

THE SHELF LIFE OF TOMATO FRUITS (*Solanum lycopersicum* L.) TREATED WITH EXTRACTS OF TWO MEDICINAL PLANTS: *Azadirachta indica* and *Vernonia amygdalina*

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Abstract

Tomato remains one of the most nutritive edible berries but challenged by incessant attack and spoilage by fungi among others. The negative effects of synthetic preservatives have shifted attention to bio-preservatives. This study investigated the shelf-life of post-harvest tomato fruits treated with the two medicinal plants: *Azadirachta indica* (neem leaf) and *Vernonia amygdalina* (bitter leaf) extracts. Fresh tomato fruits and leaves of both plants were sourced from Lokoja. The leaves were air-dried, pulverized and extracted with distilled water and absolute ethanol. The extracts were analyzed phytochemically and graded concentrations (2.5 g/mL - 10.0 g/mL) were applied to the tomato samples in five replications each. Weight loss, appearance of fungal mycelia and deteriorations on the tomato samples were monitored for 30 days. Fungal isolates from the deteriorated samples were recovered and subjected to *in vitro* inhibitory activities. Alkaloids, glycosides, saponins, flavonoids and tannins were present in both extracts, except for *A. indica*, where saponins was not detected. Both extracts significantly ($p < 0.05$) reduce the weight loss (63.4 %) and extended the shelf life of the tomato fruits to 24 days at 10.0 g/mL. *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Alternaria alternata* were recovered from the spoiled tomatoes. The most and least susceptible isolates were *R. stolonifera* (84.56 %) and *A. niger* (71.45 %), respectively. The bioactivities of both extracts were not significantly different ($p > 0.05$) from each other. These findings suggest that relatively higher concentrations of both plant extracts could be potential bio-preservatives to extend the shelf life of post-harvest tomatoes.

Keywords: Deterioration, fungi, neem leaf, phytochemicals, shelf life.

Introduction

Tomato (*Solanum lycopersicum* L.) is an edible fruit that is widely accepted and globally consumed because of their high nutritional contents (Ali *et al.*, 2020). Fruits and vegetables with high levels of nutritive biomolecules, such as antioxidant-rich phytochemicals, minerals, vitamins, proteins, essential amino acids, monounsaturated fatty acids, carotenoids (lycopene and β -carotenoids) and phytosterols have been reported to be beneficial to humans' health and wellness (Tommonaro *et al.*, 2012; Pem and Jeewon, 2015; Salehi *et al.*, 2019; Aslam *et al.*, 2020; Hou *et al.*, 2020). A previous report encouraged constant consumption of tomatoes and tomato products for healthier life and reduction in debilitating health challenges like cancer, osteoporosis and cardiovascular disease (Preedy and Watson, 2008; Freeman and Reimers, 2010). Most of the health benefits of tomatoes are attributed to its major carotenoids, lycopenes. Other uses of tomato fruits include as major raw materials in preparation of juice, salads, soup, paste or ketchups (Freeman and Reimers, 2010).

One of the major challenges faced by farmers and retailers of tomatoes is post-harvest spoilage leading to poor shelf-life of tomatoes (Ahmad *et al.*, 2020). Some of these challenges occur during transportation, storage, and marketing, thereby leading to colossal wastage of these nutritive foods. According to Yahaya and Mardiyya (2019), post-harvest handling losses of fruits and vegetables accounted for over 30 % loss of fresh agriculture produce. Studies have shown that the postharvest loss of tomato fruits is mainly by microbial attacks which remain on the rise due to the poor physiological and environmental conditions. This is especially worrisome because many food vendors use deteriorated tomato fruits in cooking to avoid economic losses. The implication is that consumers are constantly exposed to these fungi via ingestion and/or inhalation. Hence, there is need for proper storage, handling and preservation.

In recent times, environmentalists have considered technological interventions/expansion and application of preservatives to reduce post-harvest losses, particularly in developing countries in order to ensure food security and sustainability (Kumar and Kalita, 2017). Technological intervention and structural expansions are relatively capital intensive, while synthetic chemical preservatives, though effective, have been associated with carry over effects of chemical residues in stored foods and their attendant health risks. These drawbacks

have shifted the attention of scientists into considering botanicals as suitable alternative preservatives for edible foods. Plants and their derivatives are widely used as foods and medicine by man for treatment of various microbial diseases with huge successes. Traditionally, *Azadirachta indica* (neem leaf) and *Vernonia amygdalina* (bitter leaf) have been well documented for the treatment of diverse ailments of humans and other animals. Bitter leaves are widely used in folk medicine to treat diarrhoea, dysentery, recurrent fever, stomach ulcer, headaches, diabetes, joint pains, skin disorders, worm/parasitic infections, malaria, yellow fever, constipation in most African countries (Adedapo *et al.*, 2014; Danladi *et al.*, 2018). Similarly, ethnobotanical and pharmacological properties of *A. indica* have been well reported globally (Paul *et al.*, 2011; Petal *et al.*, 2016; Gupta *et al.*, 2017; Saleem *et al.*, 2018). Considering the efficacy and eco-friendly natures of medicinal plants, it is pertinent to explore their bio-preservative potentials on common perishable nutritive fruits and vegetables. Fungi are ubiquitous in soil, water, air, and processing materials/containers and their spores can easily contaminate exposed products, such as fruits and vegetables (Greco *et al.*, 2014). This study, therefore, investigated the shelf-life improvement of post-harvest tomato fruits treated with *Azadirachta indica* A. Juss. (neem leaf) and *Vernonia amygdalina* Del. (bitter leaf) extracts.

Materials and Methods

Study Area

This study was carried out in Lokoja, the capital city of Kogi State. Lokoja has geographical coordinates situated between latitudes 7°45'N and longitude 6°45'E (Figure 1). Lokoja is a unique, being a confluence town (Pittsburgh of Africa) of the River Niger and Benue as well as link zone between the South-western, South-eastern and Abuja, the Federal Capital territory of Nigeria, with an annual rainfall is between 1016 – 1524 mm and mean annual temperature of 27.7 °C (Alabi, 2009; Adetunji, 2018). According to 2006 census, the population of Lokoja local government aborigine, made up Hausa, Nupe, Kupa, Kakanda, Oworo, Ganagana, Bassa and Egbira ethnic groups is estimated at 196, 643 (Audu, 2012). The strategic nature of the State makes Lokoja a significant centre for commercial activities. The major markets within Lokoja metropolis are: New Market (International Market), Old Market, and Kpata Market among others. Cereal grains, fishes, vegetables and all-purpose domestic items are the predominant products in these markets.

