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Exploring the anti-infective properties of *Cyamopsis tetragonoloba*: A GC-MS analysis and *in-vivo* model evaluation

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Abstract: Background: Bacterial infections are sometimes dangerous leading to life threatening conditions but some bacteria are beneficial for human body. **Objectives**: Many antibacterial medicines are available in the market but there are few natural products which also have anti-bacterial properties such as Cluster beans (*Cyamopsis tetragonoloba*), commonly known as guar, belong to the Leguminosae (Fabaceae) family. Cluster beans possess many bioactive components that inhibit the growth of pathogenic bacteria. **Methods**: In this experimental study, albino rats were divided into three groups: Group I served as the control, Group II received 500 mg/kg of cluster bean extract orally and Group III received 1000 mg/kg of the extract. The treatment was administered daily for 20 days. On day 21, the animals were euthanized and blood samples were collected to evaluate infection and inflammation markers, including complete blood count (CBC), erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). **Results**: Other than this, blood test, Gas Chromatography–Mass Spectrometry (GC-MS) analysis was also performed on Cluster beans to evaluate several bioactive compounds, including hexadecanoic acid, monolinoleoylglycerol trimethylsilyl and eicosanoic acid. **Conclusion**: Our results showed a significant improvement in reducing the bacterial infection in the treated groups compared to the control.

Keywords: Anti-bacterial activity; Cluster beans; CBC; ESR; GCMS

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INTRODUCTION

Bacterial infections are the result of detrimental bacteria attacking the body, resulting in a variety of ailments that can range from mild to severe. Although a significant number of microorganisms are benign or even beneficial to human health, certain types can result in severe infections that necessitate prompt medical intervention. These may include infections such as pneumonia, tuberculosis and urinary tract infections (UTIs), all of which can have severe consequences if not promptly addressed. It is extremely important to determine the causes, side effects and available treatment options for bacterial infections, particularly in the current environment of increasing antibiotic resistance. This is because these diseases are becoming increasingly difficult to manage and more detrimental to the general health Organization (2022).

Bacterial infections represent a significant global health and economic concern, affecting millions of people each year. According to the World Health Organization (WHO), the rise of antibiotic-resistant bacteria has further exacerbated this issue, making the treatment of bacterial infections increasingly challenging and contributing to higher mortality rates associated with these diseases (Ventola 2015).

It is observed that a long term use of antibiotics or the regular and continuous use of antibiotics accelerated the development of resistant strains, rendering certain infections more difficult to treat. According to the Centers for Disease Control and Prevention (CDC) that over 2.8 million illnesses and 35,000 deaths per year in the United States are caused by antibiotic resistance.

This research strongly indicated to control the use of antibiotics and also try to find alternatives for these medicines. For hundreds of years, people have employed natural medicines, especially those made from plants, to fight illnesses. Researchers are now again attracted in their possible use as substitutions to traditional antibiotics. For instance, laboratory studies have shown that herbs like garlic, oregano and echinacea may be good in killing germs (Hudson 2012).

Cluster beans (*Cyamopsis tetragonoloba*), sometimes known as guar, are a natural treatment that has shown promise in fighting bacterial infections. Cluster beans are part of the *Leguminosae (Fabaceae*) family and are full of bioactive chemicals. They have been shown to be quite effective against many types of bacteria. Guar gum, which comes from cluster beans, has been used in Ayurvedic medicine for a long time. It is known for its health advantages, such as improving digestion and helping those with diabetes.

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Recent research indicates that cluster beans may significantly contribute to the prevention and treatment of bacterial infections. This new study shows that they might be a useful addition to the list of natural medicines that help with microbial resistance and general health (Adki, Laddha *et al*. 2020).

People have used cluster beans in traditional medicine to cure a number of various illnesses. Their high fibre content, coupled with important vitamins and minerals, helps keep people happy and in good health (Chanda and Baravalia 2010). In therapeutic contexts, cluster beans have been used to regulate glucose levels and enhance metabolic processes, so bolstering the body's defense against bacterial infections. The examination of cluster beans in both traditional and contemporary medical contexts to elucidate its potential as a natural remedy for combating bacterial infections (Huppertz, Kachhap *et al*. 2023).

