Biological Forum – An International Journal **15(11): 157-162(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Antibacterial Activity of Essential Oils Against *Enterobacter cloacae* inciting Onion Soft Rot and Molecular Docking Study of its Major constituents

Anjali V.A.*, Basavarajappa M.P., Manjunath Hubballi, Raghavendra Gunnaiah, R.K. Mesta, Venkateshalu and Namita Bhaskar Raut
College of Horticulture, University of Horticultural Sciences, Bagalkot (Karnataka), India.

(Corresponding author: Anjali V.A.)*
(Received: 04 September 2023; Revised: 02 October 2023; Accepted: 17 October 2023; Published: 15 November 2023)
(Published by Research Trend)

ABSTRACT: Although onion is a popular crop among vegetables, onion soft rot has emerged as a devastating disease affecting its production. As being a vegetable that is mostly consumed raw in salads, an ecofriendly management strategy is the need of the hour. Essential oils prove to be promising alternative to chemical bactericides. Hence, the present study evaluated essential oils (clove oil, citronella oil, thyme oil, cinnamon oil and eucalyptus oil) against *Enterobacter cloacae* inciting soft rot in onion. Among the essential oils, thyme oil was the most effective in inhibiting the pathogen with an inhibition zone of 18.72 mm, which was followed by clove oil (17.15 mm) and citronella oil (16.72 mm). To justify the antibacterial activity of essential oils against *E. cloacae*, molecular docking studies were performed which revealed that receptor protein murE had a good binding affinity with selected ligands, in the range of -4.7 to -6.2 kcal/mol. Thus, essential oils can be potential antibacterial agents against *E. cloacae* causing soft rot in onion.
Keywords: Onion soft rot, *Enterobacter cloacae*, thyme oil, molecular docking, antibacterial activity, binding affinity.

INTRODUCTION

Onion (*Allium cepa* L.) is a highly valued vegetable crop used as food and medicine since antiquity. It is cultivated globally and stands second to tomato in terms of value among vegetable crops. Onion is a short-duration horticultural crop, it is mostly known as 'Queen of Kitchen' owing to its exquisite, aroma, flavor and exclusive taste combined with medicinal properties (Griffiths *et al*., 2002). An indispensable element of every kitchen, onion is used throughout the year in the form of spices, in salads, as a condiment or cooked with other vegetables. India stands second in the production of onions in the world, after China and is the second largest exporter of onions. Being known for its pungency, Indian onions are widely in demand and available throughout the year. Despite the high production statistics, onion cultivation in India is restricted by countless biotic factors. Of these, diseases caused by phytopathogenic bacteria are gaining prevalence lately. The major bacterial diseases reported in onions are stalk rot, soft rot, bacterial streak, bulb rot, center rot, leaf blight and sour skin. Among these, bacterial soft rot is one of the destructive biotic factors causing considerable losses in onion production both under field conditions as well as in storage. Bacterial soft rot of onion initiates in the field as the bacteria enters through wounds in the neck, bulb or aged tissues. Warm, rainy weather, heavy irrigation or flooded fields are optimum conditions under which bacterial decay of onion bulbs occurs rapidly rendering the bulbs unmarketable in a short period of time. The infected bulbs become water-soaked turning pale yellow to light brown leading to the breakdown of the entire bulb. The above-ground symptoms initiate with water-soaked lesions on the leaves which then progress to complete wilting and yellowing of the leaves (Schwartz and Mohan 1995).

Among the compounds of natural origin, essential oils are getting popular as alternatives to agrochemicals in plant disease management. They contain several antibacterial constituents, making them potential bactericidal agents. An important characteristic of essential oils and their components is hydrophobicity, which enables them to partition with the lipids present in the cell membrane of bacteria and mitochondria, rendering them more permeable by disturbing the cell structures. This eventually results in the death of bacterial cell due to leakage of critical molecules and ions from the bacterial cell to a great extent (Devi *et al.*, 2010). Although the mechanism of action of a few essential oil components has been elucidated in many pioneering works in the past, detailed knowledge of most of the compounds and their mechanism of action is still lacking. Molecular docking enables the exploration of the interaction between selected receptor protein and ligand molecule. Thus, it is an approach that anticipates energetically stable configuration of ligand when it is bound to the selected target protein. Molecular docking gives information on two important factors, the right conformation of ligand-receptor complex and its binding affinity.

