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Phytochemical and fungicidal activity of selected plant extracts against *Phaeoisariopsis griseola* of common bean

Simon M. Obila[1], David M. Musyimi*[1], Phoebe A. Sikuku[1]

[1]*Department of Botany, School of Physical and Biological Sciences, Maseno University, Private Bag, Maseno University, Kenya*

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Abstract

Plant extracts are effective in plant pathogens control and are now becoming popular throughout the world. *Allium sativum*, *Azadirachta indica* and *Tithonia diversifolia* extracts have been reported to exhibit antimicrobial activity against phytopathogens. The aim of this study was to phytochemically screen and determine the fungicidal activity of *Azadirachta indica,* and *Tithonia diversifolia* extracts against *Phaeoisariopsis griseola* of common bean. Phytochemical analysis of the plants and pathogenicity test of *Phaeoisariopsis griseola* were carried out. Phytochemical screening revealed that alkaloids, tannins, terpenoids, saponins, cardiac glycosides and sterols were present in *Azadirachta indica*, and *Allium sativum* while sterols, saponins and alkaloids were absent in *Tithonia diversifolia*. Flavonoids were present in *Allium sativum* but absent in *Azadirachta indica*. All the plant extracts significantly (P≤0.05) decreased spore germination of *Phaeiosariopsis griseola* in vitro. The most effective was *Allium sativum* extract and *Azadirachta indica* at 100% extract concentrations. *Tithonia diversifolia* had moderate fungicidal effect. This study show that all the plants contain most of the active phytochemical compounds which justify their fungicidal property and extract effectiveness increased with their concentration. Therefore, there is possibility that *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extracts can be used to control *Phaeiosariopsis griseola* of common bean. Further studies are required to quantify these phytochemicals of these plants for the development of cost-effective pesticides for the control of *Phaeoisariopsis griseola*.

* **Corresponding Author:** David M. Musyimi ✉ davidmusyimi2002@yahoo.com

Introduction

Common bean (*Phaseolus vulgaris*) is among the most important plant cereals in the world (Leitich *et al*., 2016). It can be grouped into dry and green (snap) bean and in Africa majorly produced in climatic regions of temperate and sub-tropical, among other continents (Pamela *et al*., 2014). According to Damiano *et al*. (2013) common bean is consumed throughout the world and comes second in the order of importance. It is the third most important source of calories and fibre in both Eastern and Southern Africa and rich source of protein, zinc and iron (Bode *et al*., 2017; Keller *et al*., 2014; Pamela *et al*., 2014). In 2007, world's production of common bean was estimated at 417,000 Metric tomes (Mt) against a growing demand of more than 500,000 Mt, the deficit largely attributed to extreme biophysical stresses such as climate change, pest and phytopathogenic diseases, and soil fertility (Katungi *et al*., 2011). Phytopathogenic diseases make the largest proportion of the stresses leading to constant production of less than 25% of the potential yield (Ddamulira *et al*., 2014; Mauricio *et al*., 2012).

Fungus *Phaeoisariopsis griseola* is the etiologic agent of angular leaf spot of common bean and very destructive phytopathogen of common bean (Allorent & Savary, 2005). It attacks literally all the aerial plant parts such as stem, leaves and pods causing shrivelled pods, sunken seeds and premature defoliation (Bode *et al*., 2017). Angular leaf spot disease is primarily controlled using synthetic fungicide though effective has numerous shortcomings such as high input cost, human and environmental hazard and development of pathogen resistance towards the fungicide (Ddamulira *et al*., 2014). Moreover, the other effective control is by use of resistant variety which is proving to be expensive to develop and difficult to maintain due to abundant genetic variability, virulence and pathotype diversity of the pathogen (Sharma & Adikshita, 2017). The medicinal and antimicrobial activities of extracts from plants are gaining attention of researchers worldwide (Shabana *et al*., 2017). The modern synthetic fungicide has its own advantages and side effects, so the plant-based products are getting more popularity, as they are safe to use, and comparatively easily available and cheap. Many extracts possess antifungal activity (Cherkupally *et al*., 2017). Plant extracts are effective in plant pathogens and are now becoming popular throughout the world (Shabana *et al*., 2017). *Azadirachta indica* (neem) is a medicinal plant of importance with enormous phytochemical compounds such as terpenes and alkaloids on various parts of the plant such as bark, leaves and seeds, and has been reported to have biological properties against pathogenic organisms such as fungi, bacteria, viruses and pests (Al hazmi, 2013; Pankay *et al*., 2011). *Azadirachta indica* extracts have activity against phyto-pathogenic bacteria and fungi such as *Xanthomonas vesicatoria* and *Ralstonia solanacearum* (Sarawaneeyaruk *et al*., 2015). *Allium sativum* commonly known as garlic belongs to the family Alliaceae and among the important earliest known medicinal plants (Stavělíková, 2008; Byrappa, 2015). Its usage worldwide has a long history with significant role in disease prevention and control. *Allium sativum* has been reported to have fungicidal activity on *Puccinia tritina* a wheat fungus that causes wheat leaf rust (Shabana *et al*., 2017). Mexican sunflower (*Tithonia diversifolia*) is a member of Asteraceae family. It has demonstrated successful antimicrobial activity against food and human pathogens (Rejeki & Addy, 2017). The leaves extract of *Tithonia diversifolia* plant has been previously reported to show antifungal activity (Parekh & Chanda, 2007). Globally, there is growing demand for natural botanicals among common bean growers for use as bio-fungicides due to the numerous negative effects of the synthetic fungicides on the environment and human health (Cecilia & Olubunmi, 2014). Though, little is known about the success of using *Allium sativum*, *Azadirachta indica* and *Tithonia*