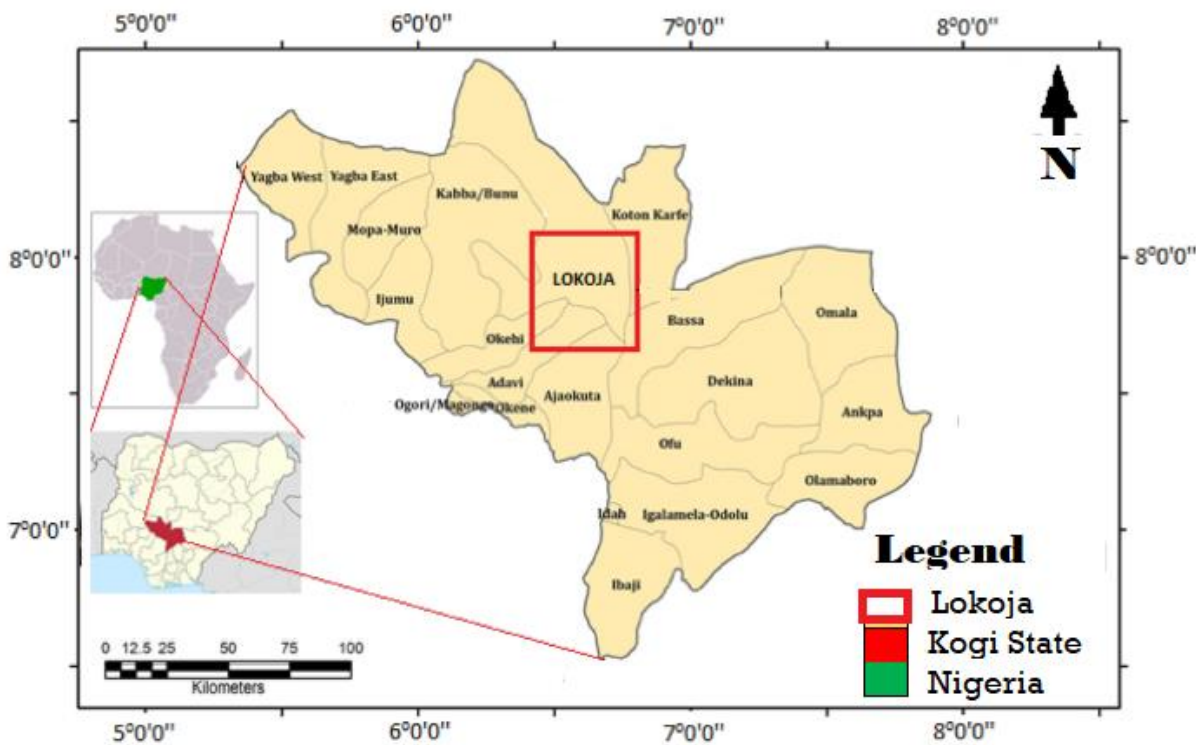


Figure 1: Location map of Nigeria showing Lokoja, Kogi State.

Collection of plant materials and tomato fruit samples

Fresh leaves of *V. amygdalina* were sourced from Lokoja main markets, while the leaves of *A. indica* were collected from the premises of Federal University Lokoja. A total of 100 fresh, ripe, firm and healthy tomato fruits were sourced randomly from five major markets in Lokoja metropolis. The samples were packaged and labelled appropriately and conveyed using a clean black polythene bag to the Microbiology Laboratory of Federal University Lokoja, for further processing.

Processing and preparation of plant extracts

The collected leaves of *V. amygdalina* and *A. indica* were air-dried on the laboratory workbench for 7 – 10 days before pulverising them. The powdered leaves were weighed separately before adding (100 g) of each into 500 mL distilled water and ethanol contained in a 1000 mL conical flask. The flasks were left to stand for 7 days and manually agitated intermittently for proper dissolution and extraction. The contents were filtered using Whatman filter paper folded within a funnel. The filtrates were concentrated to constant weigh to obtain the extracts (Ogu *et al.*, 2013).

Standardization and grading of plant extracts

The concentrated extracts were reconstituted using the extracting solvents and graded concentrations each of the aqueous and ethanol extracts (2.5 g/mL - 10.0 g/mL) were made (Ogu *et al.*, 2012) before storing them at low temperature daily for the shelf life study.

Phytochemical analysis of plant extracts

Phytochemical screening of each extract was done to detect the inherent phytochemical compounds; alkaloids, glycosides, saponins, flavonoids, and tannins, using the standard protocol described previously (Harborne, 1984; Trease and Evans, 1996).

Determination of shelf life of tomato fruits

This was determined according to previous report (Ahmad *et al.*, 2020) with slight modifications. The experimental design included a complete randomized block with seventeen treatments and five replications each. The healthy tomato fruits were divided into seventeen groups with five tomatoes in each. The surfaces of the tomatoes in groups one to sixteen were washed with 2.5 – 10.0 g/mL each of aqueous and ethanol leaf extracts of *A. indica* and *V. amygdalina* respectively. While the seventeenth group served as control with distilled water. The various treatments were left to dry before storing them in cupboard and monitored for weight loss, appearance of fungi and spoilage at ambient temperature (28 ± 2 °C) for a duration 30 days. Spoilage was rated using a 4-point hedonic scale of 4 (excellent), 3 (good), 2 (fair), 1 (poor) and 0 (very poor) (Ahmad *et al.*, 2020). Weight loss was determined using a metler beam balance (Mettler Toledo® MS303TS/00 Model, USA), and the appearance of fungal mycelia on surfaces of the tomato fruits were noted using a hand lens.

Isolation and identification fungal from samples

The prominent fungal colonies were sub-cultured onto fresh sterile potato dextrose agar (PDA) (Oxoid, UK) media to obtain pure colonies. Pure cultures were characterised using their growth pattern, pigmentation, size of colonies and microscopic morphology after staining with lactophenol cotton blue (Onuorah and Orji, 2015). According to this method, a drop of lactophenol was placed on a clean microscopic slide. A small portion of each fungal isolate was taken using a sterile needle and placed in the drop of lactophenol. A clean cover glass was gently placed over the suspension and observed microscopically. The observed cultural and microscopic morphological characteristics for each stained fungal colony were compared with standard reference keys and atlas for their probable identities as reported earlier (Fawole and Oso, 1988; Jay, 1998; De Hooget *al.*, 2000).

