Journal of Engineering and Technology
The Open University of Sri Lanka (JET-OUSL), (2021) Vol. 9, No.2, pp 12-21

In vitro Antifungal Properties of the Different Solvent Extracts of Selected Tropical Plants

P. Janarthani and A. G. B. Aruggoda*

Department of Agricultural and Plantation Engineering, Faculty of Engineering Technology, The Open University of Sri Lanka, Nawala, Nugegoda, Sri Lanka.

*Corresponding Author email: agbar@ou.ac.lk, Tele: +94112881062

Abstract - *Several species of fungi are responsible for the post-harvest deterioration. Among them, Rhizopus stolonifer, Colletotrichum gloeosporioides and Aspergillus spp. are the major causal organisms of post-harvest diseases. Synthetic fungicides are one of the most effective controlling methods for health hazards. Hence, there is an urgent need for developing a bio-safe method for the control of this pathogen without the use of chemical fungicides. The aim of the present study was to investigate* the *Antifungal effect of Adhathoda vasica - leaves, Azadirachta indica- seeds, Ricinus communis - seeds, Clerodendrum infortunatum-leaves and Pistia stratiotes- leaves via in vitro, on the growth of Rhizopus stolonifer, Colletotrichum gloeosporioides* and *Aspergillus spp. Five different solvents; methanol, ethanol, chloroform, petroleum ether, and sterilized distilled water were used for plant extraction. Disk diffusion assay was conducted to evaluate the performances of each plant extract against all three fungi. A wide range of yields among extracts was observed depending on the extraction solvent and plant material used. Ardathoda vasica leaf extract gave the highest inhibition in all five solvents; methanol, Chloroform, Petroleum ether, Ethanol, and Sterilized distilled water have shown the maximum zone of inhibition of all three fungal species. The second highest inhibition was observed in all Azadirachta indica extracted in solvents; Methanol, Chloroform, Petroleum ether, ethanol, and Sterilized Distilled Water with the higher inhibition zones of all three fungal species. Among the solvents, ethanol performed the best, having the highest inhibition zone in between 10 - 14 mm range including in the control with solvent only followed by moderate performances in the other plant extracted solvents; Methanol and Chloroform, while distilled water and petroleum ether were least effective against all fungal species. In the present study, the natural product extracts identified to inhibit fungus species with high potency are leading candidates for antifungal identification. The most effective plant extracts against the Rhizopus stolonifer are Ardathoda vasica and Azadirachta indica. The study revealed a promising prospect for the utilization of selected plant extracts in postharvest diseases control and the potential to develop bio fungicides using botanicals.*

Keywords: *antifungal effects, plant extracts, solvents, zone of inhibition*

1.0 INTRODUCTION

Post-harvest losses of fruits and vegetables mainly occur due to improper postharvest practices, diseases, and lack of facilities and technology to extend their storage life. Among all the factors for reducing the losses on food supply, postharvest diseases are a major factor that causes the losses by limiting the duration of storage (Karabulut and Baykal, 2004; Liu et al, 2005). It is estimated that post-harvest diseases destroy 10-30 % of the total yield of crops and in some perishable crops, especially in developing countries; they destroy more than 30% of the crop yield (Agrios, 2005). Several species of fungi are responsible for post-harvest deterioration. They belong to different genera including *Penicillium, Botrytis, Alternaria, Gleosporium, Mucor, Rhizopus, Fusarium, Monilinia* and *Aspergillus (*Liu et al, 2013). Generally, controlling these pathogens is quite efficiently

performed by synthetic chemical fungicides. However, it is recorded the increased resistance against the limited number of authorized chemical fungicides at present has increased the efforts of finding alternative or complementary control measures among the researchers (Ippolito et al., 2005; Smilanick et al., 2008; Droby et al., 2009; Sanzani et al., 2009; Sharma et al., 2009; Mari et al., 2010).

