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Muhammad Yaseen

Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan 29220, Pakistan

Muhammad Kamran

Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan 29220, Pakistan

Arshad Farid

Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan 29220, Pakistan,
arshadfarid@gu.edu.pk

Salmah Ismail


Institute of Biological Sciences, Faculty of Science, University Malaya, Kuala Lumpur 50603, Malaysia

Muhammad Muzammal

Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan 29220, Pakistan

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Antibacterial, Hemagglutination, and Insecticidal Activity Studies on the Solvent Extracts of the Roots of *Olea ferruginea*

Authors

Muhammad Yaseen, Muhammad Kamran, Arshad Farid, Salmah Ismail, Muhammad Muzammal, Kamal Abdul Amir, Muhammad Hashim Khan, Sohail Ahmad, and Sheikh Abdur Rashid

Antibacterial, Hemagglutination, and Insecticidal Activity Studies on the Solvent Extracts of the Roots of *Olea ferruginea*

Muhammad Yaseen¹, Muhammad Kamran¹, Arshad Farid^{1*}, Salmah Ismail²,
Muhammad Muzammal¹, Kamal Abdul Amir³, Muhammad Hashim Khan¹,
Sohail Ahmad¹, and Sheikh Abdur Rashid⁴

1. Center of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan 29220, Pakistan
2. Institute of Biological Sciences, Faculty of Science, University Malaya, Kuala Lumpur 50603, Malaysia
3. Department of Biology, Islamabad Model College F11/1, Islamabad 44000, Pakistan
4. Faculty of Pharmacy Gomal University, Dera Ismail Khan 29220, Pakistan

*E-mail: arshadfarid@gu.edu.pk

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Abstract

Olea ferruginea has been used to treat skin ailments, as well as kidney and ocular problems for a long time. The current study was designed with the aim of investigating and scientifically validating its widespread use. Chloroform, *n*-hexane, and ethyl acetate were used to assess the antibacterial, hemagglutination, and insecticidal properties of *O. ferruginea* roots. *Escherichia coli*, *Micrococcus luteus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were among the bacterial strains selected for assessing antibacterial activity. The results showed that ethyl-acetate (EtOAc) extract (56%) and chloroform (CHCl₃) extract (56%) showed the widest zone of inhibition against *K. pneumoniae*, while *n*-hexane extract (13.4%) had the lowest zone of inhibition against *P. aeruginosa*. All three extracts remained inactive against *M. luteus*. During hemagglutination activity, the CHCl₃ and EtOAc extracts, when used at different concentrations, only agglutinated the AB–ve and O+ve blood groups, respectively, while the *n*-hexane extract strongly agglutinated the A–ve and B–ve blood groups at different concentrations. The plant extracts were also checked for insecticidal activity against *Rhizopertha dominica*. The results also revealed the high mortality rate of CHCl₃ extract (70%) against *R. dominica* as compared to other extracts. The aforesaid activities suggest that the roots of *O. ferruginea* have excellent medicinal viability and contain a wide variety of agglutinins and lectins, as shown in this study.

Keywords: antibacterial, chloroform, ethyl-acetate, hemagglutination, insecticidal, *n*-hexane, *olea ferruginea*

Introduction

Medicinal plants are commonly used in both developing and developed countries due to their safety. To safeguard their health and treat diseases, the majority of Indians have access to or employ a variety of ancient medicines. These plants, such as herbs, are utilized as monotherapies and as supplements to other treatments. However, the lack of standardization, recognition, and pharmacopeial standards pose major hurdles in the use of these formulations [1]. In rural areas, herbal treatment plays a vital role as a domestic medicine for a variety of diseases in the form of local drugs derived from plants [2].