MATERIALS AND METHODS

The model was developed using albino male rats weighing between 180 and 200 grams. The rats were housed in polypropylene cages at the animal facility of the Department of Pharmacology at the Jinnah Sindh Medical University. The rats were meticulously monitored to ensure consistent conditions for sustenance, hydration and environmental variables such as temperature (25±2°C) and humidity (50-60%).

The incorporation of a 12-hour diurnal and nocturnal cycle was carefully considered. The animals were managed in accordance with the principles specified in Arrive essential.

Identification and purchasing of cluster beans

The cluster beans were purchased from the local market and were placed in the optimal temperature. The cluster beans are identified through Department of Pharmacognosy, Jinnah Sindh medical university.

Preparation of cluster beans extract

The cluster beans extract is prepared by first washing them properly then crushed them in mortal and pastel. The crushed solute is then grinding with few drops of water in the laboratory grinder. The extract is prepared in two concentrations. The first extract is prepared in 500mg/kg concentration and 2nd extract is prepared in 1000mg/kg.

Preparation of the model and dose designing

The model is prepared in albino rats that are divided in three groups. Each group consists of four rodents. Group I is considered as negative control group administered with standard diet. Group II is administered 500mg/kg cluster beans and group III is administered with 1000mg/kg cluster beans. The dosing is started 20 days prior to the infection induced.

On 20th day, the *Ecoli* broth was induced peritoneal in all three groups to introduce bacterial infection and after 24 hours blood sample is taken through cardiac puncture of the rats for laboratory testing of CBC, C- reactive protein and ESR.

C-reactive protein test

A 3 mL green/yellow-top tube, specifically developed for plasma separation, was used to take a sample on the 21st day. The process entails extracting immunoassays after pre-purification, with a preference for using the radioimmunoassay method (Paraskevas, Huizinga *et al*. 2008).

Complete blood count test

To separate the plasma, the blood sample was centrifuged for 15 minutes at 3000 revolutions per minute in a Humax 14k machine. The count of the red blood cells (RBC), white blood cells (WBC) and platelets using the Huma Count Plus Analyser, model #6400/s, made by Human Germany (Doig and Zhang 2017).

Erythrocyte sedimentation rate

A Westergren-Katz tube is filled with blood until it reaches the 200 mm mark. on order to determine the distance between the lowest point of the surface meniscus and the top limit of the red cell sediment, the tube is put on a rack in a strictly vertical position for one hour at room temperature. During this period, the measurement is taken (Kratz, Plebani *et al*. 2017).

Gas chromatography-mass spectroscopy

In the GCMS analysis, the beans were crushed and dissolved in a solution of methanol. The beans were soaked in this solution for a few hours and then subjected to sonication for three hours to remove any remaining residues. The resulting supernatant solution was then used for the evaluation of fatty acids and flavonoids using the GCMS instrument. The technique is sometimes referred to as ultrasonic extraction and analysis using GCMS in SIM (selective ion monitoring) mode (Mondello, Tranchida *et al*. 2008).

Statistical analysis

The SPSS version 18 is used to evaluate the results through one way Anova test.

