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Screening of *Warburgia Ugandensis* Crude Extracts Obtained from Different Organic Solvents against Tomato *Phytophthora Infestans* and *Alternaria Solani*

ESTHER WAITHIRA KAMAU
MWORIA G.E
Department of Agriculture
Meru University of Science and Technology, Meru, Kenya
MAINGI J.M
Department of Biochemistry, Microbiology and Biotechnology
Kenyatta University, Nairobi, Kenya
MASINDE P.W[1]
Department of Agriculture
Meru University of Science and Technology, Meru, Kenya

Abstract

Alternaria solani and Phytophthora infestans are causative agents of early and late blight of tomatoes respectively which are currently controlled using fungicides. Overuse of fungicides poses safety concerns. The research objective was to investigate the invitro efficacy of Warburgia organic solvent crude extracts on blight pathogens. Warburgia ugandensis stem bark sample was air dried at room temperature then ground. The powdered material was weighed and soaked in organic solvent then filtered and the solvent recovered using a rotary evaporator. Multiple extraction method was used with four organic solvents. Well diffusion method was used to screen Warburgia extracts against A. solani and P. infestans. All assays were performed in triplicate. Statistical analysis on inhibition zone was carried out using analysis of variance (ANOVA). Warburgia ugandensis hexane crude extract had the highest inhibition zone in A. solani while methanol crude extract gave the highest mean inhibition zone in P. infestans. All the extracts were inhibitive against P.

infestans and A. solani. Further studies are required for invivo studies and to analyze the bioactive compounds in the extracts.

Keywords: *Warburgia ugandensis*, *Phytophthora infestans*, *Alternaria solani*, invitro inhibition, tomatoes, well diffusion method.

1. INTRODUCTION

Warburgia ugandensis, commonly known as pepper bark tree, is an evergreen tree indigenous to East and South Africa. The tree has simple glossy leaves, green flowers and plum-shaped fruits. The mature bark has thick brown rhytidome with druse crystals. Parenchyma cells of secondary phloem secrete a brown sap (Kotina et al., 2014). Previously, *W. ugandensis* has been found to have antimicrobial properties and has been used in the management of animal and human disease pathogens (Were et al., 2015; Merawie et al., 2013). Its bark has been used in traditional medicine in several African countries (Wang et al., 2015). Its stem and root barks have been used in the management of ailments such as toothaches, constipation, cancer, diabetes and fever among others (Maroyi, 2014). However, information regarding the use of *Warburgia ugandensis* extract in the control of phytopathogens is still scanty.

Tomato production plays a vital role in meeting domestic and nutritional food requirements, generation of income, foreign exchange earnings and creation of employment (Sigei et al., 2014). Farmers involved in tomato production face many constraints including diseases such as blight, high cost of production arising from cost of synthetic chemicals for disease and pest control as well as poor infrastructure (Monda et al., 2003).

Alternaria solani that causes early blight in tomatoes is characterized by dark brown to black concentric rings, resulting

in a halo effect (Marak et al., 2014). The pathogen overwinters as mycelium or conidia in plant debris or in the soil and inoculum remains infective in uncultivated soil for 5-8 months (Waals et al., 2001). The conidia are disseminated by insects, wind and rain. *Phytophthora infestans* that causes late blight is characterized by necrotic lesions on stems and collar, damping off and severe root rot (Mendonca et al., 2015). Sporangia or mycelia of *P. infestans* are dispersed from infected plants and plant debris by wind or splashing raindrops and germinate releasing motile zoospores (Nelson, 2008). Zoosporangia form when relative humidity is high. Both blight pathogens affect plants in the field and after harvest leading to serious economic losses (Yao et al., 2016; Shahbazi et al., 2010). Several studies have previously reported on the control and management of these diseases (Seifu, 2017; Yao et al., 2016). Among these, inorganic chemical control has been widely used (Mendonca et al., 2015). The use of inorganic chemicals is increasingly being discouraged as a result of safety challenges, pathogens resistance, environmental pollution and accumulation of residuals in plants (Naseby et al., 2001; Laila et al., 2014). There is huge amount of literature demonstrating increased research in diverse natural products from plants that can be used as alternative bio-control chemicals (Sasidharan et al., 2011; Laware, 2015). Research on control of late blight without using fungicides has been carried out by Seifu, (2017) who applied calcium nutrients at pre-harvest to reduce severity of potato late blight caused by *P. infestans* and improved yield. Riad et al., (2016) reported that *Moringa oleifera* leaves and seed oil extracts in combination with chitosan, was effective in controlling early blight in potatoes. Nevertheless, information regarding plant extracts that can be used as biocontrol agents is inadequate.