Olea ferruginea, a native broad-leaved tree species of the subcontinent that grows up to 10 meters tall and is found in subtropical, dry temperate, and moist temperate climates, is a common evergreen tree found in the mountains of Pakistan. It is also found in Southeastern

Europe, North Africa, Western Asia, Northern Iran, and the Mediterranean region [3, 4]. There are about 80 different names for *O. ferruginea* [5]. The genus *Olea* contains about 30 species [6], but the most common is *O. europaea* L [7], which is consumed as food. It is also used to lower blood sugar, lipid, and uric acid; treat diabetes, swelling, hypertension, respiratory and urinary tract infection, diarrhea, digestive and gastral diseases, asthma, hemorrhoids, rheumatism; and also used as an antiseptic mouthwash, laxative, and vasodilator [8, 9].

Oleuropein and oleacein, two secondary metabolites found in the leaves, exhibit hypoglycemic and hypotensive properties, respectively. The fruits and oil of *Olea*, which are key components of a substantial amount of the world's daily diet, are frequently studied for their nutritional value [10, 11]. For example, several studies have shown that olive leaf extract can lower blood pressure in animals, help avoid intestinal muscle spasms,

and alleviate arrhythmia [12]. Numerous phenolic compounds, especially secoiridoids and iridoids, are extracted from olive plants [13]. In addition, its leaves, berries, and wood may also be used as fodder, oil, fuel, and building materials [14]. The antibacterial, hemagglutination, and insecticidal activities of *O. ferruginea* roots were investigated in the current work.

Materials and Methods

Plant materials. The plant roots were collected from the hilly areas of South Waziristan in 2018 and were identified in department of Botany, University of Peshawar, Pakistan. For the next step, the roots were dried under the shade at room temperature ($25\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) for 2 weeks before being sliced into little pieces.

Extraction. The plant materials were grinded using an electric grinder to obtain a fine powder. The powder was soaked with chloroform, *n*-hexane, and ethyl-acetate solvents for the process of extraction. By constant shaking after every 24 h, the maceration was carried out at room temperature for 10 days. Whatman filter papers were used to filtrate the mixture after maceration. Each filtrate contained some amount of solvent and then evaporated by a rotary evaporator to obtain the crude extracts. The final extracts were saved in labelled bottles for further use.

Hemagglutination activity. The hemagglutination activities of fractions were tested against blood groups of human erythrocytes ABO [15]. Stock solutions of the sample at a concentration of 1 mg/mL were prepared and serially diluted for each solution.

Blood samples of normal volunteer individuals were obtained. The samples were centrifuged in order to obtain erythrocytes. Then, 2% erythrocyte solution with phosphate buffer (7.4) was prepared. About 1 mL of the 2% erythrocyte was collected with 1 mL of the test sample and incubated at $\pm 25\text{ }^{\circ}\text{C}$.

Antibacterial Activity

Percent zone of inhibition. Various extracts of *O. ferruginea* were tested for antibacterial activity against *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Micrococcus luteus* by using the disc diffusion process [16]. An 18-hour-old culture of nutrient broth was transferred and spread on sterile nutrient agar plates to create bacterial lawns. Sterile 6 mm discs with test extracts were placed on the plates with broth media. Next, 3 mg of extract was dissolved in 1 mL of DMSO as a stock solution to prepare the test samples. The plates were incubated for 24 hours at $37\text{ }^{\circ}\text{C}$. The positive control was amoxicillin, and the negative control was DMSO. In comparison to the positive control, the percentage of inhibition zone was calculated in mm, as shown below.

$$\text{Percent Inhibition} = \frac{\text{Zone of Inhibition of Sample}}{\text{Zone of Inhibition of Standard}} \times 100 \quad (1)$$

