been extensively used in various countries especially in hot and sub-tropical regions for the treatment of diabetes (Rahbar and Nabipour, 2010), hypertension (Hussain *et al.*, 2014) in UAE as anti-inflammatory drug, in Saudi Arabia as purgative and antirhumetidic and in Mediterranean countries to treat urinary tract disorders (Marzouk *et al.*, 2010).The fruits of *C. colocynthis* has been utilized for the treatment of asthma, diabetes, ulcer, jaundice, sore throat, elephantiasis and tuberculosis glands while roots are mainly used to treat arthritis, joints inflammation, cough, boils and skin lesions (Hussain *et al.*, 2014).

Shigella dysenteriae is a rod shaped, gram negative, non-motile, non-sporous anaerobic bacteria that causes dysentery (Roy *et al.*, 2019). *Mycoplasma pneumoniae* is a gram negative bacteria causing respiratory tract infections including pneumonia, bronchitis and pharyngitis (Meyer *et al.*, 2020). *Helicobacter pylori* is a helical shaped, gram negative bacteria responsible for the development of ulcer in stomach (Hooj *et al.*, 2017). *Campylobacter jejuni* is a gram negative, rod shaped helical, motile, and non-fermenting bacteria responsible for food poisoning (Wallace *et al.*, 2019). *Shigella flexneri* is a non-motile, non-spore forming, gram negative bacteria responsible for diarrheal infections in humans (Mani *et al.*, 2016).The

antimicrobial activity on chloroform, ethanol and aqueous extracts of *C. colocynthis* against soil bacteria rhizospheric isolates was evaluated and it was established that chloroform extract had maximum activity while water extract had minimum activity against all tested microbial isolates (Alshammary and Ibrahim, 2014). The ethylacetate extract of *Citrullus colocynthis* has also shown potential antibacterial activity against various bacteria including *S. aureus* and *E. faecalis* and moderate antimicrobial potential towards E. coli (Chawech *et al.*, 2015).Elemental analysis of *Citrullus colocynthis* fruit by using atomic absorption spectrum has revealed that it is composed of varieties of essential elements such as calcium, copper, iron, magnesium, manganese and zinc (Verma, 2018). Its seed is composed of minerals, oils such as oleic, palmitic, stearic, myristic and linolenic, proteins, vitamins and albuminiods (Gurudeeban *et al.*, 2010). Therefore in this study a medicinal plant *C. colocynthis* has been selected to determine its antimicrobial potential against some bacterial strains. These findings highlighted the importance of medicinal plants as potential antimicrobial agents and provide scientific evidence about the usage of the medicinal plants in treating bacterial diseases.

MATERIALS AND METHODS

Plant materials and extract preparation: The fruits of the medicinal plant *Citrullus colocynthis* were obtained from thal desert, district Punjab (Pakistan). The plant was identified by Department of Botany, Arid Agriculture University, Rawalpindi. The fruits were washed and rinsed with water and then dried under the shade. These dried fruits were finally crushed into fine powder. The extracts were made by means of the solvents water, methanol, ethanol and n-hexane.10g of the dried samples were soaked into 100ml of these selected solvents separately for 48hours with constant stirring, centrifuged at 2000rpm for 20mins and filtered through Whatmann filter paper. The remainder were evaporated and further dried at 4℃ using rotatory vacuum evaporator.

Determination of antimicrobial activity:

Microbial stains: The bacterial strains that were chosen for analysis includes *H. pylori*, *S. enterica*, *C. jejuni*, *M. pneumonia*, *S. flexneri* and *S. dysenteriae* had obtained from microbiology lab of AIOU, Islamabad. All these bacteria were identified on the basis of culture, colony appearance, biochemical tests and morphological characteristics in the Department of microbiology, AIOU Islamabad. All bacteria belongs to pathogen risk group 2 and biosafety level 2 i.e. use of personal protective equipment, biohazards warning signs in lab and use of autoclave for decontamination were employed in handling pathogens. The selected strains were cultivated and stored at 37℃ in nutrient agar medium.