Abuto et al., (2016) reported that *W. ugandensis* stem bark crude extracts obtained from DCM and methanol, was

effective against *Staphylococcus aureus* and *Candida albicans*. Apart from having antimicrobial properties, Warburgia was found to be rich in sesquiterpenoid, especially terpenoids and fatty acid derivatives (Abuto et al., 2018; Mwitari et al., 2013). Other plant extracts obtained from *Azadrachta indica, Moringa oleifera, Carica papaya* and *Allium sativum* have shown inhibitory effects against *Aspergillus flavus invivo* (Tijjani et al., 2014). Sharma and Janmeda (2017) also isolated flavonoids from *Euphorbia neriifolia* which has antioxidants, useful in the prevention of many degenerative diseases in humans. However little is known about the effect of *W. ugandensis* stem bark extracts against plant associated pathogens. This study evaluated the effectiveness of Warburgia organic solvent crude extracts from stem bark in controlling invitro growth of *P. infestans* and *A. solani* which cause blight in tomatoes.

2.0 MATERIALS AND METHODS

2.1 Isolation and identification of *Phytophthora infestans* and *Alternaria solani*

Infected tomato leaves and fruits were randomly sampled from farmer's farms in Nkomo ward, Meru County, Kenya, and taken to the biological laboratory of Meru University of Science and Technology (MUST) for processing and identification. The samples were washed under running tap water to remove soil debris and surface contaminants then sterilized in 1 % Sodium hypochlorite for three minutes and rinsed in three changes of sterile distilled water. They were blot dried by using sterile blotting paper. Direct plating was carried out on sterilized PDA supplemented with chloramphenicol and streptomycin sulphate and incubated at 26 ^{0}C for 5 days. Identification of fungal isolates was carried out as described by Barnett and Hunter, (1999).

2. 2 Processing and Extraction of *Warburgia ugandensis* stem bark organic solvents crude extract

Two hundred grams of air dried powdered plant material of *W. ugandensis* stem bark was weighed and soaked in a 1000 ml conical flask using organic solvent (AR) and left to agitate on an orbital shaker for 48 hours at room temperature. The soaked material was then filtered using a Whatman filter paper fixed into a Buchner funnel using a water rotary pump. The solvent was recovered using a rotary evaporator machine (Heidolph 4000 efficient, serial number 090821594). The crude extract was put in a sealed sample container and stored at 4 ^{o}C awaiting further analysis. Multiple extraction method was used. The organic solvents used were ethyl acetate, hexane, methanol and Dichloromethane (DCM).

2.3 *Invitro* screening using plate-hole diffusion method

The bioassay screening of *W. ugandensis* crude extracts antimicrobial activity towards *P. infestans* and *A. solani* was carried out by well diffusion method on sterilized PDA growth medium as described by Abuto et al., (2016). The microbial suspension was standardized in sterile distilled water to 10^6 conidia/ml for *A. solani* or 10^6 zoospores/ml for *P. infestans*. 100 ml of each suspension was obtained using a micropipette and spread onto the surface of the PDA medium in the petri dishes. The petri dishes were allowed to rest for 10 minutes, after which 5 mm-diameter holes were punched on the PDA media in the petri dishes and the holes filled with 100 ml of the previously prepared samples of extract. The assessment was conducted by measuring the diameter of inhibition zone indicated by a clear zone to the nearest mm using a Vernier caliper. Different concentrations of the crude extracts were made by dissolving the extracts in dimethyl sulphoxide (DMSO) and the mixture agitated using a mechanical vortex mixer. The organic solvent used in extraction of each crude extract, DMSO

and Mancozeb were used as control. Concentration of Mancozeb was at the manufacturers recommendations. The plates were incubated at 27 ^{o}C for 72 hours. Each assay had 3 replicates, and the zones of inhibition were measured and recorded.

2.4 Data Analysis

Statistical analysis on inhibition zone was carried out using analysis of variance (ANOVA).