Insecticidal activity. Insecticidal activity against the insect *Rhyzopertha dominica* was performed with some modification [17]. The stored grain pests were reared in the plastic bottles containing breeding media (sterile). The stock solution was made by dissolving 200 mg of plant extract in 3 mL of methanol. The filter paper was cut on the first day and placed onto petri dishes, after which the sample solutions of each extract were placed on the dishes. The plates were left overnight to allow the organic solvents in the samples to fully evaporate. On the second day, 10 healthy insects were transferred to each plate. The plates were then incubated for 3 days at $27\text{ }^{\circ}\text{C}$ in a growth chamber with 50% relative humidity. After every 24 h of incubation, the number of insects that survived on each plate was counted and recorded. Methanol was used as a negative control, while permethrin was used as a positive control. The percentage of insects that died was estimated as follows:

$$\text{Percentage Mortality} = \frac{100 - \text{No. of Insects in Test}}{\text{No. of Insects Alive in Control}} \times 100 \quad (2)$$

Results and Discussion

Hemagglutination activity. Agglutinins and lectins are proteins found in both plants and animals with the ability to bind to definite mono or oligosaccharides. Phytolectins or phyto-agglutinins are proteins found in plants; they have an advantage over animal lectins in the sense that they are more abundant and economical [18]. Lectins can be used to determine the sugar constituents of control cells and those of tumor cells, because they specifically bind to various sugar constituents on the surface of cells or in solutions [19]. Lectins also play a role in the structure, function, and approximation of virus particles, as well as the agglutination of RBCs [20].

When *O. ferruginea* root extracts were interacted with human erythrocytes, smooth buttons formed at the bottom of the test tubes. Some extracts (at 1 mg/mL) demonstrated hemagglutination activity against human erythrocytes, while others did not. Due to the various uses of lectins listed above, the solvent extracts of *O. ferruginea* roots were selected for hemagglutination activity against the human erythrocyte blood groups (ABO). The results are shown in Table 1. As can be seen, the CHCl_3 extract revealed strong activity against the AB^- blood group at concentrations of 1:4 and 1:16, while it showed moderate and weak activity at concentrations of 1:2 and 1:8 against the same blood group. The CHCl_3 extract remained inactive against the other blood groups. Similarly, the *n*-hexane extract revealed strong agglutination activity against the A^- blood group at concentrations of 1:8 and

1:16, as well as in the B⁻ blood group at concentrations of 1:2 and 1:16. In comparison, it showed moderate activity against the same blood group (i.e., B⁻ blood group) at concentrations of 1:4 and 1:8. The *n*-hexane extract remained inactive against other blood groups. Therefore, the EtOAc fraction does not have the ability to agglutinate erythrocytes of any blood group at any concentration except O⁺, showing a weak agglutination activity against it at a concentration of 1: 8.

Antibacterial activity. Many plant extracts have shown efficacy against a wide range of pathogenic bacteria and fungi. For example, the solvent extracts of *Bunium bulbocastanum*, *Justicia zelanica*, *Thymus vulgaris*, and *Seriphidium kurramense* demonstrated significant antibacterial activities against several harmful bacteria and fungi. Antimicrobial properties have also been discovered in extracts from plants, such as lemon, *Lantana camara*, guava, *Strobilanthes urticifolia*, pomegranate, and *Acacia nilotica*, among others [21]. In the current study, various forms of *O. ferruginea* root extracts were tested for antibacterial activity, with the results indicating that the CHCl₃ and EtOAc extracts against *Klebsiella pneumonia* presented the widest zone of inhibition (56 mm). Similarly, CHCl₃ extract was more active (44.44 mm) against *E.coli*, while the EtOAc and *n*-hexane extracts showed 25 mm and Zero/No zones of inhibition, respectively. Against *P. aeruginosa*, the EtOAc, CHCl₃, and *n*-hexane extracts showed 21, 17, and 13 mm zones of inhibition, respectively. All three extracts remained inactive against *M. luteus*. Figures 1 and 2 summarize the antibacterial results.