In vitro inhibitory effect of extracts on fungal isolates

The assay was done as described previously (Ogu *et al.*, 2011). A 7-day old culture of each fungal isolate was rinsed with 10 mL normal saline in 2 % Tween 80 with the aid of glass beads to aid in separating the spores. The harvested spore suspensions were then standardized to 10^5 spores/mL. Thereafter, 1 mL of each standardized spore suspension (10^5 spores/mL) was uniformly spread on the surface of the gelled PDA plates before boring 8 mm diameter wells at the centre of each PDA culture plates. Then, 100 μ L of the graded extracts (2.5 – 10.0 g/mL) was added into each well. Distilled water was used a control. The plates were incubated at room temperature for 5 – 7 days and observed for radial mycelial growth inhibition using a transparent ruler.

Data analysis

Samples were analysed in triplicates and descriptive statistics was employed to present the data. The differences in mean of samples were analysed using ANOVA at 5 % level of significance.

Results

Qualitative phytochemical analysis of the plant extracts revealed that alkaloids, glycosides, saponins, flavonoids and tannins were present in substantial amount in both leaf extracts of *A. indica* and *A. amygdalina*, except for the aqueous extract of *A. indica*, where saponins were not detected (Table 1). However, high levels of saponins, flavonoids and tannins were detected in *V. amygdalina* extracts. Low levels of glycosides were found in both plant extracts. The results are presented in Table 1 below.

Table 1: Phytochemical analysis of *A. indica* and *A. amygdalina* leaf extracts

Phytochemicals	Aqueous extract		Ethanol extract	
	<i>A. indica</i>	<i>V. amygdalina</i>	<i>A. indica</i>	<i>V. amygdalina</i>
Alkaloids	+	++	++	++
Glycosides	+	+	+	++
Saponins	-	++	+	+++
Flavonoids	++	++	+++	++
Tannins	++	++	++	+++

Key: + = Detected; - = Not detected; +++ = High; ++ = Moderate; + = low

The values obtained from analyses of the weight loss, appearance of fungal mycelia, and deterioration of the tomato samples were presented in Figures 2 – 7. The aqueous and ethanol leaf extracts of both plants displayed significant reduction in weight loss of the treated tomato fruits when compared with the untreated controls. The ranges of weight loss for tomato samples treated with concentrations of 2.5 – 10.0 g/mL were

32.1 % - 59.1 % and 40.2 % - 61.9 % for the ethanol and the aqueous *A. indica* leaf extracts respectively (Figure 2). For the ethanol and the aqueous *V. amygdalina* leaf extracts, the values obtained for tomatoes weight loss ranged between 30.1 % - 56.3 % and 38.2 % - 63.4 % (Figure 3). Relatively lesser weight loss of the tomatoes were obtained with the ethanol extracts of both plants, though not significantly different ($p>0.05$). In general, the effect of the extracts were concentration dependent.

During the periods of shelf life evaluation, the number of days it took for fungal mycelia to appear on the surfaces of the tomato fruit samples were recorded and the findings were presented for the aqueous and ethanol leaf extracts of *A. indica* (Figure 4) and *V. amygdalina* (Figure 5). The number of days it took for mycelial appearance on the samples' surfaces ranged from 6 – 16 days and 7 – 18 days respectively for aqueous and ethanol leaf extracts of *A. indica* treated (2.5 g/mL – 10.0 g/mL) samples (Figure 4). On the other hand, aqueous and ethanol leaf extracts of *V. amygdalina* treated (2.5 g/mL – 10.0 g/mL) samples took 7 – 19 days and 8 – 20 days respectively (Figure 5). The untreated control samples took approximately 3 days for mycelial visibility.

On the effect of extracts on the duration it took for the treated and untreated tomato fruits samples to deteriorate, it was observed that 2.5 g/mL and 10.0 g/mL of the aqueous *A. indica* leaf extract treatments took 9 days and 22 days for deterioration to commence as against 10 days and 23 days recorded for its ethanol extract counterpart (Figure 6). On the other hand, the aqueous and ethanol *V. amygdalina* leaf extracts (2.5 g/mL – 10.0 g/mL) extended the shelf life of the tomatoes to 8 – 23 days and 9 – 24 days, respectively (Figure 7).

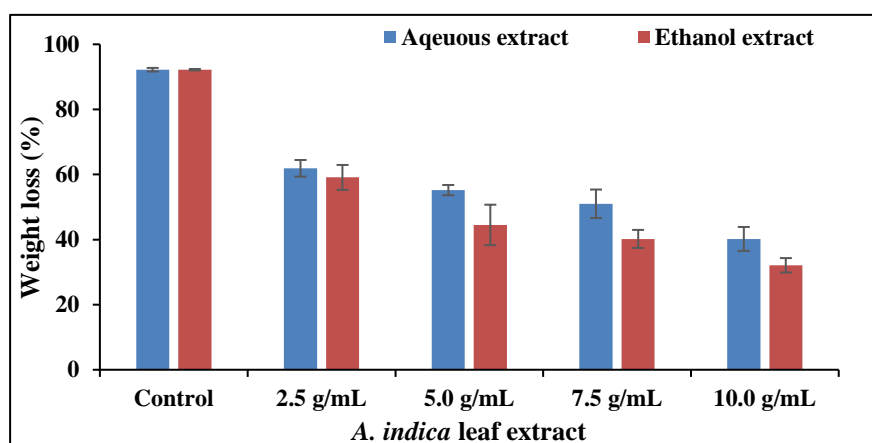


Figure 2: Effect of *A. indica* extracts on weight loss of tomato fruits.

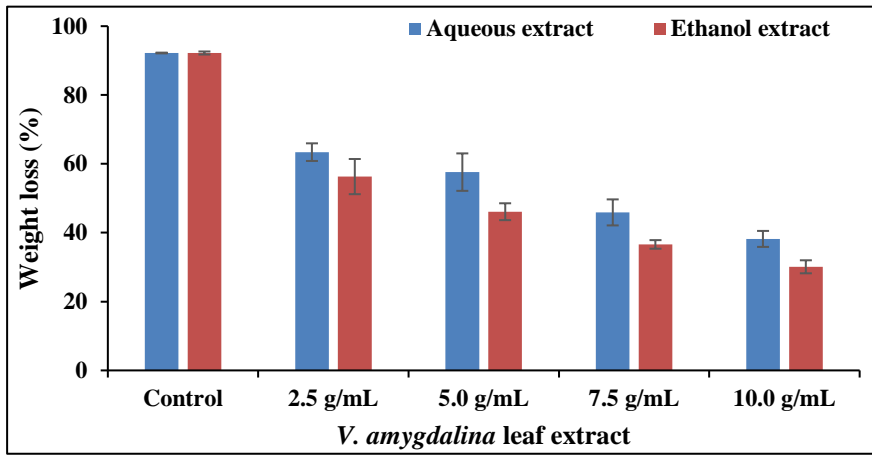


Figure 3: Effect of *V. amygdalina* extracts on weight loss of tomato fruits.