3.0 RESULTS AND DISCUSSION

3.1 Isolation and identification of *Phytophthora infestans* and *Alternaria solani*

Phytophthora infestans colonies were white in colour (Figure 1A). The mycelia lacked cross walls as observed under the light microscope and the sporangia borne at end of sporangiophores were lemon shaped, similar to observations made by Barnett and Hunter (1999). *Alternaria solani* produced deeply gray pigmented hairy colonies (Figure 1B). When viewed under the light microscope, the mycelia had haploid, septate and beaked asexual conidia similar to observations made by Barnett and Hunter (1999).

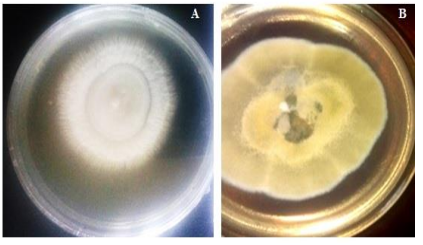

Figure 1. Cultures of tomato blight pathogens. A - Represents *P. infestans,* B – Represents *A. solani*. Both images were taken at day 5 after inoculation and incubation at 26 ^{0}C.

3.2 Screening of *W. ugandensis* stem bark ethyl acetate crude extract against *A. solani* and *P. infestans*

In both *A. solani* and *P. infestans*, concentration of 0.025g/ml showed inhibition zone of 28.33 mm (Table 1). Extract concentration of 0.05g/ml showed an inhibition zone of 29.00 mm in *A. solani* while *P. infestans* showed lower inhibition zone of 24.33 mm. At 0.1g/ml concentration, inhibition zone in *A. solani* was 27.33 mm while in *P. infestans* was 25.00 mm. Mancozeb used as a control showed lower inhibition zone in *A. solani* (16.00 mm) and *P. infestans* (11.00 mm). These results are commensulate with observations made on the plates that showed Warburgia stem bark ethyl acetate crude extract had more inhibitory effects on the pathogens than conventionally used Mancozeb fungicide (Figure 2). No inhibition zone was observed in DMSO plates.

Fungicidal activities of crude extracts obtained from *Gelliodes carnosa* using different solvents showed that extract from ethyl acetate was effective in controlling growth of *Fusarium solani* and *Fusarium sp. 2* (Khakshoor and Pazooki, 2014). Several reports have also showed that extracts obtained from Warburgia leaves and stem bark have antimicrobial properties and have been traditionally used to heal several ailments (Maroyi 2014). These results indicate that crude extract obtained from *W. ugandensis* using ethyl acetate solvent was effective in inhibiting the growth of both *A. solani* and *P. infestans*.

Table 1. Mean inhibition zones (mm) from screening different concentrations of *W. ugandensis* ethyl acetate crude extract against *A. solani* and *P. infestans*

Isolate	Mean Inhibition zone ± SE (mm)			
	0.025g/ml Extract	0.05g/ml Extract	0.1g/ml Extract	Mancozeb
A. solani	**28.33 ± 0.88**	**29.00 ± 0.58**	**27.33 ± 0.67**	**16.00 ± 1.15**
P. infestans	**28.33 ± 0.33**	**24.33 ± 0.33**	**25.00 ± 0.00**	**11.33 ± 0.33**

Data are mean ± standard error (SE) of inhibition zones (mm) by different concentrations of *W. ugandensis* stem bark ethyl acetate crude extract and the positive control Mancozeb obtained from 24 treatments (n=24).

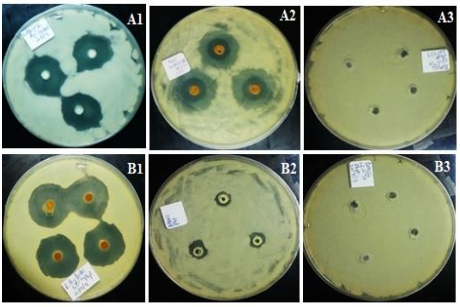

Figure 2(a) Inhibition in *A. solani*

Figure 2(b) Inhibition in *P. infestans*

Figure 2. Inhibition zones by *W. ugandensis* stem bark ethyl acetate crude extract, Mancozeb and DMSO on *A. solani* and *P. infestans*.
2(a). A1- Inhibition zone of *A. solani* by Warburgia stem bark ethyl acetate crude extract. A2 - Inhibition zone of *A. solani* by Mancozeb. A3- No inhibition of *A. solani* by DMSO. 2(b). B1- Inhibition zone of *P. infestans* by Warburgia stem bark ethyl acetate crude extract. B2- Inhibition zone of *P. infestans* by Mancozeb. B3 - No inhibition of *P. infestans* by DMSO.