diversifolia crude extracts to control *Phaeoisariopsis griseola* of common bean. This therefore, necessitates the need to search for pathogen management options that are environmentally friendly, harmless to the non-target organisms and human health, and keep the pathogen to levels below economic threshold.

Materials and methods

Collection and preservation of Allium sativum, Azadirachta indica and Tithonia diversifolia

Fresh and disease-free *Allium sativum* was purchased from Kibuye market in Kisumu County and disease free and healthy *Azadirachta indica* and *Tithonia diversifolia* leaves were collected from the Maseno University botanic garden and washed under running tap water to eliminate dust and other foreign particles according to a method by Ezeonu *et al*. (2018). Taxonomic identification and authentication of plant specimens was performed by taxonomist at the Department of Botany–Herbarium, Maseno University, Kenya. *Allium sativum* bulbs were separated, washed and sliced to await the drying process according to Baljeet *et al*. (2015). The bulbs of *Allium sativum*, leaves of both *Azadirachta indica* and *Tithonia diversifolia* were used in the experiment due to the fact that they contain high levels of active ingredient needed for pathogen control (Baljeet *et al*., 2015; Obongoya *et al*., 2010). Voucher specimens were labelled and brought to the laboratory then stored in the refrigerator at 4°C till use.

Isolation of Phaeoisariopsis griseola and inoculum preparation

Diseased leaves obtained from the different localities received similar treatments separately where they were rinsed in three changes of sterile distilled water and small sections of the common bean leaf tissue showing small, angular and dark brown spots on the leaves adjoining healthy tissue were cut using sterilized scalpel and whose surface was sterilized with 70% ethanol (Leitich *et al*., 2016). The cut portions were plated out on solidified PDA. Three cut portions were placed per plate with equal distance between them. Four replicate plates for each of the cut portions were made for each of the isolates. The plates were incubated at 24^{o}C for 7 days in a non-illuminated incubator for germinate and sub-cultured immediately until a pure culture of the isolates were obtained and stored in McCartney bottles for further use. Sub culturing of the isolates was made to obtain pure culture.

Pathogenicity test

Pathogenicity tests were carried out to establish whether the fungal isolates caused angular leaf spot disease and to determine whether they could induce similar symptoms on inoculation and be re-isolated, thus fulfilling Koch's postulates (Ezeonu *et al*., 2018). The fungal inoculum of 1ml from PDA media was serially diluted and about 5 millilitres of 10^{-4} suspension of the fungus inoculum was forced into the underside of three different leaves per pot using hypodermic syringe without a needle. Five millilitres of sterile water were also infiltrated in some common bean plants as controls and left for 48hrs to test for the virulence of the fungus organism according to Emitaro *et al*. (2017). Plants were placed in the greenhouse and observed daily. The inoculated and the uninoculated plants were covered in plastic bags to maintain humidity at its maximum (Narasimba & Srinivas, 2012). Each experiment was repeated twice, with two replications of 10 plants per treatment. Observations were made from one week after inoculation. The pathogens were re-isolated as previously described and their cultural and morphological characteristics were compared with those of the original isolates.