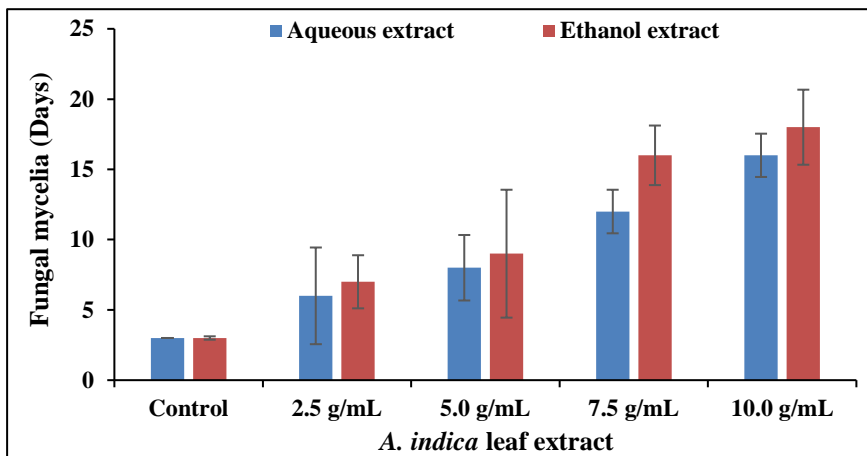


Figure 4: Effect of *A. indica* extracts on appearance of fungal mycelia on surface of tomato fruits.

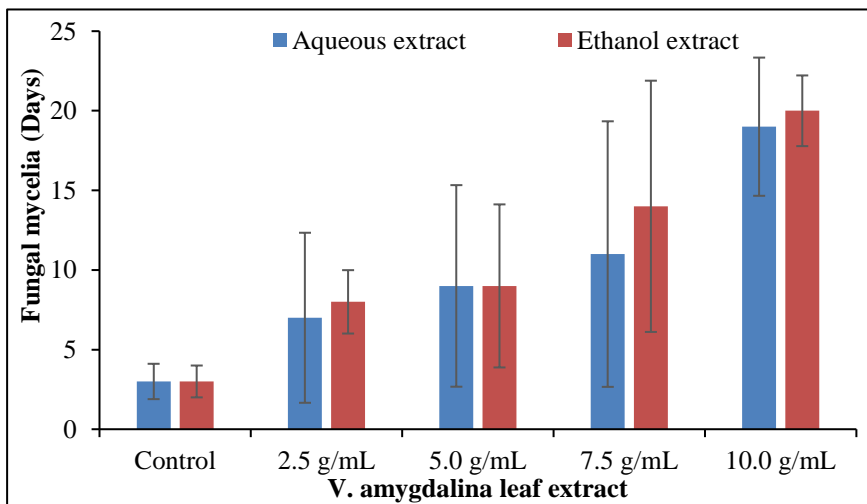


Figure 5: Effect of *V. amygdalina* leaf extracts on appearance of fungal mycelia on surface of tomato fruits.

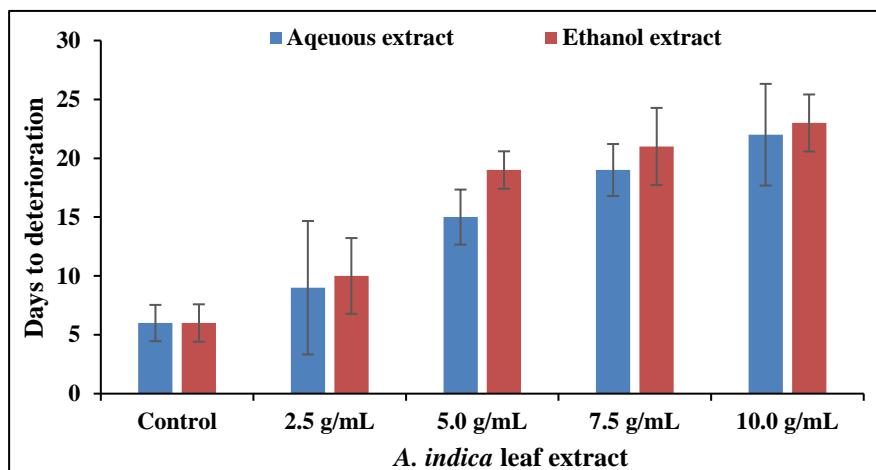


Figure 6: Effect of *A. indica* leaf extracts on shelf life of tomato fruits.

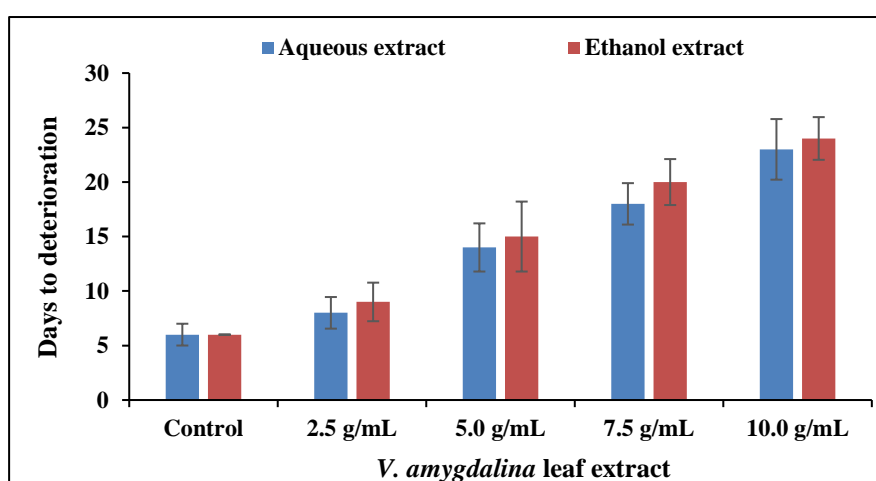


Figure 7: Effect of *V. amygdalina* leaf extracts on shelf life of tomato fruits.

