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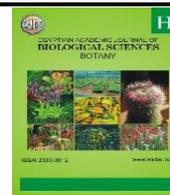
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## Ecofriendly Natural Extracts as Biobactericides Against Potato Pathogenic Bacteria

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### ABSTRACT

**Background:** The bacterial pathogens *Pectobacterium carotovorum*, *P. atrosepticum*, *Ralstonia solanacearum*, and *Streptomyces scabies* caused several diseases in potatoes. To control these pathogens, many chemical methods are used, which harm the environment and have an effect on human health. Recently, instead of these hazard methods, biocontrol agents like plant extracts were developed and had a good sense of control. **Methods:** The plant extracts were prepared in methanol and acetone solvents, and the disk diffusion method was used in the bioassay. **Results:** In this study, we tested two natural extracts as eco-friendly biobactericides against potato pathogens *P. carotovorum*, *P. atrosepticum*, *R. solanacearum*, and *S. scabies*. The *Juniperus phoenicea* 25% methanol extract showed considerable antibacterial activity in vitro, with optimal doses at 3000, 5000, 7000, and 10000 ppm. The extract effectively suppressed *P. atrosepticum* isolate, with inhibition zones ranging from 11.00 to 22.33 mm at doses of 3000-10000 ppm. However, it had little impact until reaching 3000 ppm against *R. solanacearum* (8.67–10 mm) and *P. carotovorum* (6.33–9.67 mm) isolates. The extract also greatly inhibited *S. scabies* isolate growth, with a mean inhibition zone of 3.5 mm. At doses of 7000 ppm and 3000 ppm, *Cicer arietinum* 75% acetone leaf extract showed the maximum effectiveness against *P. atrosepticum* and *P. carotovorum*, with inhibition zone values of 10 mm and 8.33 mm, respectively. However, it had less effectiveness against *S. scabies* (7.33–10.00 mm) and *R. solanacearum* (8.00–9.00 mm) isolates. Overall, these natural extracts may fight potato pathogenic microorganisms.

### INTRODUCTION

The potato, scientifically known as *Solanum tuberosum* L., holds a significant position in the global agricultural industry and food security, ranking fourth among staple food crops globally, behind wheat, rice, and maize (Saar-Reismaa *et al.*, 2020). According to Food and Agriculture Organization (FAO) statistics from 2018, Egypt has achieved an annual domestic production of roughly 5 million tons.

Potato tubers possess a significant nutritional profile, rendering them a valuable source of energy while also exhibiting notable health-promoting properties, including anti-inflammatory and anti-cancer effects (Kalita *et al.*, 2018). Nevertheless, the growth and post-

harvest storage of potatoes are substantially jeopardized by the presence of bacterial soft rot disease (Hadizadeh *et al.*, 2019). The significant economic losses incurred during the transportation and storage of crops can be attributed mostly to the pathogenic effects of *Pectobacterium carotovorum* (Charkowski, 2018).

The utilization of several chemical bactericides for the management of soft-rot bacteria has been hindered due to their potential hazardous risks to both human health and the environment. The utilization of biocontrol strategies has been investigated as a means to address several plant infections (Abd-El-Khair *et al.*, 2021). In recent times, there has been a growing need to decrease the utilization of chemical pesticides in the field of agriculture as a result of apprehensions over their ecological consequences and potential health hazards. As a result, there has been a growing recognition of the need to develop safer antimicrobial treatments, including extracts derived from plants, for the purpose of managing potato bacterial soft rot (Körpe *et al.*, 2013; Yuliar *et al.*, 2015). According to Ribera and Zuñiga (2012), plant antimicrobial metabolites, which can be either natural bioactive molecules known as phytoanticipins or those produced in response to pathogen infections called phytoalexins, have the ability to suppress pathogens.

Several studies have demonstrated that some plants possess secondary metabolites that exhibit antimicrobial capabilities. These compounds may be utilized to manage plant diseases either by inducing systemic resistance or by directly inhibiting bacterial growth (Wagura *et al.*, 2011; Malkhan *et al.*, 2012; Rahman *et al.*, 2012; Körpe *et al.*, 2013). Although water is commonly used as a universal solvent for extracting antimicrobial chemicals, organic solvents such as methanol and acetone are generally favored due to their capacity to provide more reliable antibacterial activity. These solvents are preferred due to their elevated polyphenol concentration in comparison to aqueous extracts, as well as their enhanced capacity to permeate cellular membranes, hence aiding in the extraction of intracellular constituents from plant matter. The extraction of antimicrobial components from plants is commonly carried out using acetone or methanol as solvents due to the prevalence of aromatic or saturated organic compounds in these plant sources (Prashanth *et al.*, 2011).