Extracts containing different classes of phenolic compounds from many plants have recently gained popularity as well as scientific interest for their antibacterial and antifungal activity (Lee et al., 2007; Verástegui et al., 2008; Santas et al., 2010, Rauha et al., 2000; Al-Zoreky, 2009). Phenolic compounds represent a rich source of biocides and preservatives that have been explored for a long time as postharvest alternative control means (Lattanzio, 2003). The components with phenolic structures, like carvacrol, eugenol, and thymol were highly active against the plant pathogens. With its rich biodiversity, Sri Lanka is blessed with many unexplored wild herbaceous species with different capacities and which are possible to incorporate into crop development by means of fertilizers or as pesticides. They are interesting from an ethnobotanical point of view since a lot of them are used in Sri Lanka as a source of drugs in traditional and Ayurveda medicine. In fact, they are known as a rich source of antioxidant, anti-inflammatory, diuretic, antibacterial, and antiviral active substances, with medicinal as well as cosmetic applications (Yukawa et al.,1996; Dhiman and Chawla, 2005; Wang et al., 2006; DiVenere et al., 2009). The literature claims a very low number of explorations of antimicrobial activity of phenolic obtained from wild species against postharvest fungal pathogens. Therefore, the objective of the present study was to evaluate the *in vitro* antifungal activity of different solvent extracts of five medicinal plants. Preliminary data were analyzed to study the efficacy of the different solvent extracts of selected plants in preventing the growth of three post-harvest fungal species.

2.0 METHODOLOGY

2.1 Plant Materials

Plants of five tropical plant species (*Pistia stratiotes, Adhathoda vasica, Ricinus communis, Clerodendrumin fortunatum, Azadirachta indica*) were collected from Low Country Wet Zone in Sri Lanka and classified according to botanical name and family (Table 1). Plant parts were collected and transported to the laboratory where they were cleaned washed with distilled water followed by washing with 5% of Sodium hypochlorite (NaOCl) and added with a few drops of Tween-20.

Table 1: Plant - extracts

Scientific name	Common name	Plant Part used
Pistia stratiotes	Water lettuce	Leaves
Adhathoda vasica	Adathoda	Leaves
Ricinus communis	Castor plant	Seeds
Clerodendruminfortunatum	Hill glory bower	Leaves
Azadirachta indica	Margosa	Seeds

2.2 Preparation of plant Crude Extracts

Analytical grade solvents; methanol, petroleum ether, ethanol, chloroform, and Sterilized distilled water were used as extraction solvents. Plant tissues were homogenized by following the method described by Gurjar et al., in 2012 with slight modifications. Plant materials were ground by using sterile mortar and pestle by adding sample: solvent as 1:10, subjected to shaking at 100 rpm for 24 hours at room temperature. Extracts were subjected to filtration (What-man 42 filter paper), where three-time filtration was done with the respective solvent each time followed by centrifugation at 4000 rpm for 20 minutes. The filtrate was concentrated through a rotary evaporator until a sticky dark green crude extract was obtained at 700 ppm pressure and 50°C for Methanol, Ethanol, Petroleum Ether, and Chloroform and at 0°C for distilled water. The crude extracts were kept in an airtight container and stored at 4◦C until further use.

2.3 Preparation of the fungal cultures

Rhizopus stolonifer, Colletotrichum gloeosporioides, and *Aspergillus spp* isolates were collected from the Department of Botany, Faculty of Natural Science, The Open University of Sri Lanka, Nawala, Nugegoda. The fungal cultures were subcultured from potato dextrose agar (PDA) slants into the plates of freshly prepared potato dextrose agar growth medium.

2.4 Antifungal assay

Antifungal activities were performed through the Agar Disc Diffusion method (Bauer et al, 1966; Rios et al, 1988; Alzoreky et al, 2003) and recommended by the NCCLS (National Committee for Clinical Laboratory Standards). The 100 mg/ml plant extract stock solutions were prepared by dissolving in relevant solvent for trials. Each prepared was filtered using sterilized SF13-N-22P Nylon welded syringe filter (ALWSci technologies. www.chinasepta.com) with 0.22 µm size pores.100 µg/ml concentration test solutions were prepared by adding each solvent as per the equation, $C_1V_1=C_2V_2$ where C_1 and V1 are the concentration and volume of stock solution and C_2V_2 are the concentration and volume of test solution respectively. Organisms were subcultured on Potato Dextrose Agar (PDA) at 30°C for 10 days. Conidia were harvested in sterile saline, and using a hemocytometer, the conidial suspension was adjusted to 1.0×10^6 conidia/ml. Mueller-Hinton (MH) agar plates were streaked evenly with a sterilized swab dipped into the standardized inoculums suspension. Crude extract impregnated discs were aseptically transferred on the inoculated agar plates and left to be incubated at 28 °C for 72 hrs to 7 days (Salie et al., 1996; Baris et al., 2006). The clear zones of inhibition around the test crude extract disc were measured for any indication of antimicrobial activity. Solvents were used as negative controls. All assays were carried out in triplicate.