Insecticidal activity. Plant extracts contain a variety of active chemicals that are extremely beneficial to plant defense against a variety of insect pests. For example, *Artemisia argyi*, *Cannabis indica*, and *Citrullus colocynthis* leaf extracts showed strong insecticidal effectiveness against major crop insects [22]. Using the impregnated filter paper process [23], various extracts of *O. ferruginea* roots were checked for insecticidal activity against *R. dominica*. As shown in Figure 3, the CHCl₃ extract showed strong activity (70%) on its first day and poor insecticidal activity (10%) on its second and third days. In addition, the EtOAc extract showed low insecticidal activity (20%), while the *n*-hexane extract did not have insecticidal activity at all against *R. dominica*. Positive control indicates 100% mortality and, in the case of negative control, 0% mortality.

According to the findings, the current study’s key weakness is the lack of *in vivo* trials. Furthermore, the study was only limited to certain solvent extracts, which should be expanded in the future to better investigate the therapeutic effects of the *O. ferruginea* roots. Furthermore, because our investigation was confined to only a few bacterial strains, i.e., *K. pneumonia*, *E. coli*, *P. aeruginosa*, and *M. luteus*, Future studies should be undertaken to further screen the antimicrobial activities against diverse pathogenic bacterial species. This can be achieved by further exploring the medicinal relevance of the chosen plant. The foregoing findings show that plant extracts have potent antibacterial and insecticidal properties against a variety of microorganisms and insects, which should be investigated further by isolating and characterizing compounds from these extracts.

Table 1. Hemagglutination Activity of *Olea ferruginea* Root Extracts Against Human Blood Groups

Blood Groups	CHCl ₃				n-hexane				EtOAc			
	1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16
AB ⁺	-	-	-	-	-	-	-	-	-	-	-	-
AB ⁻	++	+++	+	+++	-	-	-	-	-	-	-	-
A ⁺	-	-	-	-	-	-	-	-	-	-	-	-
A ⁻	-	-	-	-	-	-	+++	+++	-	-	-	-
B ⁺	-	-	-	-	-	-	-	-	-	-	-	-
B ⁻	-	-	-	-	+++	++	++	+++	-	-	-	-
O ⁺	-	-	-	-	-	-	-	-	-	-	+	-
O ⁻	-	-	-	-	-	-	-	-	-	-	-	-

Weak (+), Moderate (++), Strong (+++), No hemagglutination activity (-),

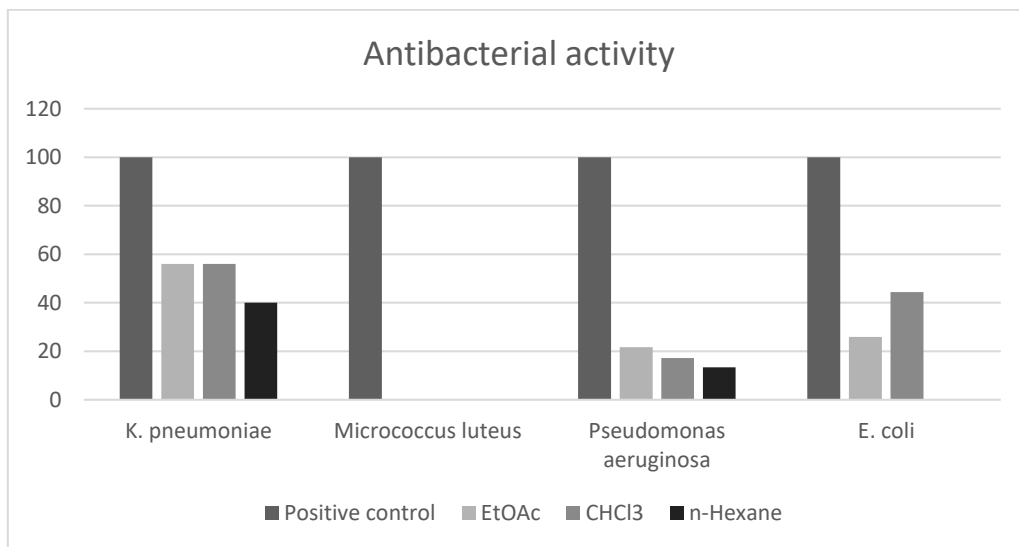


Figure 1. The Antibacterial Activity of the CHCl₃, n-Hexane and EtOAc Extracts of *Rhizopertha dominica* Against the Test Bacteria