The objective of this research is to examine the capacity of plant extracts that are readily accessible, economically feasible, and ecologically sustainable to effectively control potato bacterial soft rot through their antimicrobial capabilities. This study investigates the utilization of different organic solvents, such as methanol and acetone, in the extraction process of antimicrobial compounds from certain plant sources. The objective is to develop an environmentally friendly and sustainable method for managing phytopathogens and safeguarding potato crops.

## MATERIALS AND METHODS

### Isolation of the Pathogens:

The process of isolation was conducted on potato tubers that exhibited varied degrees of natural infection with symptoms associated with potato infection. During the 2020–2021 seasons, potato tubers were gathered from many areas throughout Alexandria governorate, Egypt. The tubers were subjected to surface sterilization by submerging them in a sodium hypochlorite solution with a concentration of 1% (v/v) for 2 minutes. Subsequently, the specimens underwent two rounds of rinsing with sterile water. Subsequently, a minute fraction of the afflicted tissues was subjected to maceration using 5 ml of nutrient broth media. Following a 24-hour duration, a single loopful of the resultant suspension was applied in a streaking manner over a nutritional agar medium (NA), as outlined by Abo-El-Dahab and El-Goorani (1969). The plates were thereafter placed in an incubator set at a temperature of 28°C for a period of 48 hours. Subsequently, an analysis

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was conducted to assess the occurrence of bacterial proliferation on the plates. The researchers utilized the single colony method in order to get a culture that is free from contamination. This involved preserving colonies that grew on slanted media at a temperature of 4 °C, which allowed for further tests to be conducted.

### **Molecular Identification Through The 16S rRNA Gene:**

In accordance with the bacterial DNA isolation protocol outlined by Ashmawy *et al.* (2020), utilizing the CTAB approach, the bacterial samples that were obtained underwent amplification of the whole 16S rRNA gene, which encompasses a length of 1550 base pairs. The amplification process was accomplished by employing the P0 and P6 primers. The PCR amplification was performed using a total volume of 20 µL, comprising 10 µL of master mix, 0.5 µL of each P0 or P6 primer, and 2 µL of bacterial genomic DNA. The remaining volume was supplemented with molecular-grade water to attain a final volume of 20 µL. The PCR protocol was executed as follows: an initial denaturation step comprising a single cycle at a temperature of 95°C for a duration of 5 minutes, followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at 50°C for 60 seconds, and elongation at 72°C for 120 seconds. The duration of the last extension stage was 7 minutes, during which the temperature was maintained at 72°C.

### **Extraction and Disk Assessment Microbiology Procedure:**

**Plant Material Preparation:** *Juniperus phoenicea* and *Cicer arietinum* plant materials were collected and subjected to a shade-drying process at room temperature conditions for four weeks. Subsequently, the materials were further dried in an oven at 40 °C for an additional two days to ensure thorough drying.

**Solvent Selection:** Based on prior research by Malkhan *et al.* (2012) and Rahman *et al.* (2012), acetone and methanol were selected as solvents for extracting polar metabolites.

**Extraction Process:** Ten grams of dried and powdered plant material were macerated in a mixture of 40 milliliters of methanol and acetone (at a 1:4 ratio). The mixture was then placed on a rotary shaker and left undisturbed for three days. Subsequently, the mixture was filtered using Whatman filter paper and placed in a petri dish. The plates were then incubated at 28 °C for two days. The resulting extracts were diluted to four different concentrations (3000 ppm, 5000 ppm, 7000 ppm, and 10000 ppm) using a 1% dimethyl sulfoxide (DMSO) solution, following the method described by Mwitari *et al.* (2013). The extracts were stored at 4 °C until further use.