Agar disc diffusion method: Antibacterial activity of water, n-hexane, ethanol and methanol extracts of fruit of *C. colocynthis* was analyzed by agar disc diffusion method (Bauer *et al.*, 1966). The sterile cellulose paper discs (5mm diameter) were prepared and then placed in cotton plugged pour tube for sterilization in autoclave. The stock solutions of the extracts were formed by mixing 1.0mg of each sample in 1.0 mL of DMSO and further diluted at the concentration of 0.75mg/ml and 0.5mg/ml. The agar plates were prepared by pouring 20ml of molten nutrient agar in sterile petri plates. After solidification of agar medium, 100µl of fresh culture of pathogens were swabbed into the respective petriplates. The cellulose discs were then placed on the surface of the inoculated plates. The petriplates were incubated at 37°C for 24 hours. The antimicrobial activity was analyzed by evaluating the zone of inhibition around the discs. The negative control was the respective solvent without plant extract while levofloxacin was used as positive control (Bauer *et al.*, 1966). The results were repeated thrice and the results were represented as 3ni± iSD.

Sequence retrieval of *Citrullus colocynthis* gene family: The available genomic resources of *C. colocynthis* was utilized to retrieve the sequence of highly conserved genes matK (ID: 33910892), rpl20 (ID: 33910875), rpl32 (ID: 33910868), and rps7 (ID: 33910887) that were further used for identification of their relevant genes in *C. rehmii*.

In silico Genome wide investigation analysis of matK, rpl20, rpl32, and rps7 genes families: The protein to protein interaction of matK, rpl20, rpl32 and rps7 genes in *C. rehmii* was done using STING database (Szklarczyk *et al.*, 2010), phylogenetic analysis using Clustal W 2.1 and ITOL (Letunicand Bork., 2016), motif distribution analysis using MEME 5.0.4 (Di *et al.*, 2010) and gene ontology pattern analysis using online web system tool Cello2go (Yu *et al.*, 2014).

RESULTS

Percentage yield and antimicrobial activity of extracts of *C. colocynthis:* In the present study, the antimicrobial activity of *Citrullus colocynthis* against six different microbial stains was investigated and recorded. Percentage yield of different extracts of fruits of *Citrullus colocynthis* is calculated (**Table 1)**.

Table 1: % yield of fruits extract of *Citrullus colocynthis.*

Extracts	water	Methanol	n-hexane	ethanol
% Yield	8.874	11.047	7.376	13.304

Antimicrobial activity of extracts at various concentration: The antimicrobial potential of the solvent extracts was performed against different bacteria and results are presented in Table 2. Among all extracts, the maximum antimicrobial activity was shown by ethanol extract against *Shigella flexneri* (32mm) when compared with standard antibiotic levofloxacin (30mm). The antimicrobial activity is due to presence of secondary metabolities soluble in ethanol, methanol, hexane and aqueous extracts of fruit of *C. colocynthis.*

Table 2: Assay of antimicrobial activities of different concentration of ethanol, methanol, n-hexane and aqueous extracts of *Citrullus colocynthis* against various pathogenic bacteria.