Phytochemical screening of Allium sativum, Azadirachta indica and Tithonia diversifolia

The plant parts were thoroughly washed then air-dried under shade until physiological dryness and then pulverised using the laboratory mill. The final product was then kept inside tight paper

bags to await phytochemical screening. *Allium sativum, Azadirachta indica* and *Tithonia diversifolia* were evaluated for the presence of alkaloids, flavonoids, sterols, cardiac glycosides, saponins, tannins and terpenoids using standard procedures as described by Mibei *et al*. (2012) & (Parithra *et al*. 2017).

Preparation of crude extracts from Allium sativum, Azadirachta indica and Tithonia diversifolia

Two hundred grams of *Allium sativum*, *Azadirachta indica* and *Tithonia diversifolia* were weighed separately using a top loading balance and each transferred into nine 500ml conical flasks. Five hundred millilitres of solvents of methanol, ethanol and distilled water was added and left at room temperature in the dark for 7 days for the extraction of the bioactive compounds according to the method of Byrappa (2015). The macerated plant tissues were separated from the aqueous solution by filtering using a muslin cloth then re-filtered using Whatman filter paper No.1. The filtrate was then sterilized by serially filtering through a 0.45 micrometre-pore-size filter and then a 0.22 micrometre-pore-size filter and the filtrate evaporated to dryness to obtain crystals by placing them in water bath at 35^{o}C overnight and then freeze-dried into fine powder for long term storage and kept at 4^{o}C in the dark (Baba & Malik, 2015). When needed the extract was reconstituted by dissolving in water at 5g extract in 10ml water to make the stock solution and stored in the dark at 4^{o}C. Sterile water was used in the experiment as negative control and kept at 4^{o}C until they were required for use according to Narayana *et al*. (2016).

Effects of Azadirachta indica, Allium sativum and Tithonia diversifolia crude extracts on spore germination obtained different solvent extracts of distilled water, methanol and ethanol at 50, 75 and 100% concentrations

Antifungal activity of *Azadirachta indica, Tithonia diversifolia* and *Allium sativum* extracts was determined using the paper disc diffusion method of Esmaeili *et al*. (2013). Ten millilitres of Potato Dextrose Agar were poured per petri dish of the 144-petri dish. The fungal growth was adjusted to 0.1 of dilution potato dextrose broth. Five millilitres of autoclaved 0.01% Tween-20 was added to scintillation vails containing the fungus and vortex thoroughly for 1minute. Wide-bore pipette tip was then used to dilute the inoculum by transferring two separate 0.1ml aliquots of spore suspension into 2ml Eppendorf tubes containing 1.8ml of 0.01% Tween-20 after which each aliquot was enumerated. Zero point one millilitres (0.1mls) of the inoculum suspension contained approximately 10^{8} fungus/ml which were poured over the agar in the petri plates and dispersed using sterile cotton swab. Sterile filter paper discs of 6mm diameter in dimension were soaked for 30 seconds in 10ml of plant extracts in sterile petri dishes at concentrations of 50, 75 & 100%, and immediately introduced into the centre using sterile mounting needle and forceps. Sterile water was used as negative control while synthetic fungicide (AMISTAR TOP) was used as the positive control. Inhibition zones were measured using ruler (mm) to determine the inhibition effect of the extract on the spore germination after incubation at 27^{o}C for 72 hours. All the treatments were replicated four times.

Data analysis

Statistical analysis of the data was conducted using R version 3.6.1 package. Data on inhibition effects of the plant extracts on in vitro spore germination of *Phaeiosariopsis griseola* were analysed using analysis of variance (ANOVA). Treatment means were separated and compared at P=0.05.