The antibacterial efficacy of *Brassica nigra* essential oil (BNEO) against bacterial wilt pathogen, *Ralstonia solanacearum* was evaluated where the essential oil exhibited 100 per cent efficacy in inhibiting the growth of the pathogen. Further, homology modelling using computational tools indicated that anti-bacterial potency of BNEO is due to preferable binding efficiency of allyl isothiocyanate (AITC), the major active ingredient of BNEO to the protein targets present in the pathogen (Mandal *et al*., 2021). In a similar study, antibacterial activity of clove essential oil was attributed to its major component eugenol which displayed higher binding affinity against enoyl-[acyl-carrier-protein] reductase [NADPH] FabI present in pathogenic bacterium (Bai *et al.*, 2023). In yet another study, the antimicrobial activity of cassia and holy basil essential oils was due to the components such as eugenol, cinnamyl acetate, caryophyllene, humulene and *trans*-calamenene which possessed high binding affinity towards chitin synthase protein in target microorganism (Kulkarni *et al*., 2021). In the present study, the antibacterial activity of selected essential oils was evaluated against *Enterobacter cloacae,* known to cause soft rot in onion. Further, molecular docking study was performed to justify the antibacterial activity and elucidate the binding affinity of major constituents present in these essential oils to target receptors in *E. cloacae.*

MATERIAL AND METHODS

The experiment was conducted under laboratory conditions at Department of Plant Pathology, College of Horticulture, Bagalkot.

A. Isolation of the bacterial pathogen and identification

The pathogen associated with the soft rot of onion was isolated from the bacterial ooze collected from the symptomatic samples. The onion bulbs showing rotting symptoms were first washed in tap water, thereafter rinsed in distilled water and air dried. The symptomatic parts of the onion bulb were sliced and disinfected with 70 per cent ethanol and suspended in 5 ml of sterile distilled water taken in test tubes. Once the suspension turned turbid, a loop full of this suspension was streaked on nutrient agar plates and incubated for 48 h at room temperature. The pathogenicity of the bacteria was carried out on onion bulbs as per the protocol given by Gore *et al.* (2020). Further, molecular confirmation of the pathogen was done using species specific primer of *Enterobacter cloacae* corresponding to the housekeeping gene hsp60 (F 5'GGTAGAAGAAGGCGTGGTTGC 3´, R 5'ATGCATTCGGTGGTGATCATCAG 3´) as given by Hoffmann and Roggenkamp (2003).

B. Evaluation of antibacterial activity of selected essential oils

A set of five essential oils (clove oil, citronella oil, thyme oil, cinnamon oil and eucalyptus oil) were selected for evaluation against *Enterobacter cloacae* by agar-well diffusion method. The pathogen was multiplied in nutrient broth overnight. The warm molten nutrient agar media was seeded with this culture broth and poured onto Petri plates for solidification. After solidification, the agar plates were punched with a 5 mm cork borer aseptically to prepare wells. About 100 µl of the above-mentioned compounds were added to the wells, and incubated in upright positions at 28°C for 48 h. The diameter of the clear zone formed around the wells was measured in millimeters. Three replications were made per treatment for each test.