3.3 Screening of *W. ugandensis* methanol stem bark crude extract against *P. infestans* and *A. solani.*

The extract concentration of 0.025g/ml showed an inhibition zone of 30.67 mm in *A. solani* and 31.00 mm in *P. infestans* (Table 2). Concentration of 0.05g/ml resulted in inhibition zone of 29.60 mm in *A. solani* while in *P. infestans*, inhibition zone was 22.00 mm. Inhibition zone of 28.33 mm was observed in *A. solani* and 14.33 mm in *P. infestans* at concentration of 0.1g/ml. Mancozeb showed an inhibiton zone of 16.00 mm and 11.33 mm in *A. solani* and *P. infestans* respectively (Figure 3) . No inhibition zone was observed in both *A. solani* and *P. infestans* while using DMSO.

Merawie et al., (2013) found Warburgia leaves and heartwood extracts obtained using methanol to be inhibitive against *S. aureus* and *S. boydii*. Abuto et al., (2016) found *W.*

ugandensis methanol stem bark crude extracts from five different populations to have higher inhibition zones against *Staphylococcus aureus* and *Candida albicans* than crude extract from leaves. These results demonstrate that *W. ugandensis* stem bark crude extract obtained using methanol solvent has antimicrobial properties against *A. solani* and *P. infestans*.

Table 2. Mean inhibition zones (mm) from screening different concentrations of *W. ugandensis* methanol crude extract against *A. solani* and *P. infestans*

Isolate	Mean Inhibition Zone ± SE (mm)			
	0.025g/ml Extract	0.05g/ml Extract	0.1g/ml Extract	Mancozeb
A. solani	**30.67 ± 0.33**	**29.6 ± 0.33**	**28.33 ± 0.33**	**16.00 ± 1.15**
P. infestans	**31.00 ± 1.15**	**22.00 ± 0.00**	**14.33 ± 1.45**	**11.33 ± 0.33**

Data are mean ± standard error (SE) of inhibition zones (mm) by different concentrations of *W. ugandensis* stem bark methanol crude extract and the positive control Mancozeb obtained from 24 treatments (n=24).

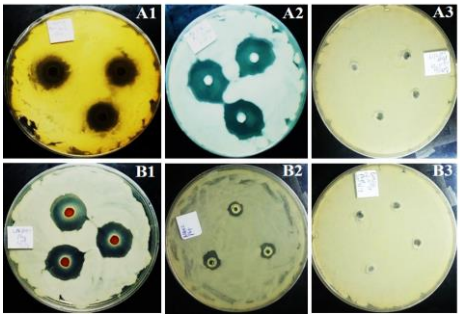

Figure 3(a) Inhibition in *A. solani*

Figure 3(b) Inhibition in *P. infestans*

Figure 3. Inhibition zones by *W. ugandensis* stem bark methanol crude extract, Mancozeb,and DMSO on *A. solani* and *P. infestans*

3(a). A1- Inhibition zones of *A. solani* by Warburgia stem bark methanol crude extract. A2 - Inhibition zone of *A. solani* by Mancozeb. A3- No inhibition of *A. solani* by DMSO. 3(b). B1- Inhibition zone of *P. infestans* by Warburgia stem bark methanol crude extract. B2- Inhibition zone of *P. infestans* by Mancozeb. B3 - No inhibition of *P. infestans* by DMSO.

3.4 Screening of *Warburgia ugandensis* dichloromethane (DCM) crude extract against *A. solani* and *P. infestans*

Extract concentration of 0.025g/ml showed an inhibition zone of 30.33 mm in *A. solani* and 26.33 in *P. infestans* (Table 3). Inhibition zone of 31.33 mm was observed in *A. solani* and 26.67 mm in *P. infestans* from concentration of 0.05g/ml. Concentration of 0.1g/ml resulted in inhibition zone of 32.33 mm in *A. solani* and 27.00 mm in *P. infestans*. There was no inhibition zone observed in the plates with DMSO for *A. solani* and *P. infestans* (Figure 4). Mancozeb showed lower inhibition zone in *A. solani* (16.00 mm) and *P. infestans* (11.33 mm) compared to Warburgia DCM crude extract. Similar observations were also made on the plates.