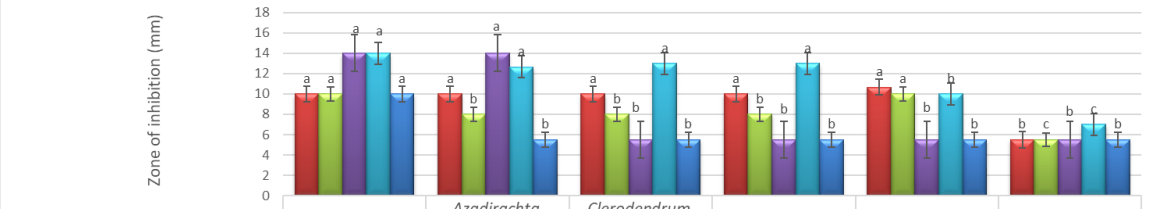
2.5 Data and statistical analysis

Microsoft Excel software (version 13) was used for basic descriptive statistical analysis. Linear growths (LG) for antifungal activities were calculated by measuring the inhibition zones' diameter in millimeters. Antifungal activities were measured by the formula described by Mahmood et al., 2012. Data were analyzed by one-way ANOVA with 95% level of confidence ($P < 0.05$).

3.0 RESULTS AND DISCUSSION

Rhizopus stolonifer, Colletotrichum gloeosporioides and *Aspergillus* sp. were very sensitive to *Adhathoda vasica* - leaves, *Azadirachta indica*- seeds, *Ricinus communis* - seeds, *Clerodendrum*

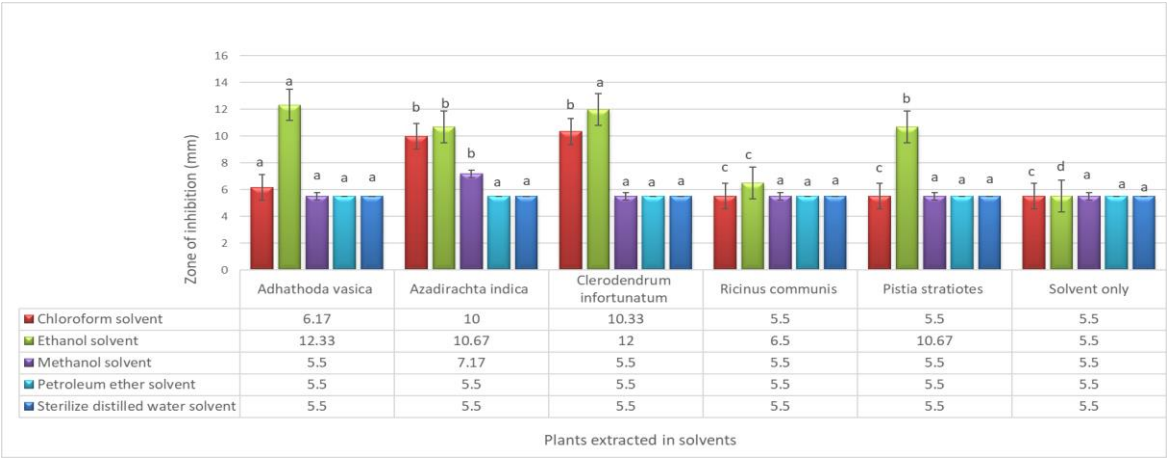
infortunatum-leaves and *Pistia stratiotes*- leaves as colony growth of this fungus was inhibited or reduced when the growth media was amended with plant extracts. The result of the *in vitro* screening tested against *Rhizopus stolonifer*, *Colletotrichum gloeosporioides,* and *Aspergillus* spp. revealed that there was a significant difference (*p<0.005) in antifungal effect among treatments when using different solvents; methanol, ethanol, chloroform, petroleum ether, and sterilized distilled water. There was a significant difference among the antifungal effect of five plant extracts; Adhathoda *vasica*, *Azadirachta indica*, *Ricinus communis*, *Clerodendrum infortunatum* and *Pistia stratiote* leaves in five different solvents; methanol, ethanol, chloroform, petroleum ether, and sterilized distilled water in inhibiting the colony growth of *Rhizopus stolonifer, Colletotrichum gloeosporioides,* and *Aspergillus* spp. Figure 1, 2, and 3 indicates the selected fungal colony inhibition in the present study. According to figure 01, *Adhathoda vasica* had the highest zone of colony inhibition (14 mm) among plant extracts in petroleum ether and Ethanol solvents. *Azadirachta indica* were also capable of inhibiting radial colony growth (14 mm, 12.67 mm) of the fungus *Rhizopus stolonifer.* Further, figure 01 indicates the *Ricinus communis* and *Clerodendrum infortunatum* ethanolic extracts strongly inhibited (13 mm) the mycelial growth of *Rhizopus stolonifer*. The inhibition of the mycelium growth of the *Rhizopus stolonifera* was visible in the negative control with solvents only having <8 mm inhibition. This could be due to chemical inhibition of fungi. However, when compared with other plant extracts *Adhathoda vasica and A. indica* had significantly higher mycelium inhibition in *Rhizopus stolonifer*. The previous studies revealed that these inhibitory activities are due to the direct toxic effects of plant extracts on the pathogens (Bhutia et al.,2015; Chowdhury et al., 2017). Mohamed and EI-Hadidy in 2008, detected those antifungal activities of the plant extracts also is a course of the presence of secondary plant metabolites such as terpenoids, phenols, flavonoids, alkaloids. Further, Tunwari and Nahunnaro revealed in 2014 that the presence of these plant metabolites indicates the fungicidal properties of natural plant products and their potential to control plant diseases. As per the Tijjani et al. (2014) and Chowdhury et al. (2017) the increase in the concentration of plant extracts implied an increase in the active ingredients of the crude extracts which act on the test pathogens thereby affecting its physiological processes, lowering the growth of the pathogens.