C. Molecular docking study

The receptor protein murE (UDP-N-acetylmuramoyl-L-alanyl-D-glutamate) was selected based on literature mining (Yadav *et al*., 2016). The biological function, molecular function and cellular localization of the selected protein was determined using Blast 2GO suite of OmicsBox 3.0. To determine the three-dimensional structure of the protein from its amino acid sequence, homology modelling was performed using SWISS-MODEL accessible via ExPasy server (https://swissmodel.expasy.org/).To ensure the quality of modeled protein, the validation of protein model was performed using the PROCHECK tool from SAVES v6.0 server (https://saves.mbi.ucla.edu/). The Ramachandran plot for the protein was constructed to confirm the stable conformation of the modeled protein. The major constituents of the selected essential oils (clove oil, citronella oil, thyme oil) were retrieved from literature and the ligand library was constituted. The ligand library consisted of ten compounds including citral, citronellal, citronellol, geraniol, geranyl acetate, eugenol, isoeugenol, caryophyllene, 1,2-benzenediol, thymol and carvacrol. The receptor was prepared for docking using UCSF Chimera 1.16 and Swiss PDB viewer. The structure filtration was done by Chimera 1.16 which involves the removal of ligands, ions, solvent molecules or residues from the structure followed by addition of hydrogen atoms and charge to the protein structure. The energy minimization was done by Swiss PDB viewer based on default parameters and the prepped structure of all proteins were saved in PDB format. The ligand structure preparation was carried out using Avogadro software where the geometry of the ligands was optimized by setting the GAFF (General AMBER Force Field) value at 5000. To determine the binding pocket sites for murE protein, the Computed Atlas of Surface Topography of proteins (CASTp 3.0) was used. The molecular docking of ligands with murE was performed using AutoDock Vina in UCSF Chimera 1.16. The grid size was adjusted to 20 and coordinates of pocket binding sites for receptor retrieved from CASTp 3.0 was entered. The visualization of docked protein-ligand complexes along with their interaction was done using BIOVIA discovery studio 2021. The different interactions between receptor-ligand complexes like alkyl interaction, Pi-Alkyl interaction, carbon- hydrogen bond interaction and conventional hydrogen bond interaction were observed.

RESULTS AND DISCUSSION

A. Isolation of the bacterial pathogen and identification

The isolation of the bacteria from symptomatic bulbs and leaves was carried out from the collected bacterial ooze. After an incubation period of 48 h, creamy white, opaque and mucoid bacterial colonies were observed on nutrient agar. Single colonies were selected and were re-streaked on nutrient agar to obtain the pure culture of the bacterium. The bacterial colonies on nutrient agar appeared creamy white, glistening and mucoid (Fig. 1), which is a typical colony morphology that can be attributed to plant pathogenic bacteria (Borkar, 2017). Similar colony morphology of bacteria isolated from soft rot symptoms was observed by various authors (Schwartz and Otto 2007; García-González *et al.*, 2018; Nataraj, 2019; Nguyen *et al.*, 2021). The pathogenicity of the bacteria was established on onion bulbs where the symptom appearance was observed three days post-inoculation. Similarly, Bishop and Davis (1990) observed similar symptoms of flaccidity and discolouration on artificially inoculated onion bulbs within 72 h of incubation. The molecular identification of the pathogen was carried out using species-specific primer corresponding to hsp60 gene where the amplification was obtained at 341 bp. Thus, the pathogen was confirmed as *Enterobacter cloacae* based on molecular characterization. The housekeeping gene hsp60 is a popular primer used in the identification of *E. cloacae*, owing to their high specificity and sensitivity. Moreover, the amplification of these two genes provides high resolution to differentiate between closely related strains of *E. cloacae* (Paauw *et al.*, 2008). Similarly, hsp60 specific primers were also employed for the molecular identification of *E. cloacae* pathogenic to chilli, shallot, cassava, paddy, *Aloe vera, etc.* (Santana *et al*., 2012; García-González *et al.*, 2018; Cao *et al.*, 2020; Nguyen *et al*., 2021).