Previous studies showed that Warbugia stem bark DCM crude extract had strong antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* (Abuto et al., 2016). Invitro studies on *A. solani* have shown inhibition by other plant extracts especially *Moringa oleifera* leaf extract (Riad et al., 2016). These results suggest that Warburgia stem bark DCM crude extract has antifungal properties that can be exploited to control *A. solani* and *P. infestans.*

Table 3. Mean inhibition zones (mm) from screening different concentrations of *W. ugandensis* DCM crude extract against *A. solani* and *P. infestans*

Isolate	Mean Inhibition Zone ± SE (mm)			
	0.025g/ml Extract	0.05g/ml Extract	0.1g/ml Extract	Mancozeb
A. solani	**30.33 ± 0.33**	**31.33 ± 0.33**	**32.33 ± 0.33**	**16.00 ± 1.15**
P. infestans	**26.33 ± 0.33**	**26.67 ± 0.33**	**27.00 ± 0.33**	**11.33 ± 0.33**

Data are mean ± standard error (SE) of inhibition zones (mm) by different concentrations of *W. ugandensis* stem bark DCM crude extract and positive control Mancozeb obtained from 24 treatments (n=24).

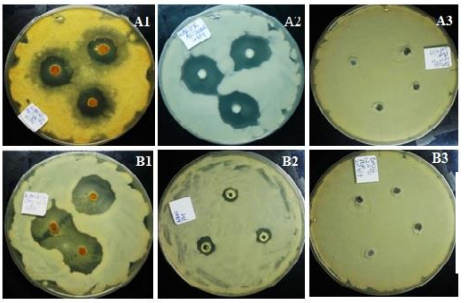

Figure 4. Inhibition zones by *W. ugandensis* stem bark DCM extract, Mancozeb, and DMSO on *A. solani* and *P. infestans.*
4(a). A1- Inhibition zone of *A. solani* by Warburgia stem bark DCM crude extract. A2 - Inhibition zone of *A. solani* by Mancozeb. A3- No inhibition of *A. solani* by DMSO. 4(b). B1- Inhibition zone of *P. infestans* by Warburgia stem bark DCM crude extract. B2- Inhibition zone of *P. infestans* by Mancozeb. B3 - No inhibition of *P. infestans* by DMSO.

3.5 Screening of *Warburgia ugandensis* hexane crude extract against *A. solani* and *P. infestans*

The inhibition zones in *A. solani* were higher than inhibition zones in *P. infestans* for all concentrations of Warburgia hexane crude extract (Table 4). The concentration of 0.025g/ml resulted in inhibition zone of 30.33 mm in *A. solani* and inhibition zone of 26.33 mm in *P. infestans*. Concentration of 0.05 g/ml showed inhibition zone of 31.33 mm and 26.67 mm in *A. solani* and *P. infestans* respectively. Inhibition zone of 32.33 mm in *A. solani* and 27.00 mm was observed from concentration of 0.1g/ml. There was no inhibition observed from DMSO in both *A. solani* and *P. infestans* (Figure 5). Inhibition zone by Mancozeb was 16.00 mm and 11.33 mm in *A. solani* and *P. infestans* respectively, which was lower than inhibition in Warburgia hexane crude extract. Ngure et al., (2009), found Warburgia stem bark hexane crude extract to have the best activity against *Leishmania major* and *Leishmania donovani* promastigotes and amastigotes. The results are similar to those obtained in these study whereby Warburgia hexane stem bark

crude extract gave the highest inhibition zone for both *A. solani* and *P. infestans*.

Table 4. Mean inhibition zones (mm) from screening different extract concentrations of *W. ugandensis* hexane crude extract against *A. solani* and *P. infestans*

Isolate	Mean Inhibition Zone ± SE (mm)			
	0.025g/ml Extract	0.05g/ml Extract	0.1g/ml Extract	Mancozeb
A. solani	**30.33 ± 0.33**	**31.33 ± 0.33**	**32.33 ± 0.33**	**16.00 ± 1.15**
P. infestans	**26.33 ± 0.33**	**26.67 ± 0.33**	**27.00 ± 0.33**	**11.33 ± 0.33**

Data are mean ± standard error (SE) of inhibition zones (mm) by different concentrations of *W. ugandensis* stem bark hexane crude extract and positive control Mancozeb obtained from 24 treatments (n=24).