	Adhathoda vasica	Azadirachta indica	Clerodendrum infortunatum	Ricinus communis	Pistia stratiotes	Solvent Only
Methanol solvent	10	10	10	10	10.67	5.5
Chloroform solvent	10	8	8	8	10	5.5
Petrolium ether solvent	14	14.0	5.5	5.5	5.5	5.5
Ethanol solvent	14	12.67	13	13	10	7
Sterilized Distilled water solvent	10	5.5	5.5	5.5	5.5	5.5

Fig. 01: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether and Sterilized Distilled water, on mycelial growth of Rhizopus stolonifera

In this study, the five plants extract with five solvents screened showed different effects on C. *gloeosporioides*. This study has demonstrated the possibility of using extracts from some plants to control the mycelial growth of *C. gloeosporiodes*. Ethanol extract of *Adhathoda vasica* showed (12.33 mm) the highest inhibition of mycelium of *Colletotrichum gloeosporioides*. *Clerodendrum infortunatum* and *Azadirachta india* also inhibited (12 mm, 10.67 mm) colony inhibition of the fungus. Ethanolic *Ricinus commuis* extract gave 6.5 mm inhibition among other solvents. Only ethanol solvent had a slightly high zone of inhibition (figure 02). According to figure 02, *A. indica* showed higher inhibition towards the *C. gloeosporiodes* than other plant extracts.