**Antimicrobial Testing:** Two separate plates were prepared for each plant extract, *Juniperus phoenicea* and *Cicer arietinum*. Each plate was coated with a nutrient agar medium (NA) inoculated with bacteria. Five paper discs were placed on the agar, consisting of one disc containing an antibiotic and four discs containing different concentrations of the plant extracts (each disc containing 20 µl). The fifth disc was soaked in distilled water, serving as the negative control. Amoxicillin at a concentration of 25 mg was employed as the positive control. The plates were then incubated for two days, and the inhibitory zone (mm) was measured after a 48-hour incubation period.

### **Statistical Analysis:**

The results of the measured parameters underwent computerized statistical analysis using the COSTAT package for analysis of variance (ANOVA). Subsequently, treatment means were compared using the LSD (Least Significant Difference) test at a significance level of 0.05.

## RESULTS AND DISCUSSION

### **Effect of *Juniperus phoenicea* Methanol Extract:**

Table 1 and Figure 1 illustrate the results of disc diffusion assays, demonstrating the antimicrobial activity of the *Juniperus phoenicea* 25% methanol extract. The extract

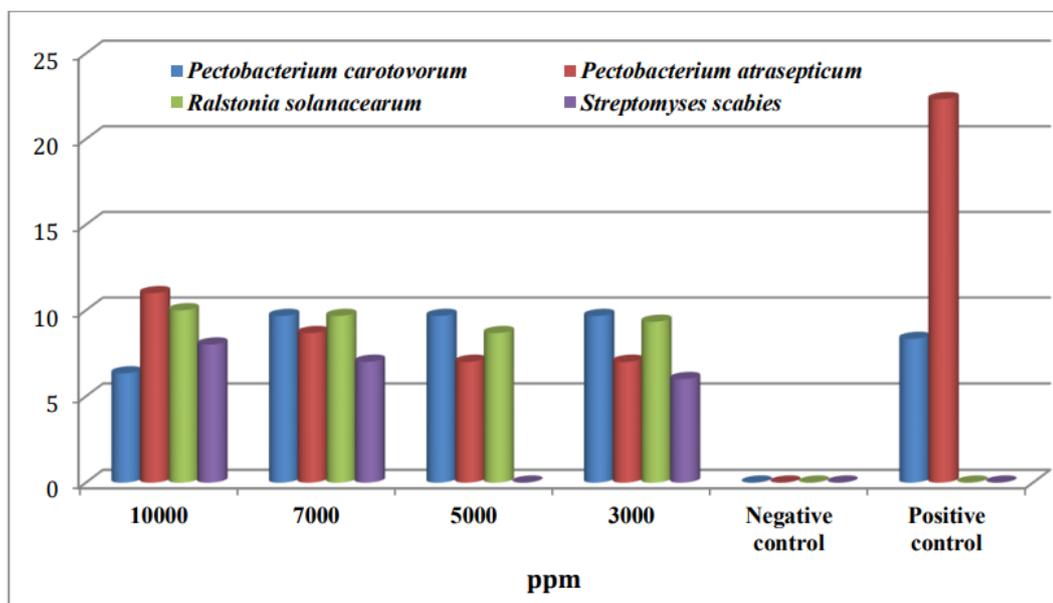
displayed significant bioactivity against *Pectobacterium carotovorum*, *P. atrosepticum*, *Ralstonia solanacearum*, and *Streptomyces scabies* at concentrations of 10,000, 7,000, 5,000, and 3,000 ppm, as well as in both negative and positive control groups.

Specifically, the 10,000-ppm concentration exhibited notable bioactivity with inhibition zone (IZ) values of 6.33, 11.00, 10.00, and 8.00 mm against the respective pathogens. At 7,000 ppm, the IZ values ranged from 9.67 to 8.67 mm, and at 5,000 ppm, they varied from 9.76 to 7.00 mm. It's worth noting that the positive control (antibiotics) displayed wider inhibition zones, especially against *P. atrosepticum*, with an impressive 22.33 mm IZ. The highest IZ value for the *J. phoenicea* extract was recorded against *P. atrosepticum*, measuring 11.00 mm.