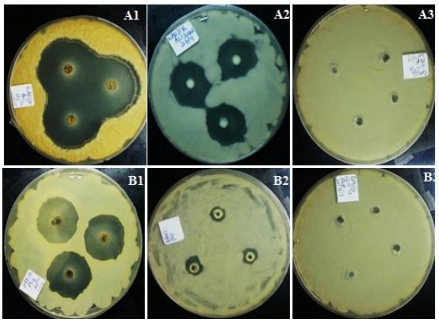

Figure 5(a) Inhibition In *A. solani*

Figure 5(b) Inhibition in *P. infestans*

Figure 5. Inhibition zones by *W. ugandensis* stem bark hexane crude extract, Mancozeb, and DMSO on *A. solani* and *P. infestans*.
5(a). A1- Inhibition zone of *A. solani* by Warburgia stem bark hexane crude extract. A2 - Inhibition zone of *A. solani* by Mancozeb. A3- No inhibition of *A. solani* by DMSO. 4(b). B1- Inhibition zone of *P. infestans* by Warburgia stem bark hexane crude extract. B2- Inhibition zone of *P. infestans* by Mancozeb. B3 - No inhibition of *P. infestans* by DMSO.

4.0 CONCLUSION

All the Warburgia stem bark organic crude extracts showed higher inhibition zones in *A. solani* than in *P. infestans*, except in 0.025g/ml concentration of Warburgia methanol crude extract, suggesting that Warburgia stem bark organic crude

extracts are more effective in invitro inhibition of *A. solani* than *P. infestans*. These results indicate that all Warburgia organic crude extracts are effective in inhibiting invitro linear growth of *P. infestans* and *A. solani*. However, further studies are required to analyze the bioactive compounds in the extracts and also to test the effectiveness of the extracts on plants invivo.

REFERENCES

1. Abuto J. O., Muchugi A., and Machocho A. K. (2018). Diversity in the Phytochemical Profiles of *Warburgia ugandensis* Sprague from Different Populations across the Kenyan Rift Valley. Journal of Pharmaceutical, Chemical and Biological Sciences. 6:41-51.
2. Abuto J. O., Muchugi A., Mburu D., Machocho A. K. and Karau G. M. (2016), Variation in Antimicrobial Activity of *Warburgia ugandensis* Extracts from Different Populations across the Kenyan Rift Valley. Journal of Microbiology Research 6: 55-64.
3. Barnett H. L. and Hunter B. B. (1999). Illustrated Genera of Imperfect Fungi. Burgess Publishing Company Mineapolis, pages 246-249.
4. Khakshoor M. S. and Pazooki J. (2014). Bactericidal and fungicidal activities of different crude extracts of *Gelliodes carnosa* (sponge, Persian Gulf). Iranian Journal of Fisheries Sciences 13:3.
5. Kotina E.L., Van Wyk B.E. and Tilney P.M. (2014). Anatomy of the Leaf and Bark of *Warburgia salutaris* (Canellaceae), an important medicinal plant from South Africa. South African Journal of Botany 94: 177-181.
6. Laila N., Umi K. Y., Ahmad I. and Kausar H. (2014). Trichoderma SPP.: A Biocontrol Agent for Sustainable

Management of Plant Diseases. Pakistan Journal of Botany. 46: 1489-1493.

