

# An effective and eco-friendly technique for control of post-harvest fungal pathogens of orange (*Citrus sinensis*) isolated from the distribution chain of Delhi NCR

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## Abstract

Orange (*Citrus sinensis*) is an important horticultural crop of India which is prone to post-harvest damage during storage, transport, and distribution due to fungal pathogens. These phytopathogenic fungi are conventionally controlled through synthetic fungicides. However, with increasing concern for health hazards and environmental pollution due to rampant usage of synthetic fungicides has forced us to devise alternative natural and eco-friendly strategies for their effective control. Natural plant-derived extracts and compounds have evoked immense interest toward their antibacterial and antifungal activities. The present investigation envisages the isolation and characterization of predominant fungal pathogens from *C. sinensis* obtained from 56 samples collected from the distribution chain of Delhi NCR. The isolated fungi included *Aspergillus niger*, *Alternaria citri*, and *Penicillium digitatum*. *In vitro* assays were conducted to assess the fungicidal/fungistatic potential of 11 bioactive plant extracts on these isolates by determination of minimum inhibitory concentrations (MIC) and minimum fungicidal concentrations (MFC). Out of 11 bioactive extracts tested 10 had fungistatic, 4 had fungicidal, and 1 had no activity on these isolates. *n*-Hex fraction of *Nepeta longibracteata* and *Actinocarya tibetica* and MeOH extract of *Zingiber officinale* and *Glycyrrhiza glabra* was found to be most active against *A. niger* with MIC and MFC values of 125 µg/mL. MeOH extract of *G. glabra* was most active against *A. citri* and *n*-Hex fraction of *N. longibracteata* and *A. tibetica* and MeOH extract of *Z. officinale* and *G. glabra* was most active against *P. digitatum*. The results obtained from the present study indicated that these extracts demonstrate antifungal effects against post-harvest phytopathogen of *C. sinensis* and can offer effective bioactive control. However, more advanced experimental studies are required to identify and pinpoint the exact bioactive compound/molecule responsible for the antifungal activities observed and further work is required to develop appropriate formulations which can be effectively integrated and applied commercially.

## 1. INTRODUCTION

Horticultural crops, particularly fruits and vegetables, play an important role in improving the nutritional status of an individual and economy of a region or country. The varied agro-climatic conditions prevalent in India are favorable for the cultivation of a variety of such fruits and vegetables. Citrus varieties, in particular, are cultivated in 0.62 million hectares area with a total production of 4.79 million tonnes [1]. The cultivation is mainly in the states of Maharashtra, Madhya Pradesh, Tamil Nadu, Assam, Orissa, West Bengal, Rajasthan, Nagaland, Mizoram, and Arunachal Pradesh. However, since these varieties are high in both water as well as nutrient content, they are very prone to attack by microbial pathogens throughout the distribution

chain, including various stages of picking, packing, storage, and transportation [2]. These post-harvest losses cause a reduction in yield as well as the quality of these fruits. In addition, they also result in causing heavy losses to producers as well as consumers. Further, at the macro level, crores of rupees are also lost. In fact, India loses fruits worth Rs. 12,700–15,876 crores (20–25% loss at an average price of Rs.10,000/t) and vegetables worth Rs. 12,588 crores (a loss of about 20% at an average price of Rs. 5000/t) per year [3].

Since the harvested citrus fruits are acidic, most of the pathogenic infection is caused by fungi and not bacteria. This infection and subsequent decay by phytopathogenic fungi can occur at any level of the distribution chain beginning right after the harvest. The infection usually follows any type mechanical injury of the fruit, disrupting the outer protective layer which facilitates the entry of the fungi [4]. Some post-harvest decays of fruit can also be due to latent infections occurring in the farms which typically include infections caused by *Alternaria alternata* pv. *citri* and *Phytophthora citrophthora* causing black and brown rots. Green and blue molds caused by *Penicillium*

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*digitatum* and *Penicillium italicum*, respectively, and anthracnose caused by *Colletotrichum gloeosporioides*. Apart from this *Geotrichum candidum*, *Diplodia natalensis*, *Penicillium* sp., *Trichoderma viride*, *Fusarium* sp., *Alternaria alternata*, *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus ochraceus* have also been implicated for their involvements in causing infection [5]. Most of these fungi are classified as wound pathogens meaning that although these might be always present on the fruit surface, they only under mechanical damage to the outer cover or favorable environmental conditions multiply to attain damaging levels and this can occur anytime during their handling and transport in the distribution chain posing serious threat [6-8]. Further deterioration in fruit quality is induced as fruit maturation occurs. Favorable temperature and humidity further enhance the process of decay [9]. Practicing hygienic packaging and sanitization methods can only to a certain extent restrict their further growth and multiplication [7].

The present regime to currently regulate post-harvest citrus diseases involves various synthetic fungicides such as imazalil, thiabendazole, and sodium ortho-phenyl phenate, fludioxonil, pyrimethanil [10]. However, recent concerns about pesticide residues, environmental pollution, and development of pathogens resistance [11] have necessitated the need to develop an alternate strategy for the management of these post-harvest losses.