RESULTS

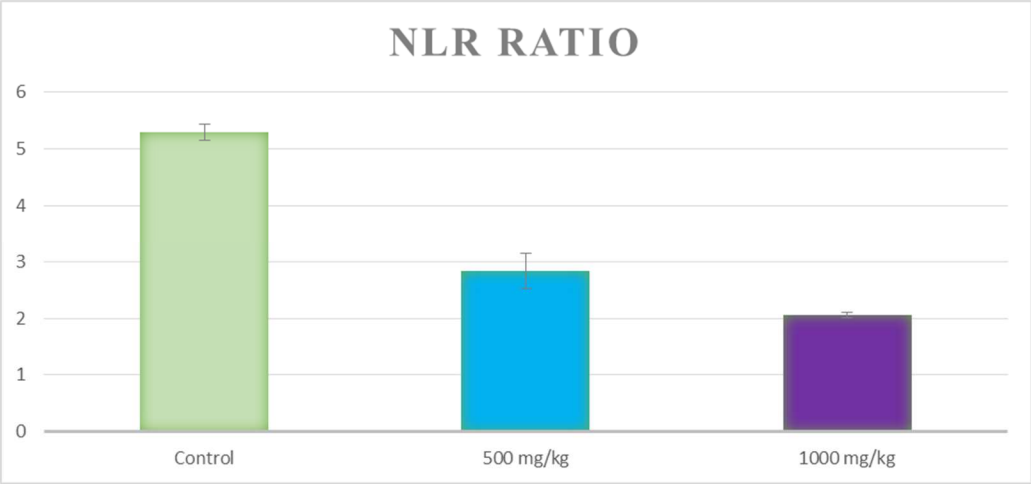
Table 1 illustrates the impact of two dosages (500mg/kg and 1000mg/kg) of *Cyamopsis tetragonoloba* for the duration of 21 days.

Post hoc analysis using Tukey's test reveals a very significant impact ($p<0.005$) in comparison to the control group for the whole treatment period for both dosages of 500mg/kg and 1000mg/kg.

Table 1: CRP value of control and treated groups.

S.No	Subjects	CRP Values (mg/dl)
1	Control	982.5±24.7
2	500 mg/kg	580±28.2@
3	1000 mg/kg	523±4.2@ $

Comparisons: @ With control $ With 500mg/kg



Graph 1: NLR ratio of control and treated groups

NLR is a biomarker for bacterial infection calculated by dividing the number of neutrophils by the number of lymphocytes in a blood sample, usually derived from a complete blood count (CBC) test.

NLR = {Neutrophil count}\{Lymphocyte count}.

Table 2: ESR of control and treated groups.

S. No	Subjects	ESR
1	Control	2 mm/hr
2	500 mg/kg	1 mm/hr @
3	1000 mg/kg	0.8 mm/hr @$

Comparisons: @ with control $ with 500mg/kg

Graph 1 indicates the value of NLR of control and treated groups. NLR formula is used to evaluate the severity of the infection. If the value is higher than the normal value of NLR, it shows the inflammation is present. The normal value is 1-2 while greater than that showed inflammation. The value of NLR in control is much greater than both the treated groups, also 1000mg/kg dose much less amount of NLR compared to 500mg/kg dose.

Table 2 illustrates the ESR of control and treated groups. Pos hoc analysis by Tukey test revealed a significant decrease ($p \leq 0.005$) in ESR in comparison with control and treated groups.

1000mg/kg showed a significant decrease ($p<0.05$) as compare to 500mg/kg of cluster beans.

DISCUSSION

Cluster beans are a rich source of many vitamins, minerals and proteins. The presence of several flavonoids and components in cluster beans confers various benefits to humans. This research used GCMS to determine that cluster beans contain several components that have positive anti-bacterial action.

The NLR (neutrophil-to-lymphocyte ratio) was assessed in all three groups of our research. The findings indicated that the NLR value in the negative control group was greater than 1000mg/kg and 500mg/kg.

This may be attributed to 7-Hexadecenal, a chemical with a long chain and unsaturated aldehyde structure. It destroys germs in three ways. The amphipathic character of the compound allows it to act on the cell's lipid bilayer. This disrupts the membrane by penetrating it and destabilizing and de-fluidizing it (Olanrewaju, Mordi *et al*. 2019). Additionally, it facilitates the exfiltration of necessary biological compounds, such as proteins, nucleotides and ions, from the cell. Depletion of these essential compounds leads to cell lysis.