7. Laware S. L., (2015). Sequential Extraction of Plant Metabolites. International Journal of Current Microbiology and Applied Sciences 4: 33-38.
8. Marak T.R., Ambesh B. S. and Das S. (2014). Cultural, Morphological and Biochemical Variations of *Alternaria solani* Causing Diseases on Solanaceous Crops. The Bioscan 9: 1295-1300.
9. Maroyi A. (2014). The Genus *Warburgia*: A Review of its Traditional Uses and Pharmacology. Pharmaceutical Biology 52: 378-391.
10. Mendonca L.B.P., Coelho L., Stracieri J., Júnior J.B.F. and Tebaldi N.D**.** **(**2015). Chemical Control of Phytophthora Wilt in Tomatoes**.** Journal of Biosciences 31: 1015-1023.
11. Merawie Y., Sahile S., Moges F. and, Husen A. (2013). Antimicrobial Activity of Crude and Semi-Purified Fractions of *Warburgia ugandensis* Against Some Pathogens. Journal of Coastal Life Medicine 1: 233-240.
12. Monda E.O., Munene, S., and Ndegwa A. (2003). French beans production constraints in Kenya. African crop science conference proceedings 6: 683-687.
13. 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 *Plectrunthus barbatus*. PLoS ONE 8: e65619.
14. Naseby, D. C., Pascual, J. A. and Lynch, J. M. (2001). Effect of Biocontrol Strains of *Trichoderma* on Plant Growth, *Pythium ultimum* Population, Soil Microbial Communities and Soil Enzyme Activities. Indian Journal of Applied Microbiology 88:161-169.

15. Nelson S.C. (2008). Late Blight of Tomato (*Phytophthora infestans*). Plant Disease. 2008: 45
16. Ngure P.K., Tonui W.K., Ingonga J., Mutai C., Kigondu E., Ng'ang'a Z., Rukunga G. and Albert Kimutai A. (2009). Invitro antileishmanial activity of extracts of *Warburgia ugandensis* (Canellaceae), a Kenyan medicinal plant. Journal of Medicinal Plants Research Vol. 3:061-066.
17. Riad S.R.E, Aboelfetoh M.A and Abdalla A. G. (2016). Field application of Chitosan and *Moringa oleifera* Extracts as Fungicides Alternatives to Control Early Blight and Improvement Growth and Yield Quality of Potatoes. Plant Pathology Journal 15: 135-143.
18. Sasidharan S., Chen Y., Saravanan D., Sundram K. M. and Yoga L. (2011). Extraction, Isolation and Characterization of Bioactive Compounds from Plants' Extracts. African Journal of Traditional Complementary and Alternative Medicines 8:1-10.
19. Seifu Y. W. (2017). Reducing Severity of Late Blight (*Phytophthora infestans*) and Improving Potato (*Solanum tuberosum* L.) Tuber Yield with Pre-Harvest Application of Calcium Nutrients. Journal of Agronomy 7: 69.
20. Shahbazi, H., Aminian, H., Sahebani, N., and Halterman, D. A. (2010). Biochemical Evaluation of Resistance Responses of Potato to Different Isolates of *Alternaria solani*. Phytopathology 100: 454-459.
21. Sharma V and Janmeda P. (2017). Extraction, Isolation and Identification of Flavonoid from *Euphorbia neriifolia* leaves. Arabian Journal of Chemistry 10:509-514.
22. Sigei K. G., Ngeno K. H., Kibe M. A., Mwangi M. M., and Mutai C. M. (2014). Challenges and strategies to improve tomato competitiveness along the tomato value

chain in Kenya. International Journal of Business and Management 9: 230-245.

23. Tijjani A, Adebitan S. A., Gurama A. U., Haruna S. G. and Safiya T. (2014). Effect of Some Selected Plant Extracts on *Aspergillus flavus*, a Causal Agent of Fruit Rot Disease of Tomato (*Solanum lycopersicum*) in Bauchi State. International Journal of Biosciences 4:244-252.
24. Waals der Van J E, Korsten L. and Aveling T. A. S. (2001). A review of early blight of potatoes. African *Plant* Protection 7: 91-102.
25. Wang X., Zhou C., Yang X., Miao D. and Zhang Y. (2015). De Novo Transcriptome Analysis of *Warburgia ugandensis* to Identify Genes Involved in Terpenoids and Unsaturated Fatty Acids Biosynthesis. Plos One Journal. 10: 1371
26. Were P.S, Waudo W., Ozwara H. S. and Kutima H. L. (2015). Correspondence Phytochemical Analysis of *Warburgia ugandensis* Sprague using Fourier Transform Infra-Red (FT-IR) Spectroscopy .International Journal of Pharmacognosy and Phytochemical Research 7:201-205.
27. Yao Y., Li Y., Chen Z., Zheng B., Zhang L., Niu B., Meng J., Li A., Zhang J. and Wang Q. (2016). Biological Control of Potato Late Blight Using Isolates of Trichoderma. American Journal of Potato Research. 93: 33–42.