**Table 1.** Effect of *Juniperus phoenicea* methanol extract against pathogenic potato bacterial isolates

Concentration ppm	<i>Pectobacterium carotovorum</i>	<i>Pectobacterium atrosepticum</i>	<i>Ralstonia solanacearum</i>	<i>Streptomyces scabies</i>	Means
10000	6.33	11	10	8	8.83
7000	9.67	8.67	9.67	7	8.75
5000	9.67	7	8.67	0	6.33
3000	9.67	7	9.33	6	8
Negative control	0	0	0	0	0
Positive control	8.33	22.33	0	0	7.67
<b>Means</b>	7.28	9.33	6.28	3.5	

LSD<sub>(0.05)</sub>: bacteria = 0.69 - LSD<sub>(0.05)</sub>: conc.= 0.84 - LSD<sub>(0.05)</sub>: interaction = 1.69



**Fig. 1.** Shows the effect of *Juniperus phoenicea* 25% methanol extract against pathogenic potato bacterial isolates.

#### Effect of *Cicer arietinum* Acetone Extract:

The results, as presented in Table 2 and Figure 2, from the disc diffusion assays, reveal a significant level of antimicrobial activity associated with the *Cicer arietinum* 75% acetone extract. The extract exhibited notable bioactivity against *P. carotovorum*, *P. atrosepticum*, *R. solanacearum*, and *S. scabies* at varying concentrations of 10,000, 7,000, 5,000, and 3,000 ppm, as well as in both negative and positive control groups.

Specifically, the 5,000-ppm concentration demonstrated robust bioactivity, resulting in inhibition zone (IZ) values of 7.00, 8.00, 9.00, and 10.00 mm against the respective

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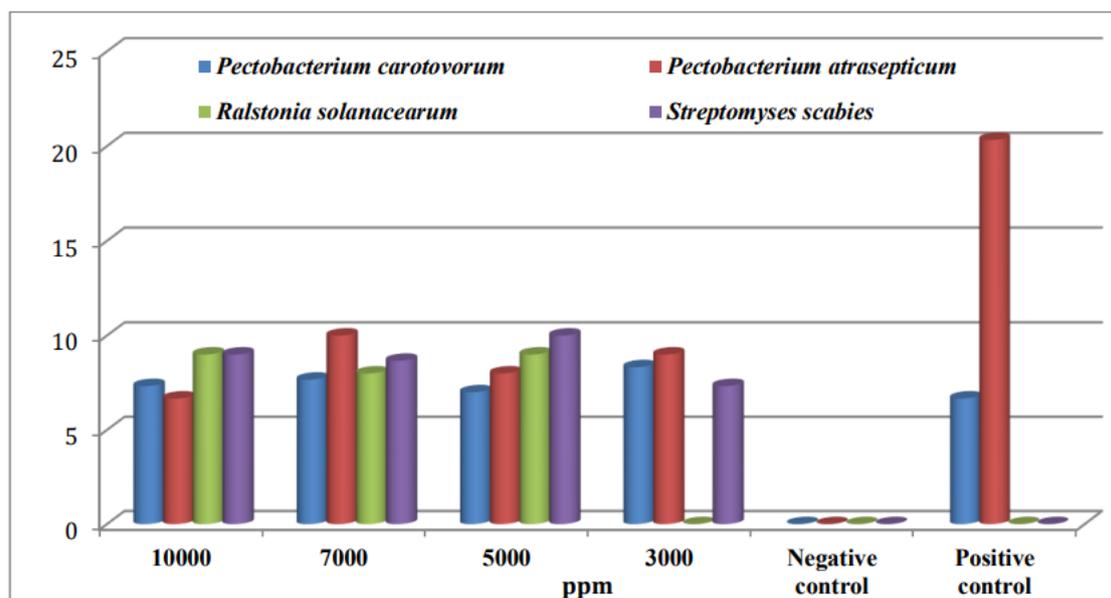
pathogens. At 7,000 ppm, the IZ values ranged from 7.67 to 8.67 mm. It's important to note that the positive control (antibiotics) exhibited wider inhibition zones, particularly against *P. atrosepticum*, with an impressive 20.33 mm IZ.

The highest IZ values for the *Cicer arietinum* extracts, specifically at a concentration of 7,000 ppm, were observed against *P. atrosepticum*, measuring 10.00 mm, and *S. scabies* at a concentration of 5,000 ppm.