B. Antibacterial activity of essential oils against Enterobacter cloacae

The antibacterial activity of selected essential oils (clove oil, citronella oil, thyme oil, cinnamon oil, eucalyptus oil) were evaluated at three different concentrations *viz.*, 500, 1000 and 1500 ppm (Table 1). Among the essential oils evaluated, citronella oil exhibited the highest inhibition zone measuring 15.43 mm, which was followed by thyme oil (14.76 mm) and clove oil (14.43 mm) at 500 ppm concentration. Whereas at 1000 ppm, thyme oil displayed the highest inhibition of 20.26 mm followed by clove oil showing an inhibition zone measuring 18.43 mm, while citronella oil exhibited an inhibition of 16.60 mm. At the highest tested concentration of 1500 ppm, thyme oil showed an inhibition zone measuring 21.28 mm, followed by clove oil (19.30 mm) and citronella oil (18.30 mm). Comparing the mean inhibition zone at all concentrations, thyme oil was the most effective (18.76) which was followed by clove oil (17.36 mm) and citronella oil (16.69 mm) (Fig. 2). Also, among the essential oils, eucalyptus oil showed no inhibition at all concentrations tested. Similar to the results obtained, Carrillo-Hormaza *et al.* (2017) evaluated three essential oils *viz*., clove, cinnamon and citronella and their respective constituents like cinnamaldehyde, thymol, carvacrol and citronellal against *E. cloacae* under *in vitro* conditions. The results revealed that the highest antibacterial activity was exhibited by clove oil, which was followed by citronella oil and cinnamon oil.

C. Molecular docking study

Molecular docking was performed with the receptor protein (murE) of *E. cloacae* with ten selected ligands (citral, citronellal, citronellol, geraniol, geranyl acetate, eugenol, isoeugenol, caryophyllene, 1,2-benzenediol, thymol and carvacrol). The essential role of the target protein (murE) in growth and survival of pathogen was evident from the biological and molecular function determined using the Blast2GO suite of OmicsBox 3.0 (Table 2). SWISS-MODEL server was employed to determine the 3-D structure of murE based on templates available in the database and the predicted models were evaluated based on three parameters *viz*., sequence identity, coverage and global mean quality estimation (GMQE). The homology model of murE (UDP-N-acetylmuramoyl-L-alanyl-D-glutamate) had a sequence identity of 86.28 per cent and 100 per cent coverage with the template (PDB ID: 1GG4) and a GMQE value of 0.90. Further, the quality of modeled proteins was validated by the construction and interpretation of Ramachandran plots using PROCHECK tool from SAVES v6.0 server. Ramachandran plots provide confirmation of protein structures based on dispersion angles present in a protein structure. Accordingly, more than 90 per cent amino acid residues in the most favored region indicate a good quality model. Ramachandran plot of the model for the protein murE revealed 92.4 per cent residues in the most favored region whereas zero per cent residues were present in the disallowed region (Fig. 3). Thus, the results of the Ramachandran plot revealed the quality of the protein model and thus was further taken as receptor for the docking studies. The docking of the receptor protein murE with ligands (citral, citronellal, citronellol, geraniol, geranyl acetate, eugenol, isoeugenol, caryophyllene, 1,2-benzenediol, thymol and carvacrol) yielded binding affinities of each receptor-ligand interaction and is given in Table 3. The lower the binding affinity, the better the ligand as it can competently bind to the receptors against other molecules and disrupt the function of the protein. The binding affinity values were in the range of -4.7 to -6.2 kcal/mol (Table 3). The best docked receptor-ligand complex was murE with caryophyllene having a binding affinity of -6.2 kcal/mol (Fig. 4). All the essential oil constituents exhibited good binding affinities with murE protein particularly 1,2-benzenediol, carvacrol, thymol and geranyl acetate showing binding affinities of -5.9 kcal/mol, -5.6 kcal/mol, -5.5 kcal/mol and -5.4 kcal/mol respectively. The target receptor murE is mainly involved in multiple pathways that leads to lysine biosynthesis and peptidoglycan synthesis in *Enterobacter cloacae.* Moreover, the protein plays a major role in regulation of cell shape and cell division in the pathogen and aids in the survival of the bacteria (Table 2). The results of molecular docking studies reveal good binding affinities for essential oil constituents with murE protein indicating that these constituents play a major role in disruption of the protein's function. Thus, the antibacterial activity of these essential oils particularly clove oil, thyme oil and citronella oil can be attributed to the constituents present in it (citral, citronellal, citronellol, geraniol, geranyl acetate, eugenol, isoeugenol, caryophyllene, 1,2-benzenediol, thymol and carvacrol) which possess high binding affinity to murE protein. Similar work was carried out by Kulkarni *et al.* (2021) where the antimicrobial activity of two essential oils *viz.*, cassia essential oil and holy basil essential oil were evaluated against postharvest pathogen of banana namely *Colletotrichum musae* and *Lasiodiplodia theobromae*. Both essential oils were effective in inhibiting the growth of the pathogen. Additionally, *in silico* analysis through molecular docking studies also ascertained that the essential oils components such as eugenol, cinnamyl acetate, caryophyllene, humulene and *trans*-calamenene, may most likely be responsible for high activity of the tested essential oils due to their binding affinity with chitin synthase protein present in the pathogen.