Several fungal genera were characterised and isolated from the spoilt tomato samples. The prominent isolates were *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Alternaria alternata*. The colonial morphology and microscopic characteristics of fungal isolates are shown in Table 2.

Table 2: Characterization and identification of fungal isolates from tomato fruits

S/N	Description of fungal isolates	Fungi isolated
1	The colony has black filaments at its centre which was surrounded by whitish, hairy edge and cream coloured base	<i>Aspergillus niger</i>
2	White thick mycelium and white colour at bottom of plate. Slender and simple conidiophores bearing a whorl of phialides	<i>Fusarium oxysporum</i>
3	The colony was a white, loose filamentous mould with black spores. Hyphae spread to cover the whole plate. The reverse side of the culture plate was whitish. The fungus resembles cotton wool appearance.	<i>Rhizopus stolonifer</i>
4	Flat white cottony growth with brown-black reverse on plate, erect conidiophores, septate hyphae with cylindrical conidia	<i>Alternaria alternata</i>

In vitro inhibitory effect of extracts on fungal isolates

Analysis of the *in vitro* inhibitory effects *A. indica* and *V. amygdalina* leaf extracts showed that all the fungal isolates were susceptible to them, though in a concentration associated pattern (Figures 8 and 9). For *A. indica*, the ethanol extracts generally produced the better mycelial growth inhibitory effect with the most susceptible fungal isolate being *R. stolonifer* (78.43 %), followed by *F. oxysporum* (77.45 %), *A. niger* (62.71 %) and *A. alternata* (45.51 %) least being at 10.0 g/mL concentration. A similar pattern was observed for the aqueous leaf extract (Figure 8). For the leaf extracts of *V. amygdalina*, *R. stolonifera* (84.56 %) was most sensitive to the ethanol fraction, followed by *F. oxysporum* (77.34 %), *A. alternata* (72.45 %) and *A. niger* (71.45 %) being the least. A similar pattern was observed with the aqueous leaf extracts (Figure 9). Although, the effects *V. amygdalina* extracts appeared to be more active against the spoilage fungi, statistical analysis revealed that the mycelia inhibition observed for both extracts were not significantly different ($p>0.05$) from each other. The least effect was observed with the 2.5 g/mL extracts.

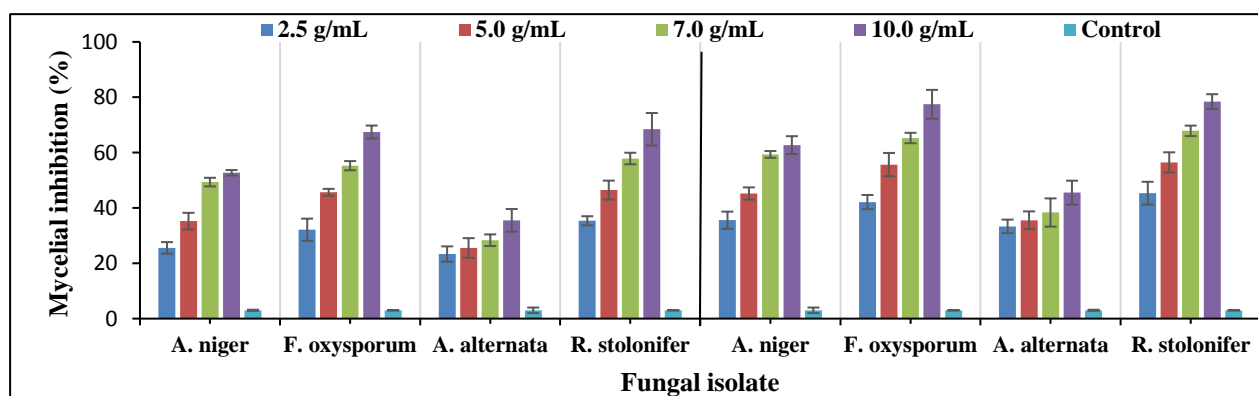


Figure 8: *In vitro* effect of *A. indica* leaf extracts on fungal isolates from tomato fruits

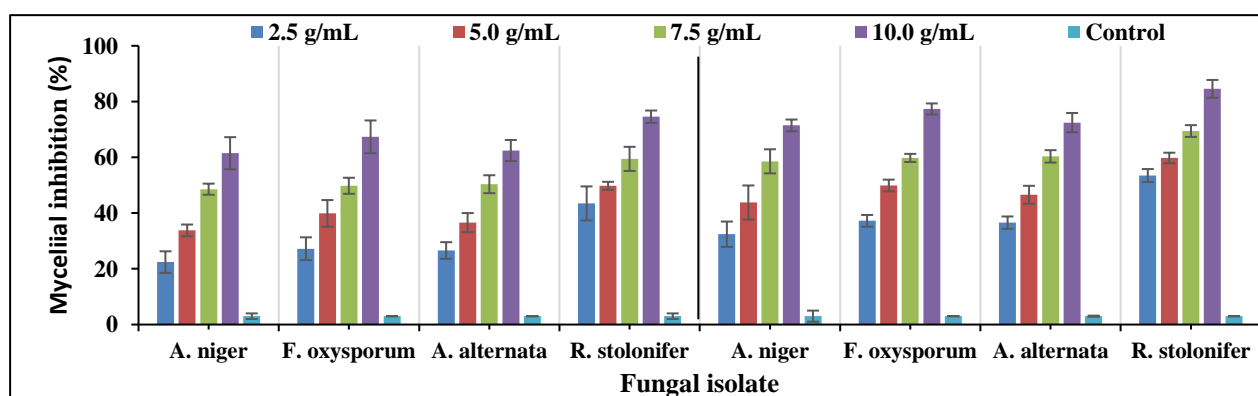


Figure 9: *In vitro* effect of *V. amygdalina* leaf extracts on fungal isolates of tomato fruits

Discussion

Plant has been an age long source of food and medicine for humans. Medicinal properties of plants are linked to the bioactive molecules they possess (Saleem *et al.*, 2018). In this study, two medicinal plants; *A. indica* (neem) and *V. amygdalina* (bitter leaf), were investigated as bio-preservatives for post-harvest tomato fruits. The presence of alkaloids, glycosides, saponins, flavonoids and tannins in both leaf extracts is in agreement with previous studies (Offor, 2014; Raimi *et al.*, 2020), who reported similar bioactive molecules.