**Table 2.** Effect of *Cicer arietinum* acetone extract against pathogenic potato bacterial isolates

Concentration ppm	<i>Pectobacterium carotovorum</i>	<i>Pectobacterium atrosepticum</i>	<i>Ralstonia solanacearum</i>	<i>Streptomyces scabies</i>	Means
10000	7.33	6.67	9	9	8
7000	7.67	10	8	8.67	8.583
5000	7	8	9	10	6.33
3000	8.33	9	0	7.33	6.167
Negative control	0	0	0	0	0
Positive control	6.67	20.33	0	0	6.75
<b>Means</b>	6.167	9	4.333	5.833	

LSD<sub>(0.05)</sub>: bacteria = 0.447 - LSD<sub>(0.05)</sub>: conc.= 0.547 - LSD<sub>(0.05)</sub>: interaction = 1.094



**Fig. 2.** Effect of *Cicer arietinum* 75% acetone extract against pathogenic potato bacterial isolates.

Hence, numerous studies have delved into the use of natural extracts to combat potato diseases. For instance, Salem (2013) uncovered that bark extracts from *Delonix regia* and *Erythrina humeana* exhibited moderate antibacterial activity against various strains of potato soft rot bacteria, including *D. dianthicola*, *P. wasabiae*, *P. carotovorum*, *P. atrosepticum*, and *D. chrysanthemi*. Furthermore, extracts derived from *Tecoma stans* leaves and branches displayed notable activity in comparison to those from *Callistemon viminalis* against the same bacterial strains.

In a related study, Salem *et al.* (2014) demonstrated that wood and bark extracts from *Picea abies* and *Larix decidua* exhibited moderate activity against the growth of *P. atrosepticum*, *P. carotovorum*, and *D. solani*. Notably, *Stenotrophomonas maltophilia*, isolated from the rhizosphere of eggplant cultivated in Egypt's Nile Delta region, emerged as a potential biocontrol agent against *R. solanacearum* (Messiha *et al.*, 2007). These

findings align with previous reports indicating that certain plant species harbor bioactive compounds with antimicrobial properties (Yuliar *et al.*, 2015; Sharma *et al.*, 2016).

Moreover, an *in vitro* experiment highlighted the potency of methanol leaf extracts from castor beans, surpassing ethanol and water extracts in their activity against both gram-positive and gram-negative bacteria (Naz and Bano, 2012).

In conclusion, the utilization of natural extracts to combat potato diseases presents a promising avenue for eco-friendly and sustainable agricultural practices. The diverse range of plant-based bioactive compounds underscores the potential of harnessing nature's arsenal in our ongoing efforts to protect vital crops like potatoes from detrimental pathogens. Further research and exploration in this field hold the key to developing effective, environmentally conscious strategies for disease management in agriculture.

### Conclusions

In summary, our research highlights the potential of natural extracts as eco-friendly biobactericides against potato pathogens. The 25% methanol extract from *Juniperus phoenicea* showed significant antibacterial activity, particularly against *Pectobacterium carotovorum*. Additionally, the *Cicer arietinum* 75% acetone leaf extract demonstrated efficacy against *P. atrosepticum* and *P. carotovorum*. These findings suggest that natural compounds could play a role in protecting potatoes from disease-causing agents in a sustainable and environmentally friendly manner.