Results and discussion

Pathogenicity test of the isolates

Common bean plants inoculated with Sabatia, Bondo and Ugenya isolates showed typical angular leaf spot symptoms of small, angular, dark brown and numerous spots on the leaves, the interaction was considered pathogenic as

shown in plate 1.1(a). There was positive pathogenicity outcome. The findings concurred with studies by Ddamulira *et al*. (2014); Satorato (2002) who obtained similar results when *Phaeiosariopsis griseola* was artificially inoculated with common bean. These results demonstrated the fact that all the fungus isolates investigated for pathogenicity were indeed pathogenic. At first, the lesions were grey, later turn brown and attain an angular shape because of limitation by veins. On all lesions, dark stroma appeared in abundance. Angular leaf spot disease causes typical symptoms on the leaves with angular shaped lesions as well as lesion multiplication and extension on the foliage (Allorent & Savary, 2005; Bode *et al*., 2017). The uninoculated common bean plants showed no symptoms of angular leaf spot disease hence served as control as shown in plate 1.1(b). There was indeed high virulence of the Bondo, Sabatia and Ugenya isolates and thus great pathogenicity of fungi examined. Data document that Sabatia isolate was highly pathogenic and caused the highest disease severity. Ugenya isolate exhibited the lowest disease severity on common bean plants followed by Bondo isolate.

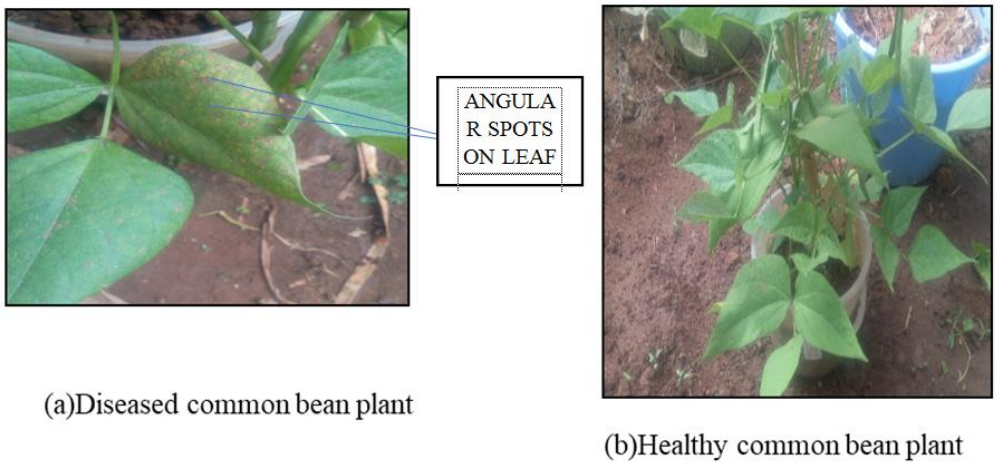

Plate 1. (**a**) Symptoms of angular leaf spot disease on diseased common bean plant and (**b**) a healthy plant. Photos courtesy of Simon Meso.

Phytochemical screening of Azadirachta indica, Tithonia diversifolia and Allium sativum

The phytochemical investigation of *Allium sativum* bulbs indicated the presence of alkaloids, flavonoids, saponin, tannins and cardiac glycoside. This is in agreement with the work done by Hunasagi *et al*. (2018).

The results are in agreement with Ramadass & Subramanian (2018). Phytochemical screening of the leaves extract of *T. diversifolia* revealed the presence of phenols, tannins and flavonoids while alkaloids, sterols and saponins were not detected which is in agreement with results of Rasaga *et al*. (2014) differing only in the presence of sterols which may be attributed to the part used to obtain the extract in the study. The difference in the phytochemical composition from these two studies could be due to variable distribution of phytochemicals compounds in different parts of *Tithonia diversifolia* plant. From the test of azadirachta indica, the following biologically active compounds were tested positive; alkaloids, saponins, tannins, terpenoids, cardiac glycosides and sterols which is partially in agreement with the findings of Ahmed (2008) on the absence of flavonoids as shown in table 1.

These class of compounds especially terpenoids, alkaloids, saponins and tannins are known to have antimicrobial activity against several pathogens Rejeki *et al*. (2018).

Table 1. Results of Qualitative phytochemical screening of Azadirachta indica, Tithonia diversifolia and Allium sativum.