The effects of leaf extracts of *A. indica* and *V. amygdalina* revealed notable findings. The aqueous and ethanol leaf extracts of both plants displayed a concentration dependent reduction in weight loss of the treated tomato fruits when compared with the untreated controls. This finding suggests that leaf extracts of *A. indica* and *V. amygdalina* could prevent high respiration rates which are responsible for fungal decay and weigh losses of the fruits. Fungi have been found to possess hydrolytic enzymes which enhance the breakdown of cell walls of fruits and vegetables (Khaleel, 2017). In the same study plant extracts possessed the potentials to modify the storage conditions, such as increase in relative humidity, decrease in respiration rates and fungal spoilages.

The extracts were also able to delay the appearance of fungal mycelia on the surfaces of the tomato fruits for over 20 days when compared with the control. This finding is in concordance with earlier works which reported that most medicinal plants possess antifungal properties and could be used in preserving fruits and vegetables from microbial and pest attack (Verghses, 2000; Hosea *et al.*, 2017; Li *et al.*, 2021). The ethanol extract of *V. amygdalina* leaf at the highest concentration, gave the most extended shelf-life observed in this study. This may be attributed to the higher contents of the bioactive molecules extracted by the ethanol. Ethanol and other organic solvents have been reported in the past as effective extracting solvents for medicinal plants (Ogu *et al.*, 2011; Ogu *et al.*, 2012). This indicates that extracts of bitter leaf will be a better bio-preservative of tomato fruits. The ethanol effects of *A. indica* also produced a comparable reduction in weight loss, delayed fungal appearance and substantially extended the shelf life of the of the studied tomato fruits. The effects of *A. indica* leaf extract in this study were in agreement with the submission of Hosea *et al.* (2017) and Ejale and Abdullah (2004), who reported that powder leaves of neem significantly extended the shelf life and market values of ripe tomato fruits. The capacity of leaf extracts of bitter leaf and neem to minimize the spoilage of tomato fruits further laid credence to their efficient bio-preservative potentials. This makes both plant good candidates for bio-protection of fruits and vegetables in many developing countries where postharvest deterioration of fruits and vegetables continue to pose a very major challenge (Ahmad *et al.*, 2020).

The fungi isolated from the spoilt tomato fruits have been reported as the major spoilage fungi of postharvest tomato fruits. This is in consonance with the work of Kutawa *et al.* (2020), who reported the presence of *Aspergillus niger*, *Rhizopus stolonifer* and *Fusarium oxysporum* in spoilt tomato fruits sold within Dutsin-Ma Metropolis, Katsina State, Nigeria. Also, similar genera of fungi were reported from Lagos (Sanyaolu, 2016) and Nsukka (Amujiet *al.*, 2013). However, our findings disagreed with the work of Onuorah and Orji (2015), who in addition to isolates of this current research, reported the presence of *Saccharomyces cerevisiae*, *Penicillium digitatum* and *Geotrichum candidum* from post-harvest tomato fruits sold in major markets within Awka, Nigeria. In the same vein, Dimphna (2016) reported the isolation of *Penicillium* sp. and *Cladosporium* sp. from post-harvest decaying tomatoes sold within Abakaliki market, Nigeria. *Penicillium* sp. and *Mucor* sp. were reported from different tomato markets in Abuja (Mailafia *et al.*, 2017; Mukhtar *et al.*, 2019). Moreover, contrary isolates; *Rhodotorula* sp., *Mucor* sp. and *Saccharomyces cerevisiae* were reported from Wukari, Nigeria (Ogodo *et al.*, 2020). The common handling practices, transportations, prevailing storage environment by the producers and marketers could have accounted for the variations with the fungal isolates from commercial tomatoes (Okojie and Isah, 2014). Generally, Greco *et al.* (2014) noted that fungi are ubiquitous in soil, water, air, and processing materials/containers and their spores can easily contaminate exposed products, such as fruits and vegetables. These might be the major sources of fungal contaminations of poorly handled tomatoes.

The fungi isolated from this study were significantly inhibited by the aqueous and ethanol extracts of both plants, as indicated by the high percentage of *in vitro* mycelial growth inhibition obtained. The bio-activities of the plant extracts could be attributed to their potent inherent phytochemicals; tannins, flavonoids, alkaloids, and saponins. The slight variations in the antifungal effects could be traced to the differences in the solvents used for the extracts process. This in line with an earlier report that extracting solvents affect the quantities and efficacies of bioactive molecules (Iloki-Assanga *et al.*, 2015). The observed results suggest that the studied plants possess the bioactive molecules needed to prevent the *in vivo* and *in vitro* activities of tomatoes spoilage fungi. Hence, they could be considered as potential bio-preservatives for tomatoes and other perishable fruits. The concentration depended effects of the extracts indicate that relatively higher concentrations should be considered when exploiting their potentials.

Conclusion

The aqueous and ethanol leaf extracts of *A. Indica* (neem) and *V. amygdalina* (bitter leaf) were investigated for their phytochemicals and abilities to extend the shelf life of fresh tomatoes. This study showed that graded aqueous and ethanol leaf extracts (2.5 g/mL – 10 g/mL) of *A. indica* (neem) and *V. amygdalina* (bitter leaf)

displayed concentration depend effected on shelf-life of the tomato samples. Both plant extracts at concentrations of 10 g/mL significantly lowered the weight loss, mycelial growth appearance, and ultimately extended the shelf life of tomato for over 20 days. The effects of both plant extracts were linked to the presence of tannins, flavonoids, alkaloids, and saponins, which were present in substantial amounts in the ethanol and aqueous leaf extracts. The plant extracts equally displayed remarkable in vitro antifungal activities against *A. niger*, *R. stolonifera*, *F. oxysporum* and *A. alternata*, which were the four prominent spoilage fungi recovered from the spoilt tomato fruits. Findings from this study, therefore, suggest that both medicinal plants at 10.0 g/mL could be considered as bio-preservatives to extend the shelf life and reduce economic losses of post-harvest tomatoes. Further studies on the effects of both plant extracts on the nutritive values of tomatoes can be recommended.

Authors' contribution statement

This work was carried out in collaboration among all authors. 'G. I. Ogu and G. U. Jonah' designed the study, wrote the protocol, and wrote the first draft of the manuscript. 'J. C. Okolo and G. U. Jonah' performed the statistical analysis and managed the analyses of the study. 'G. I. Ogu, J. C. Igborgbor and E. M. Eze' managed the literature searches. All authors read and approved the final manuscript.

Conflict of interest statement

Authors have declared that no competing interests exist.