### REFERENCES

- Abd-El-Khair, H., Abdel-Gaied, T. G., Mikhail, M. S., Abdel-Alim, A. I., Seif El-Nasr, H. I. 2021. Biological control of *Pectobacterium carotovorum* subsp. *carotovorum*, the causal agent of bacterial soft rot in vegetables, *in vitro* and *in vivo* tests. *Bulletin of the National Research Centre*, 45: 37.
- Abo El-Dahab, M.K., El-Goorani, M. 1969. Antagonistic effect of *Bacillus subtilis* strain upon *Erwinia amylovora*. *Phytopathology*, 54: 10, 1285 -1286.
- Ashmawy, N.A., El-Bebany, A.F., Shams, A.H., Shoeib, A.A. 2020. Identification and differentiation of soft rot and blackleg bacteria from potato using nested and multiplex PCR. *Journal of Plant Diseases and Protection*, 127: 141–153.
- Charkowski, A.O. 2018. The Changing face of bacterial soft-rot diseases. *Annual Review of Phytopathology*, 56: 269–288.
- Food and Agriculture Organization Statistics. 2018. World Food and Agriculture – Statistical Pocketbook. Retrieved from FAOSTAT: <http://www.fao.org/statistics/en/>
- Hadizadeh, I., Peivastegan, B., Hannukkala, A., van der Wolf, J.M., Nissinen, R., Pirhonen, M. 2019. Biological control of potato soft rot caused by *Dickeya solani* and the survival of bacterial antagonists under cold storage conditions. *Plant Pathology*, 68: 297–311.
- Kalita, D., Holm, D.G., LaBarbera, D.V., Petrash, J.M., Jayanty, S.S. 2018. Inhibition of  $\alpha$ -glucosidase,  $\alpha$ -amylase, and aldose reductase by potato polyphenolic compounds. *PLoS ONE*, 13: e0191025.
- Körpe, D.A., Özlem, D., Feride, I.S., Cabi, E., Haberal, M. 2013. High-antibacterial activity of *Urtica* spp. Seed extracts on food and plant pathogenic bacteria. *International Journal of Food Sciences and Nutrition*, 64: 3, 355-362.
- Malkhan, S.G., Shahid, A., Masood, A., Kangabam, S.S. 2012. Efficacy of plant extracts in plant disease management. *Agricultural Sciences*, 3: 3, 425-433.
- Messiha, N.A.S., van Diepeningen, A.D., Farag, N.S., Abdallah, S.A., Janse, J. D., van Bruggen, A.H.C. 2007. *Stenotrophomonas maltophilia*: A new potential biocontrol agent of *Ralstonia solanaceum*, causal agent of potato brown rot. *European Journal of Plant Pathology*, 118: 3, 211-225.

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- Mwitari, P.G., Ayeka, P.A., Ondicho, J., Matu, E.N. and Bii, C.C. 2013. Antimicrobial activity and probable mechanisms of action of medicinal plants of Kenya: *Withania somnifera*, *Warbugia ugandensis*, *Prunus africana* and *Plectranthus barbatus*. *PLoS ONE*, 8: 6, 1-9.
- Naz, R., Bano, A. 2012. Antimicrobial potential of *Ricinus communis* leaf extracts in different solvents against pathogenic bacterial and fungal strains. *Asian Pacific Journal of Tropical Biomedicine*, 2:12, 944-947.
- Prashanth, T., Bimlesh, K. Mandeep, K., Kaur G., Kaur, H. 2011. Phytochemical screening and extraction: A Review. *Internationale Pharmaceutica Scientia*, 1:98-106.
- Rahman, A.A., Khan, K., Ali, M.E., Mian, I.H., Akanda, A.M., Abed Hamid, S.B. 2012. Botanical control of soft rot bacteria of potato. *The Scientific World Journal*, 6: 1-6.
- Ribera, A.E., Zuñiga, G. 2012. Induced plant secondary metabolites for phytopathogenic fungi control. *Journal of Soil Science and Plant Nutrition*, 12: 4, 893-911.
- Saar-Reismaa, P., Kotkas, K., Rosenberg, V., Kulp, M., Kuhtinskaja, M., Vaher, M. 2020. Analysis of total phenols, sugars, and mineral elements in colored tubers of *Solanum tuberosum* L. *Foods*, 9: 1862.
- Salem, M.Z.M. 2013. Evaluation of the antibacterial and antioxidant activities of stem bark extracts of *Delonix regia* and *Erythrina humeana* grown in Egypt. *Journal of Forest Products and Industries*, 2:2, 48-52.
- Salem, M.Z.M., Ali, H.M., Mohamed, N.H. 2014. Evaluation of extracts from different parts of some tree species against the growth of some human bacterial pathogens. *Journal of Pure and Applied Microbiology*, 8: (Spl. Edn. 1), 149-154.
- Sharma, Y., Dua, D., Nagar, A., Srivastva, S.N. 2016. Antibacterial activity, phytochemical screening and antioxidant activity of stem of *Nicotiana tabacum*. *International Journal of Pharmaceutical Sciences and Research*, 7: 3, 1156-1167.
- Wagura, A.G., Wagai, S.O., Mangulo, L., Gichimu, B.M. 2011. Effects of selected plants' extracts on *in vitro* growth of *Ralstonia solanacearum* (Smith), the causal agent of bacterial wilt of Irish potatoes. *Journal of Plant Pathology*, 10: 2, 66-72.
- Yuliar, Y., Yanetri, A.N., Koki, T. 2015. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum* L. *Environmental Microbiology*, 30: 1, 1-11.