Phytochemical	Plant material *Azadirachta indica*	*Tithonia diversifolia*	*Allium sativum*
Tannins	+	+	+
Saponins	+	_	+
Flavonoids	_	+	+
Terpenoids	+	+	+
Sterols	+	_	+
Cardiac glycosides	+	+	+
Alkaloids	+	-	+

Key= **+** present; **-** absent

Effect of Azadirachta indica, Allium sativum and Tithonia diversifolia crude extracts on spore germination obtained using different solvent extracts of distilled water, methanol and ethanol

The effect of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* crude extracts on spore germination is presented in table 2. Three types of concentration 50%, 75% and 100% of the extracts were used to test the spore germinate of *Phaeiosariopsis griseola* obtained from methanol, ethanol and water as solvents. *Allium sativum* methanolic extract had the highest effect on spore germination at 100% concentration followed by *Tithonia diversifolia* ethanoic extract at 100% concentration, both methanolic and ethanoic extracts of *Azadirachta indica* at 100% concentration also showed high efficacy on fungus spore germination inhibition compared to synthetic fungicide (AMISTAR TOP) and distilled water (control) as presented in table 2. All the extracts at 50%, 75% and 100% concentrations performed better than the synthetic fungicide (AMISTAR TOP).

Sterile water (control) had no effect on fungus spore germination. All the plant extracts at 100% concentration showed the highest activity on spore germination followed with 75% while 50% extract concentration had the least fungicidal activity. Methanolic extracts of all the plants showed more effectiveness than ethanoic extract. Water extracts had the least activity. The findings showed that all the treatments differed significantly compared to the controls on spore germination *in vitro*; the most effective was methanolic *Allium sativum* extract at 100% concentration.

Table 2. Effect of Azadirachta indica, Allium sativum and Tithonia diversifolia crude extracts on spore germination using different solvent extracts of distilled water, methanol and ethanol at different extract concentration of 50%, 75% and 100%, sterile water and synthetic fungicide (AMISTAR TOP).

Plant extract	Concentration (%)	Water	Ethanol	Methanol
		Inhibition zones (mm)		
Allium sativum	50	6.63±0.03d	9.09±0.04cd	9.03±0.04c
	75	14.34±0.05c	14.89±0.05c	20.70±0.07b
	100	16.77±0.06ab	20.95±0.06ab	26.10±0.11a
Azadirachta indica	50	7.97±0.0cd	6.79±0.03cd	7.30±0.04cd
	75	16.08±0.0b	14.89±0.05b	19.70±0.06b
	100	18.5±0.0ab	22.28±0.12a	22.95±0.12a
Tithonia diversifolia	50	8.74±0.04cd	10.59±0.05c	11.18±0.05c
	75	17.00±0.06b	16.21±0.06b	21.01±0.06b
	100	16.77±0.06ab	21.29±0.10ab	24.62±0.09a
Control (sterile water)	0%	6.0±0e	6.0±0e	6.0±0e
Fungicide (amistar top) MP		6.4±0.07d	6.4±0.07d	6.4±0.07d

Legend: Means followed by different letters down the columns differ significantly at P=0.05 by Duncan test. ± standard error. Each value is an average of four replicates.

The findings showed the inhibition of spore germination in vitro with all tested extracts with the most effective being methanolic *Allium sativum* extract at 100% concentration. *Allium sativum* is a spice with global recognition.

In this study, *Allium sativum* had inhibited spore germination when tested. The inhibitive effect was proportional to the concentration for all the methanolic plant extracts of *Allium sativum*, *Azadirachta indica* and *Tithonia diversifolia*;

the higher the concentration of the extracts showed more inhibitive effects. These effects are in accordance with the results of Singh *et al*. (2014) who reported that garlic extract had effective spore germination inhibition on *Alternaria dauci*. The fungicidal action of *Allium sativum* is due to the compound allicin. It has strong antimicrobial and antifungal activities (Keerio *et al*., 2017). Thus, inhibition of fungi observed in this study may be related to allicin or ajoene which curbs the performance of some enzymes that are important to fungi.