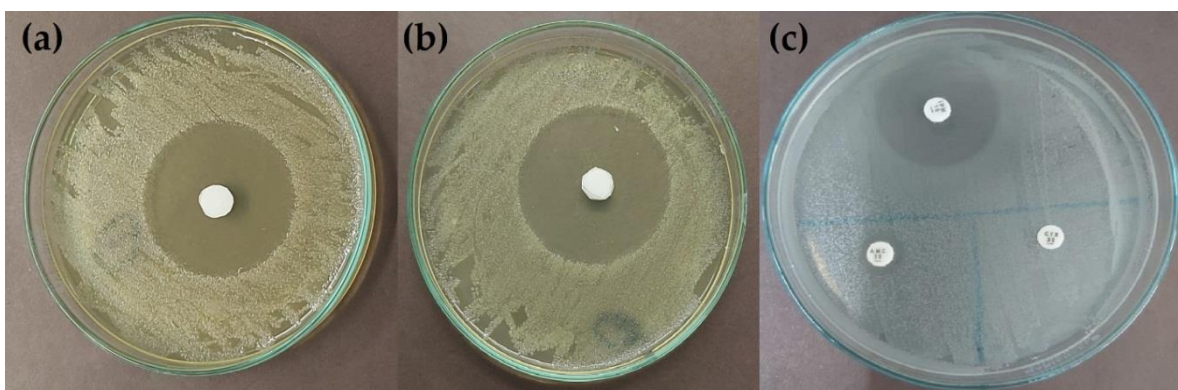


Figure 2. (a, b) The CHCl₃ and EtOAc Extracts with the Widest Zone of Inhibition (56 mm) Against *Klebsiella pneumoniae*, (c) The EtOAc and n-hexane Extracts with 25 mm and Zero/No Zones of Inhibition, Respectively

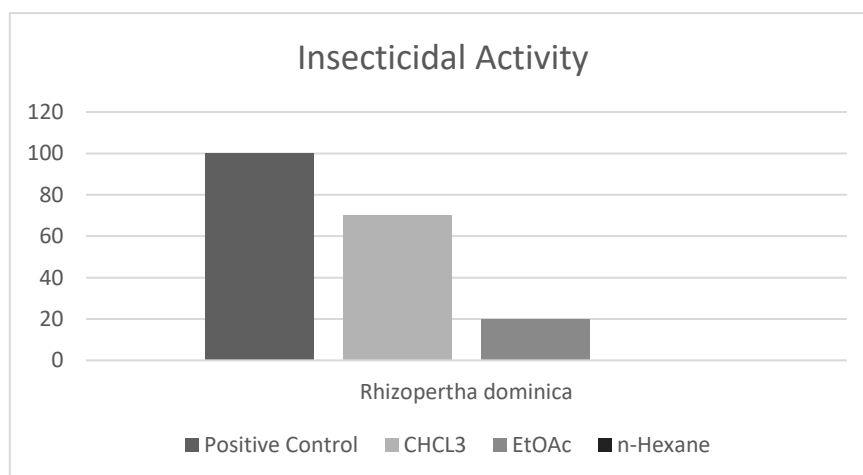


Figure 3. Insecticidal Activities of the CHCl₃, n-hexane and EtOAc Extracts Against *Rhizopertha dominica*

Conclusion

The current study clearly demonstrated that the roots of *O. ferruginea* contained lectins and can be a beneficial source of phytolectins in small amounts. This material was also able to agglutinate the blood when tested at various concentrations. Furthermore, *O. ferruginea* plant extracts have powerful insecticidal and antibacterial properties. Based on the above findings, it can be inferred that the study should be expanded to include the separation of biochemical compounds, which can provide researchers with a new avenue to explore the plant's other medicinal properties.