Extracts	Concentration mg/ml	Zone of inhibition (mm)					
		Helicobacter pylori	***Salmonella enterica***	***Campylobacter jejuni***	***Mycoplasma pneumoniae***	***Shigella flexneri***	***Shigella dysenteriae***
Ethanol	**1**	26±0.63	19±0.37	27±0.43	21±0.28	32±0.71	22±0.39
	0.75	17±0.52	11±0.23	18±0.57	14±0.13	20±0.83	15±0.15
	0.5	8±0.32	7±0.79	10±0.65	10±0.57	16±0.31	10±0.56
	0.25	-	-	-	-	12±0.53	-
Methanol	**1**	21±0.71	16±0.25	23±0.88	20±0.21	26±0.65	19±0.41
	0.75	15±0.42	9±0.31	15±0.54	13±0.33	17±0.41	11±0.23
	0.5	9±0.33	-	11±0.58	9±0.46	12±0.76	7±0.42
	0.25	5±0.67	-	7±0.78	5±0.19	9±0.53	-
Aqueous	**1**	19±0.65	12±0.98	15±0.44	17±0.26	16±0.53	10±0.85
	0.75	12±0.45	7±0.23	8±0.78	10±0.66	8±0.32	-
	0.5	7±0.58	-	-	6±0.87	-	-
	0.25	-	-	-	-	-	-
n-hexane	**1**	18±0.72	8±0.44	11±0.62	10±0.17	13±0.97	8±0.28
	0.75	11±0.36	-	7±0.55	5±0.76	7±0.95	-
	0.5	-	-	-	-	-	-
	0.25	-	-	-	-	-	-
Levofloxacin	**1**	30±0.12	20±0.51	30±0.64	25±0.39	30±0.54	28±0.19
	0.75	25±1.36	16± 0.84	26±0.95	22±0.83	27±0.59	25±0.36
	0.5	22±0.84	15±0.45	23±0.87	19±0.76	23±0.36	21±0.98
	0.25	19±0.78	12±0.53	18±0.64	16±0.39	20±0.45	15±0.23

Activity is presented in millimeter (mm), No activity (-)
Results were indicated as 3n±SD

The methanol extract showed extreme inhibition towards *S. flexneri* (26mm) while the least inhibition was observed on *S. dysenterae* (19mm) and *S. enterica* (16mm). The wet ater extract showed maximum inhibition zone against *H. pylori* (19mm) and least effect was observed on *S. dysenterae* (10mm). n-hexane extract had shown maximum antimicrobial activity against *H. pylori* (18mm) while on *S. dysenterae* (8mm) and *S. enterica* (8mm) it had shown least activity. The ethanol extract showed maximum activity against *Shigella flexneri* (32mm) while the least effect was observed on *M. pneumoniae* (21mm) and *S. enterica* (19mm). It had shown that high zone of inhibition is associated with the concentration of different extracts of fruits of *C. colocynthis*. Higher the zone of inhibition greater is the antimicrobial activity. The effective lower concentration of ethanol extract against *S. flexneri* is 0.25mg/ml while for all other tested bacteria it was 0.5mg/ml. The effective lower concentration of aqueous extract against *H. Pylori* and *M. pneumoniae* was 0.5mg/ml, for *C. jejuni*, *S. enterica* and *S. flexneri* it was 0.75mg/ml. The n-hexane extract had not shown any activity against tested bacteria at the concentration of 0.25mg/ml and 0.5mg/ml, at the concentration of 0.75mg/ml it had shown activity against *C. jejuni*, *H. Pylori* and *Mycoplasma pneumoniae* while for *S. dysanteriae* and *S. enterica* 1mg/ml had been reported as effective lower concentration.

Identification and insilico analysis of *Citrullus rehmii* gene family: Four genes matK (33910892), rpl20 (33910875), rpl32 (33910868), and rps7 (33910887) were identified with high conservation based on motif distribution and phylogenetic relations in *Citrullus rehmii* during the insilico genome wide distribution analysis. The gene ontology analysis showed that matK gene was localized in plasma membrane (70.5%) with molecular function 100% and biological progression 33.3%. While the rpl20 protein was found to be highly contained in the nuclear and mitochondrial part (42.4 and 29%) with molecular functionality 25% and biological progression 50%. The rpl32, protein had shown to be localized in the nuclear and mitochondrial part (46.5 and 24.4 %) with molecular functionality 50% and biological progression 50% while gene rps7 gene was contained in the nucleus