The results clearly indicate that the *Allium sativum* methanolic extract showed the highest inhibitory activity. The aqueous extracts of *Allium sativum* showed least fungicidal activity than the ethanolic extract against the test organism, which is in agreement with earlier report by Abdulaziz *et al*. (2018) which reported that methanol had higher extraction ability than ethanol and water. *Allium sativum* has been reported to have fungicidal activity on *Puccinia tritina* a wheat fungus that causes wheat leaf rust (Shabana *et al*., 2017). Similar results were reported by Keerio *et al*. (2017) with *Allium sativum* being effective in controlling *Fusarium oxysporum* fungus. The fungicidal activity of *Azadirachta indica* leaves extract against fungal plates showed considerably spore germination inhibition activity similar findings were also presented by Ravishankar *et al*. (2018) that alcoholic extracts of *Azadirachta indica* had high inhibitory effect on fungus; the fungus targeted were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus terreus*. The leaves extract of *Azadirachta indica* and *Tithonia diversifolia* plant has been previously reported to show antifungal activity (Allorent & Savary, 2005). Effectiveness of *Azadirachta indica* leaves extract in this experiment also agree with the work of Pankaj *et al*. (2013) which reported its antifungal activity against *Trichophyton mentagrophytes*. The results also in agree with the findings of Keerio *et al*. (2017) in which it was found to have fungicidal activity against *Fusarium oxysporum* fungus. The results also conform to Ezeonu *et al*. (2018) who reported that 5% aqueous leaf extract of neem was shown to cause inhibition in growth of six tested fungal pathogens (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Candida albicans* and *Microsporum gypseum*) and further confirmed that aqueous neem extracts inhibited *A. niger* more than *C. albicans*, while alcohol neem extract inhibited *C. albicans* better than *A. niger*. The highest extraction potential effect was observed with methanol solvent while water solvent had the least effect. Similar results were reported by Sultana *et al*. (2009) that aqueous methanol was found to be more effective in recovering highest amounts of phenolic compounds from rice bran and *Moringa oleifera* leaves than ethanol and water. Extracts obtained using methanol as solvent had higher activity followed by ethanol and then water. This shows that the extraction yield increases with increasing polarity of the solvent used in extraction. The results of this study are in agreement with the findings of Quy *et al*. (2014).

From the experiment done, about three quarter of the results showed that as the concentrations of the plant extracts increases the inhibition effectiveness of the extracts also increased which is partly in agreement with the findings of Effiong *et al*. (2016). These results were also in agreement with the findings of Onaran & Saglam (2016) that concluded; the plant extracts showed a different level of antifungal activities in a dose depend manner.

The phytochemical screening confirmed that *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* plants are rich in phytochemicals. The findings confirmed the presence of phytochemicals like alkaloids, saponins, tannins, cardiac glycosides, terpenoids, flavonoids and sterols in nearly all the plants. These plants extracts have confirmed to possess fungicidal

activity on spore germination of *Phaeiosariopsis griseola* fungus but there was some degree of variation in their fungicidal activities. This study showed that all the plants contained most of the active phytochemical compounds which justify their fungicidal property and extract effectiveness increased with the concentration. Methanol solvent was confirmed to have the highest extraction potential followed with ethanol and lastly sterile water. These findings confirm that the more polar a solvent is the more its extraction potential and vice versa. All the various types of plant extracts were found to be effective against *Phaeiosariopsis griseola*. Therefore, there is possibility that *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extracts can control *Phaeiosariopsis griseola* of common bean. *Allium sativum* methanolic extracts at high concentrations should be used in controlling and management of angular leaf spot disease of common bean. There is need to harness the potential of these plant extracts which are eco-friendly and biologically degradable to control *Phaeiosariopsis griseola* pathogen as it would help ameliorate the cost and negative effects of continuous use of synthetic fungicides. Considering the high fungicidal activity of *Azadirachta indica, Allium sativum* and *Tithonia diversifolia* extracts under the test conditions, the extracts may be strong candidates for future field tests. The study revealed significant findings that would be beneficial for the common bean producers. Extensive research still needs to be done on phytochemicals of these plants for the development of cost-effective drugs for the future. More so, since many of the existing synthetic drugs cause various side effects, drug development using plant-based compounds could be useful in meeting this demand for newer drugs with minimal side effects.

References

Abdulaziz BK, Musa DD, Aisha H. 2018. Antifungal activity of garlic (*Allium sativum*) extract on some selected fungi. Journal of Medicinal Herbs and Ethnomedicine **4,** 12-14.

Ahmed IM. 2008. Bioactivity of neem (*Azadirachta indica*) callus extract. Biosciences, Advance Technology and Environment Council **2,** 223-238.

Al hazmi RH. 2013. Effect of neem (*Azadirachta indica*) leaves and seeds extract on the growth of six of the plant diseases causing fungi. Global Advanced Research Journal of Microbiology **2,** 89-98.

Allorent D, Savary S. 2005. Epidemiological characteristics of angular leaf spot of bean: A systems analysis. European Journal of Plant Pathology **113,** 329–341.

Baba SA, Malik SA. 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. Integrative Medicine Research **9,** 449-454.