References

- Ahmad, M., Abubakar, M. M., Sale, S., 2020. Enhancing the shelf life of tomato fruits using plant material during storage. *Journal of Horticulture and Postharvest Research*, 3(2): 347 – 354. <https://doi.org/10.22077/jhpr.2020.2946-1109>.
- Adetunji, M., 2018. Travelling preferences of the elderly and their perception of transport services in Lokoja, Kogi State, Nigeria. *Economic and Environmental Studies*, 18(1): 9 – 29. <https://doi.org/10.25167/ees.2018.45.1>.
- Adedapo, A. A., Aremu, O. J., Oyagbemi, A. A., 2014. Anti-oxidant, anti-inflammatory and antinociceptive properties of the acetone leaf extract of *Vernonia amygdalina* in some laboratory animals. *Advances in Pharmaceutical Bulletin*, 4(Suppl 2): 591 – 598. <https://doi.org/10.5681/apb.2014.087>.
- Alab, M. O., 2009. Urban sprawl, pattern and measurement in Lokoja, Nigeria. *Theoretical and Empirical Researches in Urban Management*, 4(13): 158 – 164.

- Ali, M. D., Sina, A. A. I., Khandker, S. S., Neesa, L., Tanvir, E. M., Kabir, A., Khalil, M. I., Gan, S. H., 2020. Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A Review. *Foods* 10 (1): 45. <https://doi.org/10.3390/food10010045>.
- Amuji, C. F., Uguru, M. I., Ogbonna, P. E., Ugwuoke, K. I., Eze, I. E., Mbadianya, J. I., 2013. Isolation and identification of fungal pathogens associated with tomato genotypes lines in Nsukka South-Eastern Nigeria. *International Journal of Scientific Research*, 2(6): 5 – 8. <https://doi.org/10.15373/22778179/JUNE2013/2>.
- Aslam, T., Maqsood, M., Jamshaid, I., Ashraf, K., Zaidi, F., Khalid, S. Hassan, S. F.U., Saba, S., Maria, N., 2020. Health benefits and therapeutic importance of green leafy vegetables (GLVs). *European Academic Research*, 8(7): 4213 – 4229.
- Audu, E. B., 2012. Descriptive analysis of rainfall for agricultural planning in Lokoja Local Government Area of Kogi State, Nigeria. *International Journal of Science and Technology*, 2(12): 850 – 855.
- Danladi, S., Hassan, M. A. Masa'ud, I. A., Ibrahim, U. I., 2018. *Vernonia amygdalina* Del: A mini review. *Research Journal of Pharmacy and Technology*, 11(9): 4187 – 4190. <https://doi.org/10.5958/0974-360X.2018.00768.0>.
- De Hoog, G. S., Guarre, J., Gene, J. F., 2000. *Atlas of Clinical Fungal*, 2nd Edition, the Netherland Publishers, p. 450 – 453.
- Dimphna, N. E., 2016. Isolation and identification of fungi associated with postharvest decay of *Lycopersicon esculentum* Mill. sold in Abakaliki, Nigeria. *IOSR Journal of Agriculture and Veterinary Sciences*, 9(7): 87 – 89. <https://doi.org/10.9790/2380-0907018789>.
- Ejale, A., Abdullah, H., 2004. Preservation of ripe tomato (*Lycopersicon esculentum* Mill.) fruits with dried leaf powder of Neem (*Azadirachta indica* A. Juss). *Nigerian Journal of Applied Science* 22: 344 – 350.
- Fawole, M. O., Oso, B. A., 1988. *Laboratory Manual of Microbiology*. Spectrum books limited, p. 127.
- Freeman, B. B., Reimers, K., 2010. Tomato consumption and health: emerging benefits. *American Journal of Lifestyle Medicine*, 5: 24 – 30. <https://doi.org/10.1177/1559827610387488>.
- Greco, M. V., Franchi, M. L., Golba, S. L. R., Pardo, A. G., Pose, G. N., 2014. Mycotoxins and mycotoxigenic fungi in poultry feed for food-producing animals. *The Science World Journal*, 14: 1 – 9. <https://doi.org/10.1155/2014/968215>.
- Gupta, S. C., Prasad, S., Tyagi, A. K., Kunnumakkara, A. B., Aggarwal, B. B., 2017. Neem (*Azadirachta indica*): An Indian traditional panacea with modern molecular basis. *Phytomedicine*, 34: 14 – 20. <https://doi.org/10.1016/j.phymed.2017.07.001>.
- Harborne, J. B., 1984. *Phytochemical methods*, 2nd Edition, Chapman and Hall, London-New York, p. 279.

- Hosea, Z. Y., Liamngee, K. A. L. Owoicho, A. L. and Agatsa, T. D. 2017. Effect of neem leaf powder on post-harvest shelf life and quality of tomato fruits in storage. *International Journal of Development and Sustainability*, 6(10): 1334 – 1349.
- Hou, X., Zhang, W., Du, T., Kang, S., Davies, W. J., 2020. Responses of water accumulation and solute metabolism in tomato fruit to water scarcity and implications for main fruit quality variables. *Journal of Experimental Botany*, 71: 1249 – 1264. <https://doi.org/10.1093/jxb/erz526>.
- Iloki-Assanga, S. B., Lewis-Luján, L. M., Lara-Espinoza, C. L., Gil-Salido, A. A., Fernandez-Angulo, D., Rubio-Pino, J. L., Haines, D. D., 2015. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucidabuceras* L. and *Phoradendron californicum*. *BMC Research Notes*, 8: 396. <https://doi.org/10.1186/s13104-015-1388-1>.
- Jay, J. M., 1998. *Food Spoilage in Modern Food Microbiology*, 4th Edition, Chapman and Hall Inc. New York, p. 195.
- Khaleel, R., 2017. Efficacy of some plant extracts and biodegradables wrapping materials as an alternative to fungicides in increasing shelf life Citrus Fruits. *Open Journal of Ecology*, 7: 621 – 630. <https://doi.org/10.4236/oje.2017.712042>.
- Kumar, D., Kalita, P., 2017. Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. *Foods*, 6(1): 8. <https://doi.org/10.3390/foods6010008>.
- Kutawa, A. B., Ahmadu, T., Jibrin S. M., 2020. Assessment of fungal species associated with tomato spoilage sold in Dutsin-Ma metropolis, Katsina State, Nigeria. *International Journal of Agriculture, Forestry and Plantation*, 10: 262 – 266.
- Li, L., Yan, X., Li, J., 2021. The prolonging effect of natural plant extracts on the storage period of postharvest litchi. *Horticultural Science and Technology*, 39(2): 254 – 262. <https://doi.org/10.7235/HORT.20210023>.
- Mailafia, S., Okoh, G. R., Olabode, H. O. K., Osanupin, R., 2017. Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria. *Veterinary World*, 10(4): 393 – 397. <https://doi.org/10.14202/vetworld.2017.393-397>.
- Mukhtar, M. F., Wusa, N. R., Danladi, U. I., Salisu, N., 2019. Isolation and characterization of fungal species from spoilt fruits in Utako Market, Abuja, Nigeria. *Journal of Applied Sciences*, 19: 15 – 19. <https://doi.org/10.3923/jas.2019.15.19>.
- Ogodo A. C., Agwaranze D. I., Kalu A. C., Sabo, I. A., Aso, R. E., Okachi, M. A., 2020. Mycological Assessment of deteriorated *Lycopersicum esculentum* fruits sold in Wukari, Nigeria. *Journal of Biotechnology Research*, 6(7): 84 – 89. <https://doi.org/10.32861/jpr.67.84.89>.