and mitochondria (48.9 and 27%) with minimum molecular functionality and 48.7%. biological progression. The network analysis showed that matK and rpl20 genes had 21, 21 nodes, 100, 97 edges, 9.52, 9.24 average nodes and 0.876, 0.539 average local cluster coefficient respectively. The network of rpl32 gene is taking from String database. The rpl32 genes confirming statistics of nodes is 31, total of ends is 128, the averages nodes 7.52, the value of average local cluster coefficient reported is 0.776, predictable number of edges is 24 and the network of rps7 gene is confirming statistics of nodes is 23, total of edges is 100, the averages nodes 9.52, the average number of local cluster coefficient is 0.876, predictable number of edges is 24. This study about insilco genome-wide investigation of gene ontology patterns generated the valuable evidence on all identified gene in both plant families that will be helpful for improving crop productivity.

DISCUSSION

Result of this study provide valuable information that the extracts withdrawn the growth of very tested bacterial germs. The inhibition is chiefly due to the occurrence of active complexes that prevent enzymatic activity in cytoplasmic membrane of tested pathogens by their target on lipid bilayer **(**Amudha and Manikandan, 2018). The active compounds of the extract therefore strive for the active sites of different enzymes responsible for bacterial replication and hence inhibit the bacterial growth (Aljasim and Barkat, 1973).The components found in the medicinal plant Citrullus colocynthis are accountable for antibacterial activity. Alkaloids are mainly found in ethanol extract and had been known for its antihypertensive and detoxifying properties (Jasim *et al.,* 2015). Tannins have been recognized for its capacity to inhibit the progression and development of bacteria by precipitating the proteins responsible for bacterial growth and nutrition (Rubens *et al.,* 2015). Saponins are glycosidic compounds responsible for their active antifungal and antibacterial properties (Qin *et al.,* 2012). Flavonoids have been reported for their various biochemical activities including antiviral, anti-cancerous, antimicrobial, anti-allergic and anti-inflammatory properties (Khameneh *et al.,* 2016).

Citrullus colocynthis is an also very effective and valuable herbal plant which is used to cure various diseases and infections. Results of this investigation provide a scientific understanding of the importance of different extracts of fruits of *C. colocynthis* to cure various bacterial diseases. Results of this study are in link with the studies of Gurudeeban *et al.,* (2010) about the antimicrobial activity of ethanol extract of leaves and fruits of *Citrullus colocynthis* against *S. aureus*, *S. typhi* and *S. pyogenes*. Similarly Rodge and Biradar, (2012) also reported the effective antibacterial activity of ethanol, methanol, acetone and aqueous extracts extracts of *Citrullus colocynthis* towards *Staphylococcus aureus*, *Shigella shigella*, *S. typhi* and *Esterichia coli.* The usefulness of plant extracts towards different bacterial pathogens revealed the usefulness of this plant as an alternative to chemotherapy and antibiotics (Seow *et al.,* 2014).The antimicrobial potential of *C. colocynthis* is dependent on the capacity of different solvents to extract various phytochemicals present in the plant. The plant contains various medicinally important bioactive substances which are responsible for its antimicrobial potential (Gacem *et al.,* 2013). This also elucidate the application of this plant as a potential source of new drugs that can solve the problems of drug resistance. To understand the particular components responsible for antibacterial activity, detailed study is required (Bnyan *et al.,* 2013).

Conclusion: The practice of utilization of herbal products in medical industry underrates the hazards associated with chemical based medicines. This is the first report that described the antibacterial potential of different extracts of fruits of *C. colocynthis* against six different bacteria causing various gastrointestinal, respiratory and nervous system disorders. All the extracts had given good antimicrobial activity and maximum zone of inhibition which are effective and wide. So, it can be employed in handling diseases produced by these tested bacteria. Hence it can also have great potential towards multi-drug unaffected strains of bacteria. The results of this study can be beneficial for numerous research groups interested in exploring the natural sources of useful bioactive ingredients.

REFERENCES

Amudha, M. & R. Manikandan (2014). Photochemical analysis and antimicrobial activity of *Citrullus colocynthis*. Int. J. Sci. Res. Rev. 7(2): 341-348.