Baljeet SY, Simmy G, Ritika Y, Roshanlal Y. 2015. Antimicrobial activity of individual and combined extracts of selected spices against some pathogenic and food spoilage microorganisms. International Food Research Journal **22,** 2594-2600.

Bode S, Tryphone GM, Chilagane LA, Protas D, Kusolwa PM., Nchimbi-msolla S. 2017. Marker assisted selection for common bean diseases improvements in Tanzania. Prospects and Future Needs **2,** 34-45.

Byrappa M. 2015. Antimicrobial activity of *Allium sativum* against *Escherichia coli* bacterium. International Journal of Institutional Pharmacy and Life Sciences **5,** 215.

Cecilia P, Olubunmi F. 2014. Management of foliar and soil-borne pathogens of cowpea (*Vigna unguiculata* L. Walp) with two garlic varieties (*Allium Sativum* L.), African Journal of Biotechnology **13,** 1791-1795.

Cherkupally R, Kota SR, Amballa H, Reddy BN. 2017. In vitro antifungal potential of plant extracts against *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Annals of Plant Sciences **6,** 1676-1680.

Damiano AH, Romero AJ, Simon BA, Huerta ML, Cabrara EH. 2013. The nutritional value of beans (*Phaseolus vulgaris* L.) and its importance for feeding of rural communities in Puebla-Mexico, International Research Journal of Biological Sciences **2,** 59-65.

Ddamulira G, Mukankusi C, Ochwo-Ssemakula M, Sseruwagi P, Edema R, Gepts P. 2014. Distribution and variability of *Phaeoisariopsis griseola* in Uganda. Journal of Agricultural Science **6,** 16-29.

Effiong EB, Gwana AM, Halima MB, Bagudu BY. 2016. Phytochemical analysis and antimicrobial activity of methanolic, ethanolic and acetonic extracts of stem bark and leaf of neem plant (*Azadirachta indica*) Journal of Diseases and Medicinal Plant **2,** 14-25.

Emitaro WO, Musyimi DM, Otiato DA, Onyango B. 2017. Morphological, biochemical and molecular characterization of *Xanthomonas campestris* pv*. vesicatoria* of African nightshades (*Solanum scabrum* Mill.). International Journal of Biosciences **11,** 190-197.

Esmaeili AK, Taha RM, Banisalam B, Mohajer S, Zalina N. 2013. Antimicrobial activities of extracts derived from in vivo and in vitro grown *Trifolium pratense* (Red clover), International Journal of Environmental Science and Development **4,** 475–478.

Ezeonu CS, Chinedu I, Dawn IA, Assumpta I, Abraham J. 2018. Antifungal effect of aqueous and ethanolic extracts of neem leaves, stem bark and seeds on fungal rot diseases of yam and cocoyam. Chemical, Biological Technologies in Agriculture **5,** 18.

Hunasagi BS, Somashekhar M, Kalyane NV, Gaviraj EN. 2018. Phytochemical investigation & wound healing activity of *Jasminum grandiflorum,* Journal of Pharmacognosy & Phytochemistry **7,** 31-34.

Katungi E, Sperling L, Karanja D, Farrow A, Beebe S. 2011. Relative importance of common bean attributes and variety demand in the drought areas of Kenya, Journal of Development & Agricultural Economics **3,** 411-422.

Keerio A, Nizamani ZA, Hussain S, Rafiq M, Iqbal S, Ahmed S. 2017. To evaluate different botanical extracts against *Fusarium oxysporum* in-vitro condition, the causal agent of sunflower wilt. European Journal of Biotechnology and Bioscience **2,** 25-29.

Keller B, Manzanares C, Jara C, David J, Bruno L. 2015. Fine - mapping of a major QTL controlling angular leaf spot resistance in common bean (*Phaseolus vulgaris* L.). Theoretical and Applied Genetics **4,** 813-826.

Leitich RK, Omayio DO, Mukoye B, Mangeni BC, Wosula DW, Arinaitwe W, Abang MM. 2016. Pathogenic variability of angular leaf spot disease of common bean in Western Kenya, International Journal of Applied Agricultural Sciences **2,** 92-98.

Mauricio J, Hoyos Á, Alves E, Rozwalka LC, Souza EA, De, Zeviani WM. 2012. Antifungal activity and ultrastructural alterations in *Phaeoisariopsis griseola* treated with essential oils, Agricultural Sciences **36,** 270-284.