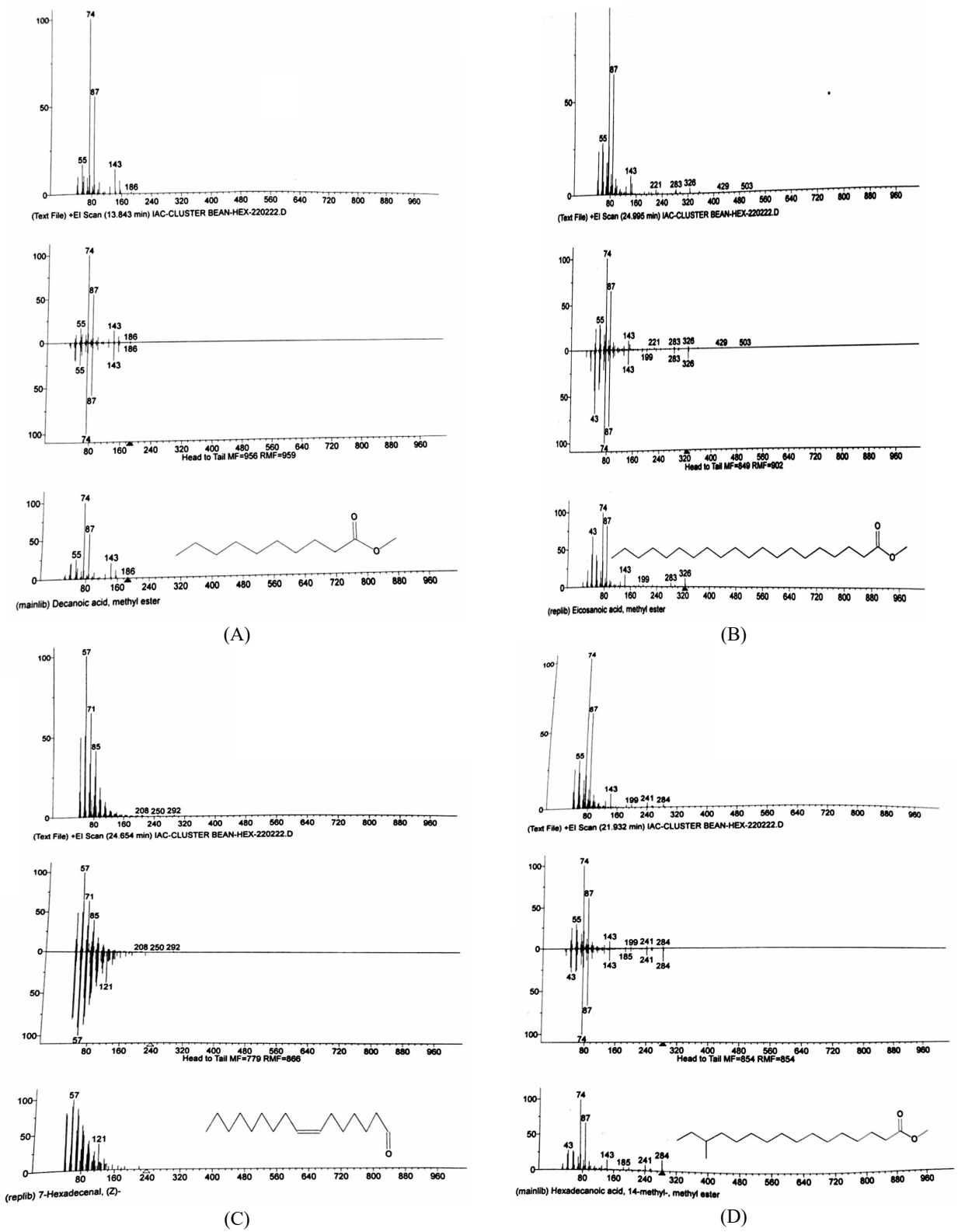


Fig. 1: GC-MS evaluation of cluster beans.

The constituents present in cluster beans are reported through GCMS. The RT value of Monolinoleoylglycerol trimethylsilyl is 22.2 minutes, (A) decanoic acid is 13.8 minutes; (B) eicosanoic acid is 24.9 minutes; (C) 7-hexadecenal is 24.6 minutes and; (D) hexadecanoic acid is 21.9 minutes.