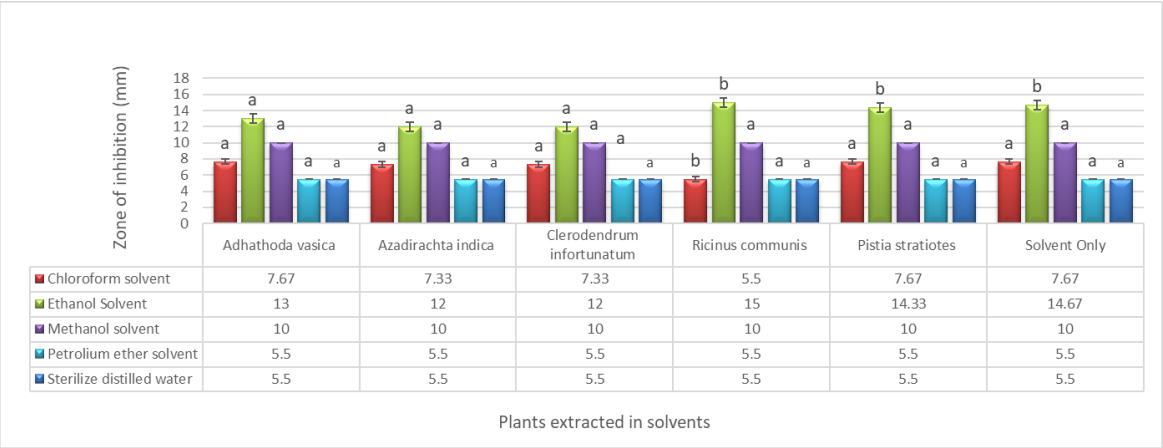


	Adhathoda vasica	Azadirachta indica	Clerodendrum infortunatum	Ricinus communis	Pistia stratiotes	Solvent only
Chloroform solvent	6.17	10	10.33	5.5	5.5	5.5
Ethanol solvent	12.33	10.67	12	6.5	10.67	5.5
Methanol solvent	5.5	7.17	5.5	5.5	5.5	5.5
Petroleum ether solvent	5.5	5.5	5.5	5.5	5.5	5.5
Sterilize distilled water solvent	5.5	5.5	5.5	5.5	5.5	5.5

Fig 02: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether, and Sterilized Distilled water, on mycelial growth of *Colletotrichum gloeosporioides*

Ethanolic *Ricinus communis* seeds extraction gave 15 mm inhibition of *Aspergillus* sp. as well as ethanolic *Adhathoda vasica* leaves *Azadirachta indica seeds, Clerodendrum infortunatum leaves,* and *Pistia stratiotes*- leaves extracts were capable of 13 mm,12 mm, 12 mm, 14.33 mm inhibition of mycelium growth of *Aspergillus* sp. Only consider about solvent Ethanol also gave the highest inhibition (14.67 mm) than the other four solvents. This has to be further studied to clarify the effects of ethanol on growth inhibition on *Aspergillus* sp. In addition, chloroform, as well as methanol showed higher inhibition in control, were required further studies or clarifications. Moreover, none of the extracts of *Adhathoda vasica,* as well as *Azadirachta indica* showed significant inhibition on *Aspergillus* sp.

Adhathoda vasica and *Azadirachta indica* extracted in ethanol showed significantly higher growth inhibition of all three fungal species, *R. stolonifer C. gloeosporioides* and *Aspergillus* sp. beyond that, *Ardathoda vasica and Azadirachta indica* gave higher mycelium growth inhibition on *Rhizopus stolonifer.* Asdaq and Inamdar in 2010 reported that the aqueous extracts of plants generally showed antimicrobial activities. As per the findings of Belewa et al, in 2011, extract of *A. indica* and *Chromolalenaodonata* inhibited the growth of *A. niger*, *F. oxysporum, R. stolonifer* and *Geotrichum candidum*. Anukworji et al, 2012 stated that the ability of the leaf extract of *A. indica, R. comunis*, and *M. indica* to inhibit growth and spore germination of *R. stolonifer* and *F. oxysporum* could be due to the presence of fungi toxic compounds in the extracts of the three plant species. In the present study, *A. indica* showed high effectiveness in Aspergillus sp. Like *Aspergillus niger* and *R. stolonifer.* Among the five solvents, moderate antifungal activity was shown by plants, *Ricinus communis,* and *Clerodendrum infortunatum* extracted in all solvents other than ethanol extracted.



	Adhathoda vasica	Azadirachta indica	Clerodendrum infortunatum	Ricinus communis	Pistia stratiotes	Solvent Only
Chloroform solvent	7.67	7.33	7.33	5.5	7.67	7.67
Ethanol Solvent	13	12	12	15	14.33	14.67
Methanol solvent	10	10	10	10	10	10
Petrolium ether solvent	5.5	5.5	5.5	5.5	5.5	5.5
Sterilize distilled water	5.5	5.5	5.5	5.5	5.5	5.5

Fig 03: Total Effect of leaf and seed extracted in different solvents; Chloroform, Ethanol, Methanol, Petroleum ether, and Sterilized Distilled water, on mycelial growth of *Aspergillus* spp