Mibei KE, Ojijo KN, Karanja SM, Kinyua KJ. 2012. Phytochemical and antioxidant analysis of methanolic extracts of four African indigenous leafy vegetables. Food Science and Technology, **13,** 37-42.

Narasimha MK, Srinivas C. 2012. *In vitro* screening of bio antagonistic agents and plant extracts to control bacterial wilt (*Ralstonia solanacearum*) of tomato (*Lycopersicon esculentum*). Journal of Agricultural Technology **8,** 999-1015.

Narayana N, Kola B, Mallapuram N, Yamparala K, Naidu N. 2018. Antioxidant activity of the combined ethanolic extracts of bark of the plants *Hemidesmus indicus* and *Ficus religiosa*, World Journal of Pharmacy & Pharmaceutical Sciences **7,** 1807-1814.

Obongoya BO, Wagai SO, Odhiambo G. 2010. Phytotoxic effect of selected crude plant extracts on soil-borne fungi of common bean. African Crop Science Journal, **18,** 15-22.

Onaran A, Sağlam HD. 2016. Antifungal activity of some plant extracts against different plant pathogenic fungi, International Journal of Advances in Agriculture and Environmental Engineering, **3,** 284-287.

Pamela P, Mawejje D, Ugen M. 2014. Severity of angular leaf spot and rust diseases on common beans in Central Uganda, Uganda Journal of Agricultural sciences **15,** 63-72.

Pankaj S, Lokeshwar T, Mukesh B, Vishnu B. 2011. Review on neem (*Azadirachta indica*): Thousand problems one solution, International Research Journal of Pharmacy **2,** 97-102.

Parekh J, Chanda S. 2007. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. African Journal of Biomedical Research **31,** 53-58.

Parithra CS, Suchiritha D, Jessie WS, Anila BK, Rani VD. 2017. Antioxidant activity of papaya reel & developed chapatis, International Journal of Current Microbiology & Applied Sciences **6,** 636-640.

Quy DD, Artik EA, Phuong LT, Lien, HH, Felycia ES, Suradi I, Yi-Hsu J. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, & antioxidant activity of *Limophila aromatica*, Journal of Food and Drug Analysis **22,** 296-302.

Ramadass N, Subramanian N. 2018. Study of phytochemical screening of neem (*Azadirachta indica*), International Journal of Zoology Studies **3,** 209-212.

Rasaga CY, Tepora MM, Rideout JA. 2014. Terpenoids from *Tithonia diversifolia*, Journal of Research in Science Computing and Engineering **4,** 1-7.

Ravishankar TL, Ramneek K, Sukirat K, Shyamalima B. 2018. Neem (*Azadirachta indica*): An elixir in dentistry. Chronicles of Dental Research, **8,** 7-13.

Rejeki D, Addy HS. 2017. Antimicrobial activity of *Tithonia diversifolia*, *Elephantopus scaber*, and *Kigelia africana* against plant pathogens, Frontiers in Environmental Microbiology **3,** 56-61.

Sarawaneeyaruk S, Krajangsang S, Pringsulaka O. 2015. The effects of neem extract and *Azadirachtin* on soil microorganisms, Journal of Soil Sciences and Plant Nutrition **15,** 1071-1083.

Sartorato A. 2002. Identification of *Phaeoisariopsis griseola* pathotypes from five states in Brazil, Fitopatologia Brasileira **27,** 1-4.

Shabana YM, Abdalla ME, Shanin AA, El-Sawy MM, Draz IS, Youssif AW. 2017. Efficacy of plant extracts in controlling wheat leaf rust disease caused by *Puccinia triticina*. Egyptian Journal of Basic and Applied Sciences **4.** 67-73.

Sharma M, Adikshita P. 2017. Integrated approaches for management of angular leaf spot (*Phaeoisariopsis griseola*) of French bean. International Journal of Science, Environment and Technology **6,** 1555-1559.

Singh KP, Pandey V, Singh H, Shukla DN. 2014. Effect of plant leaf extract on fungal diseases of carrot in spore germination. European Journal of Experimental Biology **4,** 138-142.

Stavělíková H. 2008. Morphological characteristics of garlic (*Allium sativum* L.) genetic resources collection- Information, Horticultural Science, **35,** 130-135.

Sultana B, Anwar F, Ashraf M. 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts, Molecules **9,** 2167-2180.