- Ogu, G. I., Madagwu, E. C., Eboh, O. J., Ezeadila, J. O., 2011. Anti-fungal evaluation of *Diodia scandens* SW leaf extracts against some dermatophytes in Ukwuani Region of Delta State, Nigeria. *International Research Journal of Plant Science*, 10: 311 – 316.
- Ogu, G. I., Tanimowo, W. O., Nwachukwu, P. U., Igere, B. E., 2012. Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of *Cyathula prostrata* L. Blume against some human pathogens. *Journal of Intercultural Ethnopharmacology*, 1(1): 35 – 43. <https://doi.org/10.5455/jice.20120407085952>.
- Ogu G. I., Ezeadila, J., Ehiobu, J. M., 2013. Antioxidant and antimicrobials activities of *Dialium guineense* (Willd) leaf extract. *Pharmacy and Pharmacology Research*, 1(1): 1 – 7.
- Ogu, G. I., Madar, I. H., Igborgbor, J. C., Okolo, J. C. 2017. Mycological quality of fresh and frozen chicken meat retailed within Warri metropolis, Delta State, Nigeria. *Jordan Journal of Biological Sciences*, 10(4): 303 – 304.
- Okojie, P. W., Isah, E. C., 2014. Sanitary conditions of food vending sites and food handling practices of street food vendors in Benin City, Nigeria: Implication for food hygiene and safety. *Journal. Environmental Public Health*, 2014: 1–6. <https://doi.org/10.1155/2014/701316>.
- Onuorah, O., Orji, M. U., 2015. Fungi Associated with the spoilage of post-harvest tomato fruits sold in major markets in Awka, Nigeria. *Universal Journal of Microbiology Research*, 3(2): 11 – 16. <https://doi.org/10.13189/ujmr.2015.030201>.
- Paul, R., Prasad, M., Sah, N. K., 2011. Anticancer biology of *Azadirachta indica* L. (neem): A mini review. *Cancer Biological Therapy*, 12: 467 – 476. <https://doi.org/10.4161/cbt.12.6.16850>.
- Patel, S. M., Nagulapalli-Venkata, K. C., Bhattacharyya, P., Sethi, G., Bishayee, A., 2016. Potential of neem (*Azadirachta indica* L.) for prevention and treatment of oncologic diseases. *Seminars in Cancer Biology*, 40: 100–115. <https://doi.org/10.1016/j.semcancer.2016.03.002>.
- Pem, D., Jeewon, R., 2015. Fruit and vegetable intake: Benefits and progress of nutrition education interventions-Narrative Review Article. *Iranian Journal of Public Health*, 44(10): 1309 – 1321.
- Preedy, V. R., Watson, R. R., 2008. Tomatoes and tomato products nutritional, medicinal and therapeutic properties. CRC Press, p. 1 – 672. ISBN 9780367452759.
- Raimi, C. O., Oyelade, A. R., Adesola, O. R., 2020. Phytochemical screening and in-vitro antioxidant activity on *Vernonia amygdalina* (ewuro-bitter leaf). *European Journal of Agriculture and Forestry Research*, 8(2): 12 – 17.
- Offor, C. E., 2014. Comparative chemical analyses of *Vernonia amygdalina* and *Azadirachta indica* leaves. *IOSR Journal of Pharmacy and Biological Sciences*, 9(5): 73 – 77. <https://doi.org/10.9790/3008-09527377>.

- Saleem, S., Muhammad, G., Hussain, M. A., Bukhari, S. N. A., 2018. A comprehensive review of phytochemical profile, bioactive for pharmaceuticals, and pharmacological attributes of *Azadirachta indica*. *Phytotherapy Research*, 32: 1241 – 1272. <https://doi.org/10.1002ptr.6067>.
- Salehi B., Sharifi-Rad R., Sharopov, F., Namiesnik J., Roointan, A., Kamle, M., Kumar, P., Martins, N., Sharifi-Rad, J., 2019. Beneficial effects and potential risks of tomato consumption for human health: An overview. *Nutrition*, 62: 201 – 208. <https://doi.org/10.1016/j.nut.2019.01.012>.
- Sanyaolu, A. A. A., 2016. Postharvest fungal deterioration of tomato (*Lycopersicon esculentum* Mill) and pepper (*Capsicum annum* L): The “ESA” connection. *Science World Journal*, 11(3): 1 – 10.
- Tommonaro, G., De Prisco, R., Abbamondi, G. R., Marzocco, S., Saturnino, C., Poli, A., Nicolaus, B., 2012. Evaluation of antioxidant properties, total phenolic content, and biological activities of new tomato hybrids of industrial interest. *Journal of Medicine and Food*, 15(5): 483 – 489. <https://doi.org/10.1089/jmf.2011.0118>.
- Trease, G. E., Evans, W. C., 1996. Pharmacognosy. 15th Edition, London J and A, Churchill Limited, p. 234 – 492.
- Verghese, A., 2000. Potential of botanicals in the management of the pomegranate aphid, *Aphis punica* Passerini. *Insect Environment*, 5(4): 147 – 148.
- Yahaya, S. M., Mardiyya, A. Y., 2019. Review of post-harvest losses of fruits and vegetables. *Biomedical Journal of Scientific & Technical Research*, 13: 10192 – 10200. <https://doi.org/10.26717/BJSTR.2019.13.002448>.