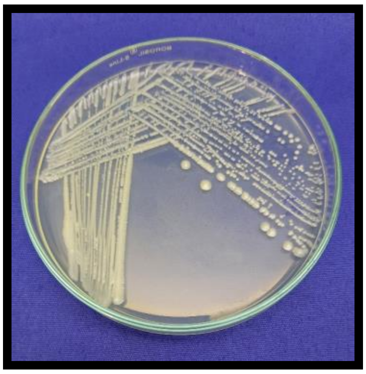

Fig. 1. *Enterobacter cloacae* colonies on nutrient agar.

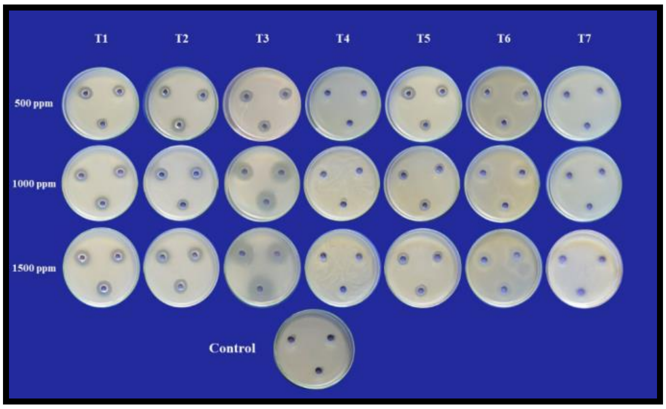

Fig. 2. *In vitro* evaluation of essential oils against *Enterobacter cloacae.*

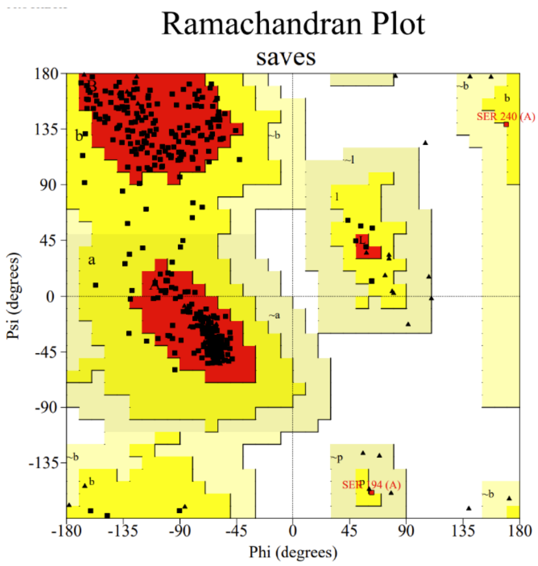

Fig. 3. Ramachandran plot for murE protein model.

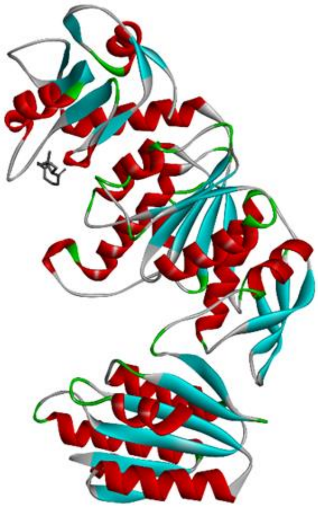

Fig. 4. Molecular docking of murE with caryophyllene (-6.2 kcal/mol).

Table 1: Effect of essential oils against *Enterobacter cloacae* under *in vitro* conditions.