Alshammary, A. S. and N. A. Ibrahim (2014) Antimicrobial Activity of *Citrullus colocynthis* Extracts against Soil Bacteria. Global J. Pure Appl. Sci. 3(4):71-73.

Al-Snafi, A. E. (2016). Chemical constituents and pharmacological effects of *Citrullus colocynthis*-A review. J. Pharm. Sci. 6(3): 57-67.

Bauer, A. W., W. M. Kirby, J. C. Sherris and M. Turck (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45(4): 493-496.

Bnyan, I., H. Hasan and M. Ewadh (2013). Antibacterial activity of *Citrullus colocynthis* against different types of bacteria. Adv. Life Sci. Technol. 7: 48-51.

Chawech, R., D. M. Mhalla, M. Trigui, M. Mihoubi, N. Fabre and R. Jarraya (2015). Chemical

composition and antibacterial activity of extracts and compounds isolated from *Citrullus colocynthis* (L.) Schrad. J. Pharma. Phytochem. 4(4): 197.

Di, C., W. Xu, Z. Su and J. S. Yuan (2010). Comparative genome analysis of PHB gene family reveals deep evolutionary origins and diverse gene function. 11(6): 22).

Elkamali, H. H. and S. E. Mahjoob (2015). Evaluation of antibacterial activity of some medicinal plants used in Sudanese folk medicine for treatment of gastrointestinal tract infections. Am. J. Life Sci. 3(3): 230-237.

Gacem, M. A., A. O. El Hadj-Khelil, S. Hadef, A. Boudharem and A. N. Djerbaoui (2013). Phytochemical screening and antibacterial activity of aqueous extracts of *Citrullus colocynthis* seeds. Photochem. Photobiol. 7(3): 90-94.

Gupta, A., D. K. Sharma, N. S. Gill and A. Goyal (2012). A review on the medicinally important plants of the family Cucurbitaceae. Asian J. Clin. Nutr. 4(1): 16-26.

Gurudeeban, S., E. Rajamanickam, T. Ramanathan and K. Satyavani (2010). Antimicrobial activity of *Citrullus colocynthis* in Gulf of Mannar. Int. J. Curr. Res. 2: 78-81

Hooi, J. K., W. Y. Lai, W. K. Ng, M. M. Suen, F. E. Underwood, D. Tanyingoh, P. Malfertheiner, D. Y. Graham, V. W. Wong, J. C. Wu and F. K. Chan (2017). Global prevalence of *Helicobacter pylori* infection: systematic review and meta-analysis. Gastroenterology. 153(2): 420-429.

Hussain, A. I., H. A. Rathore, M. Z. Sattar, S. A. Chatha, S. D. Sarker and A. H. Gilani (2014). *Citrullus colocynthis* (L.) Schrad (bitter apple fruit): A review of its phytochemistry, pharmacology, traditional uses and nutritional potential. J. Ethnopharmacol. 155(1): 54-66.

Idan, S. A., A. H. Al-Marzoqi and I. H. Hameed (2015). Spectral analysis and anti-bacterial activity of methanolic fruit extract of *Citrullus colocynthis* using gas chromatography-mass spectrometry. Afr. J. Biotechnol. 14(46): 3131-3158.

Jasim, H., A. O. Hussein, I. H. Hameed and M. A. Kareem (2015).Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry. J. Pharmaco. Phytother. 7(4): 56-72.

Khameneh, B., R. Diab, K. Ghazvini and B. S. F Bazzaz (2016). Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them. Microbiol. Res. 95: 32-42.

Letunic, I. and P. Bork (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res. 44(1): 242-245.

Mani, S., T. Wierzba and R. I. Walker (2016). Status of vaccine research and development for Shigella. Vaccine. 34(26): 2887-2894.