Further, as per figures 02 and 03, ethanol had the highest fungal inhibition, with respect to the solvents used for extraction in the present study. Figure 02, explain the higher inhibition zone in between 10 - 14 mm range including the control with solvent only having the inhibition zone of 7 mm followed by other extracts; Methanol and Chloroform, while distilled water and petroleum ether were least effective against *R. stolonifer*. This is an indication that the active principles in these mosses are predominantly polar. These results are in agreement with those of Alam et al. (2011) as well as the results of Femi-Adepoju et al, (2014) that aqueous extract of the liverwort *Dumortiera hirsute* was found to inhibit a number of phytopathogenic fungi mediated by different modes of action such as spore germination inhibition, development of anomalies in the hyphae, formation of the flaccid cell wall and granulated cytoplasm. The present results also partially agree with those of Basile et al. (1998) in which acetone extract of *Lunularia cruciate* (a bryophyte) showed no activity against *Candida albicans* and *Aspergillus niger* as well as the findings of Amadioha (2001) who investigate the effects of *Cymbopogon citratus*, *Azadirachta indica* (Neem) and *Ocimum gratissimum* extracts on controlling of the growth of *Rhizopus oryzae in vitro* and *in vivo*. In the tests involving acetone and petroleum ether as solvents, the absence of antifungal activity is suspected to be due to the presence of non-polar molecule(s) in the extracts resulting in the inability of the molecules to cross the fungal cell wall (Basile et al., 1998). Therefore, in the present study the highest mycelium inhibition was achieved by the *Ardathoda vasica* and *Azadirachta indica* plant extracts against the *Rhizopus stolonifer*.

4.0 CONCLUSION

In the present study, the natural product extracts identified to inhibit *Rhizopus stolonifer, Colletotrichum gloeosporioides* and *Aspergillus* sp with high potency are leading candidates for antifungal identification. The most effective plant extracts against *all three fungal species* are *Ardathoda vasica* leaves and *Azadirachta indica seeds*. The present study also confirms the in-vitro synergistic effect of *Ardathoda vasica* leaves and *Azadirachta indica against the Rhizopus stolonifer. In-vitro* experiment showed that ethanolic *Ardathoda vasica* leaves and *Azadirachta indica seeds* resulted in highest colony inhibition on *Rhizopus stolonifer*. Isolation of the active compounds from ethanolic extracts could lead to improved antifungals for use in agriculture to preserve food crops as well as in the pharmaceutical industry for the treatment of mycoses. Moreover, the results of the study will form the base for the selection of plant species for further investigation in the potential discovery of new natural bioactive

compounds. Further studies that aim at the isolation of antibacterial active constituents from the plant have to be initiated while estimating the Minimum Inhibitory Concentration (MIC) of the plant extracts in different solvents.

REFERENCES

Agrios G. N. 2005. Plant pathology. Fifth edition, Elsevier Acad Press; Amsterdam: 2005.

Alam, A., Tripathi, A.,Vats, S., Behera, K. and Sharma, V., 2011. *In vitro* antifungal efficacies of aqueous extract of *Dumortiera hirsute* (Swaegr.) Neems against sporulation and growth of post-harvest phytopathogenic fungi. Arch. Bryol., 10: 3-1 9.

Al-Zoreky N. S. 2009. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. International journal of food microbiology, 134(3), 244–248. https://doi.org/10.1016/j.ijfoodmicro.2009.07.002

Amadioha A. C., 2001. Fungitoxic effects of some leaf extract against *Rhizopus oryzae* causing tuber rot of potato, Archives of Phytopathology and Plant Protection,33:6,499-507, DOI: 10.1080/03235400109383372.

Anukworji, C. A., Ramesh, R. P., Okigbo, R. N., 2012. Isolation of fungi causing rot of cocoyam (*Colocasia esculenta* (l.) schott) and control with plant extracts: (*Allium sativum, l., Garcinia kola, heckel., Azadirachta indica, l.* and *Carica papaya*, l.). Global Advanced Research Journal of Agriculture Science, 1(2), 33-47.

Asdaq S. and Inamdar M., 2010. Potential of garlic and its active constituent, S-allyl cysteine, as antihypertensive and cardioprotective in presence of captopril. Phytomedicine; 17:1016-1026.