Treatments	Essential oils	Mean inhibition zone (mm)			Mean
		500ppm	1000 ppm	1500 ppm	
T_1	Clove oil	14.36	18.43	19.30	17.36
T_2	Citronella oil	15.43	16.60	18.30	16.79
T_3	Thyme oil	14.73	20.26	21.28	18.76
T_4	Eucalyptus oil	0.00	0.00	0.00	0.00
T_5	Cinnamon oil	13.36	14.43	14.46	14.08
Mean		11.58	13.94	14.67	
		SEm±		**CD at 1 %**	
Treatment (T)		0.08		0.23	
Concentration (C)		0.06		0.18	
Interaction (T × C)		0.13		0.40	

Table 2: Biological function, molecular function and cellular localization of the selected protein determined using Blast 2GO.

Target protein	Biological function	Molecular function	Cellular localization
UDP-N-acetylmuramoyl-L-alanyl-D-glutamate (murE)	Anatomical structure development, cell wall organization or biogenesis, carbohydrate derivative metabolic process	Ligase activity	Cytosol

Table 3: Binding affinity of target protein (murE) with selected ligands.

Sr. No.	Ligands	Target proteins
1.	Citral	-5.0
2.	1,2-Benzenediol	-5.9
3.	Carvacrol	-5.6
4.	Caryophyllene	-6.2
5.	Citronellal	-4.8
6.	Citronellol	-4.7
7.	Eugenol	-5.1
8.	Geraniol	-4.9
9.	Geranyl acetate	-5.4
10.	Isoeugenol	-5.2
11.	Thymol	-5.5

CONCLUSIONS

Although India stands second in the production of onions, its productivity is often affected by many biotic factors among which soft rot is an emerging issue. The use of essential oils which is of plant origin can be a promising alternative for the management of this disease both under field and storage conditions. The study reveals the efficacy of mainly thyme oil, clove oil and citronella oil in managing the bacterial soft rot. Moreover, the mechanism of action of major constituents of these essential oils against target protein in the pathogen *Enterobacter cloacae* has been validated through molecular docking studies. Thus, the efficacy of essential oils against *E. cloacae* is backed by molecular docking studies and the study proposes essential oils as a suitable eco-friendly alternative to chemical pesticides for managing soft rot in onion.

FUTURE SCOPE

The study reveals the efficacy of essential oils against *Enterobacter cloacae* inciting bacterial soft rot in onion. The molecular docking studies reveal the potency of essential oil constituents in binding with the target protein in pathogenic bacterium. Thus, the future scope involves development of formulations based on these components which can effectively manage bacterial plant diseases.

Acknowledgement. The authors are thankful to University of Horticultural Sciences for the facilities used in the conduction of the experiments.

Conflict of interest. None.

REFERENCES

Bai, J., Li, J., Chen, Z., Bai, X., Yang, Z., Wang, Z. and Yang, Y. (2023). Antibacterial activity and mechanism of clove essential oil against foodborne pathogens. *LWT, 173*,114-129.

Bishop, A. L. and Davis, R. M. (1990). Internal decay of onions caused by *Enterobacter cloacae*. *Plant Disease, 74*(9), 692-694.

Borkar, S. G. (2017). Laboratory techniques in plant bacteriology. CRC Press, Pune pp. 457.

Cao, P., Li, C., Tan, K., Liu, C., Xu, X., Zhang, S., Wang, X., Zhao, J. and Xiang, W. (2020). Characterization, phylogenetic analyses, and pathogenicity of *Enterobacter cloacae* on rice seedlings in Heilongjiang Province, China. *Plant Dis*ease, *104*(6), 1601-1609.

Carrillo-Hormaza, L., Mora, C., Alvarez, R., Alzate, F. and Osorio, E. (2015). Chemical composition and antibacterial activity against *Enterobacter cloacae* of essential oils from Asteraceae species growing in the Páramos of Colombia. *Industrial Crops Production*, *77*, 108-115.

Devi, K. P., Nisha, S. A., Sakthivel, R. & Pandian, S. K. (2010). Eugenol (an essential oil of clove) acts as an antibacterial agent against *Salmonella typhi* by disrupting the cellular membrane. *Journal of ethnopharmacology*, *130*(1), 107-115.