Marzouk, B., Z. Marzouk, E. Haloui, N. Fenina, A. Bouraoui and M. Aouni (2010). Screening of analgesic and anti-inflammatory activities of *Citrullus colocynthis* from southern Tunisia. J. Ethnopharmacol. 128(1): 15-19.

Meyer Sauteur, P. M., J. Trück, A. van Rossum and C. Berger (2020). Circulating antibody-secreting cell response during Mycoplasma pneumoniae childhood pneumonia. J. Infect. Dis. 3 (2): 121-124

Najafi, S., N. Sanadgol, B. S. Nejad, M. A. Beiragi and E. Sanadgol (2010). Phytochemical screening and antibacterial activity of *Citrullus colocynthis* (Linn.) Schrad against *Staphylococcus aureus*. J. Med. Plants Res. 4(22): 2321-2325.

Nora, N. B., K. Hamid, M. Snouci, M. Boumediene and M. Abdellah (2015). Phytochemical and antibacterial screening of *Citrullus colocynthis* of South-west Algeria. J. Chem. Pharm. Res. 7(5): 1344-1348.

Peng, J., J. Yang and Q. Jin (2011). An integrated approach for finding overlooked genes in Shigella. PLoS One. 6(4).

Qin, X. J., D. J. Sun, W. Ni, C. X. Chen, Y. Hua, L. He and H. Y. Liu (2012). Steroidal saponins with antimicrobial activity from stems and leaves of Paris polyphylla var. yunnanensis. Steroids. 77(12): 1242-1248.

Rahbar, A. R. and I. Nabipour (2010). The hypolipidemic effect of *Citrullus colocynthis* on patients with hyperlipidemia. Pak. J. Biol. Sci. 13(24): 1202.

Rodge, S. V., & Biradar, S. D. (2012). Preliminary Phytochemical screening and antimicrobial activity of *Citrullus colocynthis* (Linn.) Shared. Indian J. Plant Sci, 2(1), 19-23.

Roy, B., S. T. Ahamed, B. Bandyopadhay and N. Giri (2019). Development of Quinolone resistance and prevalence of different virulence genes among *Shigella flexneri* and *Shigella dysenteriae* in environmental water samples. Letters in Applied Microbiology.

Rubens, D. M., O. O. Constantin, A. A. Moevi, G. K. Nathalie, T. Daouda, N. J. David and D. A. Joseph (2015). Anti-Staphylococcus aureus activity of the aqueous extract and hexanic fraction of Thonningia sanguinea (Cote ivoire). Int. J. Pharmacogn. Phytochem. Res. 7(2): 301-6.

Szklarczyk, D., A. Franceschini, M. Kuhn, M. Simonovic, A. Roth, P. Minguez and L. J. Jensen (2010). The STRING database in 2011:

functional interaction networks of proteins, globally integrated and scored. Nucleic acids research. 39(1): 561-568.

Seow, Y. X., C. R. Yeo, H. L. Chung and H. G. Yuk (2014). Plant essential oils as active antimicrobial agents. Crit. Rev. Food Sci. Nutr. 54(5): 625-644.

Sharma, A., S. Singh and T. N. Nag (2010). Antibacterial activity of *Citrullus colocynthis* and Tribulus terrestris against some pathogenic bacteria. Asian J. Microbiol. Biotechnol. Environ.Sci. 12: 633-637.

Verma, V. N. (2018). Elemental analysis of Citrullus colocynthis (L.) using atomic absorption spectrometer. World Sci. News. 95: 64-74.

Wallace, R. L., D. Bulach, A. McLure, L. Varrone A. V. Jennison, M. Valcanis and M. D. Kirk (2019). Status of antimicrobial resistance in clinical isolates of Campylobacter jejuni and Campylobacter coli in Australia. J. Clin. Microbiol.

Yu, C. S., C. W. Cheng, W. C. Su, K. C. Chang, S. W. Huang, J. K. Hwang and C. H. Lu (2014). CELLO2GO: a web server for protein subCELlular LOcalization prediction with functional gene ontology annotation. PloS one. 9(6).