Baris, 2006. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Online available at https://www.academicjournals.org/JMPR.

Basile, A., Giordano, S., Sorbo, S., Vuotto, M. L., Ielpo, M. T. L. and Cobianchi, R. C., 1998. Antibiotic effects of *Lunularia cruciata* (Bryophyta) extract. Pharmaceut. Biol., 36: 25-28.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., Turck, M., 1966. Antibiotic Susceptibility Testing by a Standardized Single Disk Method, *American Journal of Clinical Pathology*, Volume 45, Issue 4_ts, April 1966, Pages 493–496, https://doi.org/10.1093/ajcp/45.4_ts.493

Belewa, X., Baijnath, H., Frost, C., Somai, B., 2017. *Tulbaghia violacea* Harv. plant extract affects cell wall synthesis in Aspergillus flavus. Journal of Applied Microbiology. 122. 10.1111/jam.13405.

Bhutia, D. D., Zhimo, Y., Kole, R. and Saha, J. 2016. Antifungal activity of plant extracts against Colletotrichum musae, the post harvest anthracnose pathogen of banana cv. Martaman, Nutrition & Food Science, Vol. 46 No. 1, pp. 2-15. https://doi.org/10.1108/NFS-06-2015-0068

Chaudhuri, S. K., Malodia, L., 2017. Biosynthesis of zinc oxide nanoparticles using leaf extract of *Calotropis gigantea*: characterization and its evaluation on tree seedling growth in nursery stage. Appl Nanosci 7, 501–512. https://doi.org/10.1007/s13204-017-0586-7

Dhiman, R.K., Chawla, Y.K., 2005. Herbal medicines for liver diseases. Dig. Dis. Sci.50, 1807–1812.

Di Venere, D., Sergio, L., Linsalata, V., Pieralice, M., Cardinali, A., Cascarano, N., Bianco,V. V., 2009. Antioxidant properties of wild edible herbaceous species. Ital. J. Agron./Riv. Agron. 4 (4 Suppl.), 635–640.

Droby, S., Wisniewski, M., Macarisinb, D., Wilson, C. 2009. Twenty years of postharvest biocontrol research: Is it time for a new paradigm? Postharvest Biology and Technology 52, 137–145. https://doi.org/10.1016/j.postharvbio.2008.11.009

Femi-Adepoju, A., Adepoju, A., Ogunkunle, T., 2014. Assessment of fungicidal and fungistatic properties of some Nigerian moss plants. Global Journal of Biology, Agriculture and Health Sciences. 3. 126-131.

Gurjar, M., Ali, S., Akhtar, M. and Singh, K., 2012. Efficacy of plant extracts in plant disease management. *Agricultural Sciences*, **3**, 425-433. doi: 10.4236/as.2012.33050.

Ippolito, A., Schena, L., Pentimone, I. and Nigro. F. 2005. Control of postharvest rots of sweet cherries by pre- and postharvest applications of Aureobasidium pullulans in combination with calcium chloride or sodium bicarbonate. Postharvest Biology and Technology, 36: 245-252. ISSN 0925-5214, https://doi.org/10.1016/j.postharvbio.2005.02.007.

Karabulut, O. and Baykal, N. 2004. Integrated control of postharvest diseases of peaches with a yeast antagonist, hot water and modified atmosphere packaging. Crop Protection - CROP PROT. 23. 431-435. 10.1016/j.cropro.2003.09.012.

Lattanzio, V. 2003. Bioactive Polyphenols: Their Role in Quality and Storability of Fruit and Vegetables. Journal of Applied Botany - Angewandte Botanik 77, 128 – 146.

Lee, S.-O., Choi, G.-J., Jang, K.-S., Lim, H.-K., Cho, K.-Y., & Kim, J. C. 2007. Antifungal Activity of Five Plant Essential Oils as Fumigant Against Postharvest and Soilborne Plant Pathogenic Fungi. The Plant Pathology Journal. Korean Society of Plant Pathology. https://doi.org/10.5423/ppj.2007.23.2.097

Liu, H. X., Jiang, W. B., Bi, Y., Luo, Y. B. 2005. Postharvest BTH treatment induces resistance of peach (*Prunus persica* L. cv. Jiubao) fruit to infection by *Penicillium expansum* and enhances activity of fruit defense mechanisms. Postharvest Biol. Technol. 2005, 35, 263–269. https://doi.org/10.1016/j.postharvbio.2004.08.006.