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References

- [1] Vaidya, A.D., Devasagayam, T.P. 2007. Current status of herbal drugs in India: an overview. *J. Clin. Biochem. Nutr.* 41(1): 1–11, <https://doi.org/10.3164/jcbrn.2007001>.
- [2] Bano, A., Ahmad, M., Hadda, T.B. 2014. Quantitative ethnomedicinal study of plants used in the skardu valley at high altitude of Karakoram-Himalayan range, Pakistan. *J. Ethnobiol. Ethnomed.* 10: 43, <https://doi.org/10.1186/1746-4269-10-43>.
- [3] Zohary, D., Hopf, M., Weiss, E. 2012. *Domestication of Plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin.* Oxford University Press on Demand.
- [4] Ahmad, B., Ali, N., Bashir, S., Choudhary, M.I., Azam, S., Khan, I. 2009. Parasitocidal, antifungal and antibacterial activities of *Onosma griffithii* Vatke. *Afr. J. Biotechnol.* 8: 19.
- [5] Medail, F., Quezel, P., Besnard, G., Khadari, B. 2001. Systematics, ecology and phylogeographic significance of *Olea europaea* L. ssp. *maroccana* (Greuter & Burdet) P. Vargas *et al.*, a relictual olive tree in south-west Morocco. *Bot. J. Linn. Soc.* 137(3): 249–266, <https://doi.org/10.1111/j.1095-8339.2001.tb01121.x>.
- [6] Bracci, T., Busconi, M., Fogher, C., Sebastiani, L. 2011. Molecular studies in olive (*Olea europaea* L.): overview on DNA markers applications and recent advances in genome analysis. *Plant. Cel. Rep.* 30(4): 449–462, <https://doi.org/10.1007/s00299-010-0991-9>.
- [7] Kaniewski, D., Van Campo, E., Boiy, T., Terral, J.F., Khadari, B., Besnard, G. 2012. Primary domestication and early uses of the emblematic olive tree: palaeobotanical, historical and molecular evidence from the Middle East. *Biol. Rev.* 87(4): 885–899, <https://doi.org/10.1111/j.1469-185X.2012.00229.x>.
- [8] Sarwar, M.F., Sarwar, M.H., Sarwar, M., Qadri, N.A. and Moghal, S., 2013. The role of oilseeds nutrition in human health: A critical review. *J. Cereals Oilseeds.* 4(8): 97–100, <https://doi.org/10.5897/JCO12.024>.
- [9] Deng, J., Xu, Z., Xiang, C., Liu, J., Zhou, L., Li, T., Yang, Z., Ding, C. 2017. Comparative evaluation of maceration and ultrasonic-assisted extraction of phenolic compounds from fresh olives. *Ultrason Sonochem.* 37: 328–334, <https://doi.org/10.1016/j.ultsonch.2017.01.023>.
- [10] Boskou, D. 2008. Phenolic compounds in olives and olive oil. *Olive Oil Minor Constituents Health.* 1, <https://doi.org/10.1201/9781420059946.ch3>.
- [11] Susalit, E., Agus, N., Effendi, I., Tjandrawinata, R.R., Nofiarny, D., Perrinjaquet-Mocchetti, T., Verbruggen, M. 2011. Olive (*Olea europaea*) leaf extract effective in patients with stage-I hypertension: comparison with Captopril. *Phytomed.* 18(4): 251–258, <https://doi.org/10.1016/j.phymed.2010.08.016>.
- [12] Mehmood, A., Murtaza, G. 2018. Phenolic contents, antimicrobial and antioxidant activity of *Olea ferruginea* Royle (*Oleaceae*). *BMC Complem. Altern. Med.* 18(1): 1–6, <https://doi.org/10.1186/s12906-018-2239-0>.
- [13] Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A.M., Segura-Carretero, A., Fernández-Gutiérrez, A., Lercker, G. 2007. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade Alessandra. *Mol.* 12(8): 1679–1719, <https://doi.org/10.3390/12081679>.
- [14] Ahmed, M., Khan, N., Wahab, M., Hamza, S.A.L.M.A., Siddiqui, M.F., Nazim, K., Khan, M.U. 2009. Vegetation structure of *Olea ferruginea* Royle forests of lower Dir district of Pakistan. *Pak. J. Bot.* 41(6): 2683–2695.
- [15] Farid, A., Ahmad, B., Yasin, M., Haq, F., Khan, M. B., Khan, S. 2017. Haemagglutination and Antibacterial activities of *Strobilanthes urticifolia* Wall. ex Kuntze. *Int. J. Biosci.* 11(4): 35–40, <http://dx.doi.org/10.12692/ijb/11.4.35-40>.
- [16] Farid, A., Shah, A.H., Ayaz, M., Amin, A., Yaseen, M., Ullah, H., Haq, F. 2012. Comparative study of biological activity of glutathione, sodium tungstate and glutathione-tungstate mixture. *Afr. J. Biotechnol.* 11(45): 10431–10437.
- [17] Ali, A., Tabanca, N., Demirci, B., Raman, V., Budel, J.M., Baser, K., Khan, I.A. 2020. Insecticidal and biting deterrent activities of *Magnolia grandiflora* essential oils and selected pure compounds against *Aedes aegypti*. *Mol.* 25(6): 1359, <http://dx.doi.org/10.3390/molecules25061359>.