Its second method of working is protein inhibition, which interrupts necessary proteins or enzymes and thus prevents cell division. It is not possible to pinpoint a target protein for this chemical because it is broad-spectrum. The compound causes the synthesis of reactive oxygen species like hydrogen peroxide and superoxide radicals, which cause cellular damage via DNA mutations, protein denaturation and lipid peroxidation (Lykholat, Khromykh *et al*. 2021).

The current study demonstrates that the levels of C-reactive protein in the negative control were significantly higher than those in the treated groups. This indicates that the severity of infection in the negative control group exceeds those in the treated groups. The compound Monolinoleoylglycerol trimethylsilyl ether (MLG-TMS), assessed using GCMS, could be responsible for this, as it acts by inhibiting the Quorum Sensing Interference mechanism (Zhang and Dong 2004). Quorum sensing is a process that allows bacteria to communicate and coordinate their actions using signaling molecules called autoinducers. These molecules spread and build up in the bacteria's surroundings, enabling the bacteria to sense their population density and regulate growth and behavior accordingly (Zhang and Dong 2004). Bacteria have specific receptors that recognize and bind to auto inducers. When these receptors are activated, they trigger a chain of signaling events that affect gene expression. This communication system allows bacteria to coordinate their behavior as a group. However, the process can be disrupted through a mechanism known as quorum sensing interference, which blocks the activation of certain pathways and gene expressions. As a result, bacteria are prevented from forming biofilms or producing virulence factors. One example of an autoinducer inhibitor is monolinoleoylglycerol trimethylsilyl, which disrupts this communication system.(Khattak, Ahmad *et al*. 2024).

Hexadecanoic acid, also known as palmitic acid, is a compound identified through GC–MS analysis of cluster beans. Although it functions similarly to eicosanoid acids, its unique mechanism involves altering the pH of the environment but in spite the fact that in carboxylic acid, the pH is lowering by donating the proton by hexadecanoic acid thus creating an environment which is not favorable for bacterial growth (Shaaban, Ghaly *et al*. 2021).

The results showed that the disease control group has higher value of ESR as compare to treated groups, this may indicating lower intensity of infection in the treated groups.

This difference may be attributed to the presence of Eicosanoic acid in cluster beans, also known as arachidic acid, which is a saturated fatty acid found in cluster beans and other plant species. Eicosanoic acid exhibits antibacterial activity through various mechanisms, similar to 7-hexadecenal. One unique mechanism of action for this compound is Micelle Formation. At low concentrations, Eicosanoic acid exists as individual molecules (Zheng, Yoo *et al*. 2005). However, as the concentration increases, the hydrophobic tails of these molecules start to interact with each other, forming micelles. These micelles disrupt the bacterial cell membrane, extract lipids from the membrane, or denature proteins, ultimately leading to cell lysis (Das 2018).

Through membrane disruption, protein inhibition and pH alteration, dodecanoic acid, also known as lauric acid, functions in a manner analogous to eicosanoic acid. The accumulation of this compound in the membrane of bacterial cells causes a change in the pH of the intracellular environment, disrupting the optimal conditions for bacterial growth and survival, including disrupting their metabolism and denaturing proteins by rendering them inactive (Lieberman, Enig *et al*. 2006).

These findings highlight the promising antibacterial potential of cluster beans and support their possible use as a natural therapeutic option for managing various infectious diseases. However, further clinical studies are warranted to validate their efficacy and safety in humans.

Limitations of methodology

The sample size was relatively small, which may limit the statistical power and generalizability of the results. Additionally, the study was conducted using an animal model and therefore the findings may not be directly extrapolated to humans. Only two doses of the extract were evaluated and the duration of treatment was limited. Furthermore, the study focused primarily on selected hematological and inflammatory markers without assessing detailed molecular mechanisms of antibacterial activity.

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Author's contributions

Rabia munawwar: Conception, analysis, interpretation; Humera answer: Proof read; Maliha afridi: Experimental research; Bisma nafees: Research and write up; Javeria Iftikhar: GCMS; Fatima hussain: Introduction write up.

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Data availability statement

Data will be provided on request by the corresponding author.