Liu, J. Y., Wisniewski, S. M., Droby, S., Liu., Y. 2013. Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. International Journal of Food Microbiology, 2013, 167: 153–160.

Mari, M., Griffith, J., Rieter, E., Krishnappa, L., Klionsky, D. J., Reggiori, F. 2010. An Atg9-containing compartment that functions in the early steps of autophagosome biogenesis.

Journal of Cell Biology, 190(6), 1005-1022. 190(6):1005-22. doi: 10.1083/jcb.200912089. PMID: 20855505; PMCID: PMC3101592.

Mohamed, N., El-Hadidy, A., 2008. Studies of Biologically Active Constituents of Verbascum eremobium Murb. and its Inducing Resistance against some Diseases of Cucumber. 36.

Mahmood, A., Mahmood, A. and Qureshi, R.A., 2012. Antimicrobial activities of three species of family Mimosaceae. Pak. J. Pharm. Sci., Vol.25, No.1, January 2012, pp.203–206

Rauha, J., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H., Vuorela, P. 2000. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. International Journal of Food Microbiology. 56. 3-12. 10.1016/S0168-1605(00)00218-X.

Rios, J. L., Recio, M.C., Villar, A. 1988. Screening methods for natural products with antimicrobial activity: A review of the literature, Journal of Ethnopharmacology, Volume 23, Issues 2–3, Pages 127-149, ISSN 0378-8741, https://doi.org/10.1016/0378-8741(88)90001-3.

Santas, J., Almajano, M. P., Carbó, R., 2010. Antimicrobial and antioxidant activity of crude onion (Allium cepa, L.) extracts. International Journal of Food Science & Technology. 45. 403 - 409. 10.1111/j.1365-2621.2009.02169.x.

Sanzani, S. M., De Girolamo, A., Schena, L., Solfrizzo, M., Ippolito A. and Visconti. A. 2009. Control of *Penicillium expansum* and patulin accumulation on apples by quercetin and umbelliferone. European Food Research and Technology, 228 (3): 381-389. 10.1007/s00217-008-0944-5.

Salie, F., Eagles, P. F. K. and Lens, H. M. S., 1996. Preliminary Antimicrobial Screening of Four South African Asteraceace species. J. Ethnopharmacol. 52(1):27-33.

Sharma, R. R., Singh D., Singh R. 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review, Biological Control, 50 (2009) 205–221. ISSN 1049-9644, https://doi.org/10.1016/j. biocontrol. 2009.05.001.

Smilanick, J., Mansour, M., Mlikota G. F., Sorenson, D., 2008. Control of postharvest green mold and sour rot by potassium sorbate combined with heat and chemicals. Postharvest Biology and Technology. 47. 226-238. 10.1016/j.postharvbio.2007.06.020.

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(2), pp.244-252.

Tunwari, B. A., Nahunnaro, H., 2014. Effects of Botanical Extracts and a Synthetic Fungicide on Severity of Cercospora Leaf Spot (*Cercospora Sesame* Zimm) On Sesame (Sesamum Indicum L.) Yield Attributes Under Screen House Condition In Ardo-Kola, Taraba State, Nigeria. International Journal of Scientific & Technology Research. Volume 3, Issue 1, January 2014

Verástegui, Á., Verde-Star, M., García, S., Heredia, N., Oranday, A., Rivas-Morales, C. 2008. Species of Agave with antimicrobial activity against selected pathogenic bacteria and fungi. World Journal of Microbiology and Biotechnology. 24. 1249-1252. 10.1007/s11274-007-9563-8.

Wang, K. A., Lin, R. D., Hsu, L. F., Huang, Y. H., Chang, H. C., Huang, C. Y., Lee, M. H., 2006. Cosmetic application of selected traditional Chinese herbal medicines.J. Ethnopharmacol. 106, 353–359.

Yukawa, T. A., Kurokawa, M., Sato, H., Yoshida, Y., Kageyama, S., Hasegawa, T., Namba, T., Imakita, M., Hozumi, T., Shiraki, K., 1996. Prophylactic treatment of cytomegalovirus infection with traditional herbs. Antivir. Res. 32, 63–70