- [18] Lannoo, N., Van Damme, E.J. 2014. Lectin domains at the frontiers of plant defense. *Front. Plant. Sci.* 5: 397, <https://doi.org/10.3389/fpls.2014.00397>.
- [19] Jiang, Q.L., Zhang, S., Tian, M., Zhang, S.Y., Xie, T., Chen, D.Y., Chen, Y.J., He, J., Liu, J., Ouyang, L., Jiang, X. 2015. Plant lectins, from ancient sugar-binding proteins to emerging anti-cancer drugs in apoptosis and autophagy. *Cel. Prolif.* 48(1): 17–28.
- [20] Jena, J., Gupta, A.K. 2012. *Ricinus communis* Linn: a phytopharmacological review. *Int. J. Pharm.* 4(4): 25–29, <https://doi.org/10.1111/cpr.12155>.
- [21] Ahmad, K., Ali, A., Afridi, W.A., Somayya, R., Ullah, M.J. 2018. Antimicrobial, hemagglutination and phytotoxic activity of crude ethanolic and aqueous extracts of *Seriphidium kurramense*. *J. Tradit. China Med.* 38(3): 433–438, [https://doi.org/10.1016/S0254-6272\(18\)30635-6](https://doi.org/10.1016/S0254-6272(18)30635-6).
- [22] Ahmed, M., Peiwen, Q., Gu, Z., Liu, Y., Sikandar, A., Hussain, D., Javeed, A., Shafi, J., Iqbal, M.F., An, R., Guo, H. 2020. Insecticidal activity and biochemical composition of *Citrullus colocynthis*, *Cannabis indica* and *Artemisia argyi* extracts against cabbage aphid (*Brevicoryne brassicae* L.). *Sci. Rep.* 10(1): 1–10, <https://doi.org/10.1038/s41598-019-57092-5>.
- [23] Maliha, M., Tan, B., Wong, K., Miri, S., Brammananth, R., Coppel, R.L., Werrett, M., Andrews, P.C., Batchelor, W. 2021. Bismuth phosphinato incorporated antibacterial filter paper for drinking water disinfection. *Colloid. Surf. A Physicochem. Eng.* 627: 127167, <https://doi.org/10.1016/j.colsurfa.2021.127167>.