Ethical approval

This study is approved by the Department of Pharmacology, Institute of Pharmaceutical Sciences and Karachi, Pakistan with the approval number IPS/DRC/043/2024.

Conflict of interest

No potential conflict of interest was reported by the author(s)

REFERENCES

Adki KM, AP Laddha, AB Gaikwad and YA Kulkarni (2020). Potential role of seeds from India in diabetes. *Nuts and Seeds in Health and Disease Prevention, Elsevier*. pp.365-391.

Chanda S and Y Baravalia (2010). Screening of some plant extracts against some skin diseases caused by oxidative stress and microorganisms. *Afr. J. Biotechnol.,* **9**(21): 3210-3217.

Das UN (2018). Arachidonic acid and other unsaturated fatty acids and some of their metabolites function as endogenous antimicrobial molecules: A review. *J. Adv. Res.*, **11**: 57-66.

Doig K and B Zhang (2017). A methodical approach to interpreting the red blood cell parameters of the complete blood count. *ASCLS.*, **30**(3): 173-185.

Hudson JB (2012). Appl ications of the phytomedicine *Echinacea purpurea* (Purple Coneflower) in infectious diseases. *BioMed Res. Int.*, **2012**(1): 769896.

Huppertz M, D Kachhap, A Dalai, N Yadav, D Baby, MA Khan, P Bauer and KC Panigrahi (2023). Exploring the potential of mung bean: From domestication and traditional selection to modern genetic and genomic technologies in a changing world. *J. Agric. Food Res.*, **14**: 100786.

Khattak SU, M Ahmad, J Ahmad, S Ikram, S Ahmad, FM Alshabrmi and EA Alatawi (2024). Purification of potential antimicrobial metabolites from endophytic *Fusarium oxysporum* isolated from *Myrtus communis*. *Appl. Biochem. Biotechnol.*, **196**(12): 8940-8964.

Kratz A, M Plebani, M Peng, Y Lee, R McCafferty, S Machin and ICfSi Haematology (2017). ICSH recommendations for modified and alternate methods measuring the erythrocyte sedimentation rate. *Int. J. Lab. Hematol.*, **39**(5): 448-457.

Lieberman S, MG Enig and HG Preuss (2006). A review of monolaurin and lauric acid: natural virucidal and bactericidal agents. *Altern. Complement. Ther.*, **12**(6): 310-314.

Lykholat Y, N Khromykh, O Didur, S Okovytyy, T Sklyar, V Davydov, T Lykholat and I Kovalenko (2021). Soluble cuticular wax composition and antimicrobial activity of the fruits of Chaenomeles species and an interspecific hybrid. *Biosyst. Divers.,* **29**(4): 334-339.

Mondello L, PQ Tranchida, P Dugo and G Dugo (2008). Comprehensive two-dimensional gas chromatography-mass spectrometry: A review. *Mass Spectrom. Rev.*, **27**(2): 101-124.

Olanrewaju IO, RC Mordi, JO Echeme, OP Bolade, M Bashir, S Ekwuribe and JI Ayo-Ajayi (2019). *In-vitro* anti-microbial studies and GC/MS analysis of the leaf extract and fractions of *Polyalthia longifolia* (Engl. & Diels) Verde. *J. Phys. Conf. Ser.*, IOP Publishing.

Organization WH (2022). Global antimicrobial resistance and use surveillance system (GLASS) report 2022, World Health Organization.

Paraskevas S, JD Huizinga and BG Loos (2008). A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *J. Clin. Periodontol.*, **35**(4): 277-290.

Shaaban MT, MF Ghaly and SM Fahmi (2021). Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. *J. Basic Microbiol.*, **61**(6): 557-568.

Ventola CL (2015). The antibiotic resistance crisis: Part 1: Causes and threats. *P. T.*, **40**(4): 277.

Zhang LH and YH Dong (2004). Quorum sensing and signal interference: Diverse implications. *Mol. Microbiol.*, **53**(6): 1563-1571.

Zheng CJ, JS Yoo, TG Lee, HY Cho, YH Kim and WG Kim (2005). Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS letters*, **579**(23): 5157-5162.