García-González, T., Sáenz-Hidalgo, H. K., Silva-Rojas, H. V., Morales-Nieto, C., Vancheva, T. and Koebnik, R. (2018). *Enterobacter cloacae*, an emerging plant-pathogenic bacterium affecting chili pepper seedlings. *Plant Pathology Journal*, *34*(1), 1-18.

Gore, P. M., Ingle, R. W. and Rakhonde, P. N. (2020). Management of bulb rot of onion caused by *Erwinia carotovora*pv. *carotovora*. *Journal of Pharmacognosy and Phytochemistry, 9*(4), 3294-3301.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B. and Smith, B. (2002). Onions: a global benefit to health. *Phytotherapy Research*, *16*(7), 603–615.

Hoffmann, H., and Roggenkamp, A. (2003). Population genetics of the nomenspecies *Enterobacter cloacae*. *Applied and Environmental Microbiology*, *69*(9), 5306-5318.

Kulkarni, S. A., Sellamuthu, P. S., Anitha, D. P. M. and Madhavan, T. (2021). *In vitro* and *in silico* evaluation of antifungal activity of cassia (*Cinnamomum cassia*) and holy basil (*Ocimum tenuiflorum*) essential oils for the control of anthracnose and crown-rot postharvest diseases of banana fruits. *Chemical Papers*, *75*, 2043-2057.

Mandal, A., Kundu, A., Shanmugam, V., Kumar, R., Siddiqui, A., Chaudhary, A., Dutta, A., Patanjali, N., Saha, S., Kumar, A. and Dash, S. (2021). *Brassica nigra* essential oil: *In-vitro* and *in-silico* antibacterial efficacy against plant pathogenic and nitrifying bacteria. *Journal of Environmental Science and Health, Part B*, *56*(12), 1007-1022.

Nataraja, K. D. (2019). Etiology and management of leaf rot disease of onion caused by *Enterobacter cloacae. M. Sc. (Hort) Thesis*, Univ. Hort. Sci., Bagalkot (India).

Nguyen, T. N., Nguyen, T. V., Ho, T. C. N., Ngo, H. P. T. and Van Nguyen, H. M. (2021). A New Bacterial Soft Rot Disease of *Aloe vera* in Vietnam Infected by *Enterobacter cloacae*. *International Journal of Agricultural Biology*, *26*(5), 561-568.

Paauw, A., Caspers, M. P., Schuren, F. H., Leverstein-van Hall, M. A., Deletoile, A., Montijn, R. C., Verhoef, J. and Fluit, A. C. (2008). Genomic diversity within the *Enterobacter cloacae* complex. *PLoS One*, *3*(8), 30-48.

Santana, M. A., Rodriguez, M., Matehus, J., Faks, J., Bocsanczy, A., Gerstl, A., Romay, G., Montilla, J., Fernande, C.E., Moreno Zambrano, N. and Marval, D. (2012). A new bacterial disease of cassava in Venezuela caused by *Enterobacter cloacae*. *International Journal of Agricultural Biology*, *14*(2), 1-16.

Schwartz, H. F. and Mohan, S. K. (1995). Compendium of onion and garlic diseases, APS Press, New York pp. 256.

Schwartz, H. F. and Otto, K. (2007). First report of a bulb decay of onion by *Enterobacter cloacae* in Colorado. *Plant Disease, 84*(7), 808-808.

Yadav, P. K., Shukla, S. and Malhotra, S. K. (2016). *In Silico* Putative Drug Target Identification in *Enterobacter cloacae* and Homology Modelling of a Candidate Drug Target. *Journal of Bioinformatics and Proteomics Review*, *2*(1), 1-10.

How to cite this article: Anjali V.A., Basavarajappa M.P., Manjunath Hubballi, Raghavendra Gunnaiah, R.K. Mesta, Venkateshalu and Namita Bhaskar Raut (2023). Antibacterial Activity of Essential Oils Against *Enterobacter cloacae* inciting Onion Soft Rot and Molecular Docking Study of its Major constituents. *Biological Forum – An International Journal, 15*(11): 157-162.