Due to the aforesaid limitations of synthetic fungicides, it becomes imperative to explore other safe and eco-friendly approaches to control citrus post-harvest diseases, which present lower risks to both human health as well as the environment [12]. In recent times various promising alternatives like the use of antagonistic organisms, induction of plant's natural resistance through signaling pathways have been proposed, but these are not of much significance for the control of post-harvest phytopathogenic fungi. A more suitable approach is to explore the possibility of application of naturally derived bioactive compounds and indeed, this strategy has gained much popularity and scientific interest [2,8,13,14] due to their recently reported antifungal activities non-phytotoxicity and biodegradability [15]. To cite an example, Askarne *et al.* have reported antifungal activities of 50 different species of plants collected from regions of southern Morocco with demonstrated high antifungal activity of *Anvillea radiata* and *Thymus leptobotrys* against *P. italicum* even at concentrations of 10% m/v [16]. Various other medicinal plants such as garlic [17], neem [18], *Withania somnifera* L., and *Acacia seyal* L. [19] are also reported inhibitors *P. digitatum*. Very recently, Zhu *et al.* have reported *in vivo* antifungal activity of tannic acid on *P. digitatum* by significantly decreasing mycelial growth and spore germination by as much as 70% [20]. Extracts of *Capsicum annuum* and *Zingiber officinale* have also been reported to control or inhibit post-harvest diseases in citrus [21].

Previous studies in our lab have demonstrated significant activity of various Indian medicinal plant extracts and fractions against standard fungal strains *Aspergillus*, *Penicillium*, and *Fusarium* [22-24]. In the present study, we are extrapolating the effect of these extracts, specifically on the post-harvest fungal pathogens of citrus fruits in our region.

Present investigation involves the isolation and identification of post-harvest fungal pathogens of citrus varieties from the distribution chain of Delhi NCR and to develop a strategy to control the growth of the phytopathogenic fungi using a variety of natural plant extracts. Further, a methodology is developed to determine the optimum concentrations for fungicide applications based upon minimum inhibitory concentrations (MIC) and minimum fungicidal

concentrations (MFC) values. All these investigations will be ultimately very helpful for the management of post-harvest diseases of citrus and minimize post-harvest losses. An additional step of covering the fruit surface with bioactive extracts can be a good step for citrus fruit preservation.

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection

A survey was conducted in the Mother Dairy Safal stores and domestic markets of Delhi NCR and orange samples which showed visible signs of fungal infection were picked from wooden box or pile of fruits. Fifty-six such samples were collected from 15 locations in paper bags as described by Rasool *et al.* [5]. These were brought to the Chemical Biology Lab of Amity Institute of Biotechnology, Amity University, Noida, India, and categorized according to the visible symptoms of post-harvest diseases.

### 2.2. Isolation of Fungi

Fungi present in the diseased fruits and vegetables were isolated on potato dextrose agar (PDA) medium. Samples with visible disease symptoms were removed by a sterilized knife in such a manner as to contain the lesion edges too. These were then immersed in HgCl<sub>2</sub> (1:10,000) for 2–3 mins for surface sterilization and washed thoroughly in sterile distilled water until all the HgCl<sub>2</sub> was washed away. The surface-sterilized tissues were further cut into smaller pieces using a sterile blade and placed on PDA medium using disinfected forceps. The plates were incubated at 28°C for 4–5 days. To obtain pure culture of the fungal isolate, repeated subculturing was performed on PDA plates with incubation at 28°C for 5 days. The fungi were sub-cultured on fresh agar plates and identified by their morphological and cultural characteristics. The purified isolates were maintained on PDA slants for further studies.

### 2.3. Identification of Phytopathogenic Fungi

Phenotypic identification was performed on standard growth conditions, as described previously. Genus level identification was carried out based upon macro as well as microscopic characteristics. These included visible colonial morphology, color, texture, shape and appearance, and microscopic characteristics such as conidia shape, hyphal color, septation, pigmentation, fruiting bodies, or any other visible structures by observing lactophenol cotton blue-stained slides under the compound microscope at the magnification of ×10, ×45, and ×100 [25]. For this purpose, a small quantity of the aerial mycelia with representative spores was placed on a drop of lactophenol cotton blue stain on a clean slide. A mounting needle was used to evenly spread the mycelia and the spores and subsequently a coverslip was gently placed with little pressure to eliminate air bubbles. The slide was then observed under a binocular compound light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified as per Onuorah *et al.* [26].

### 2.4. Preparation of Extracts

Medicinal plants *Orchis latifolia* and *Alpinia galanga* were purchased from authorized vendor and *Nepeta longibracteata*, *Actinocarya tibetica*, *Rhodiola imbricata*, *Lepidium latifolium*, *Perovskia abrotanoides*, and *Echinacea purpurea* were collected from different regions of Ladakh as described in our previous works [24]. Ginger rhizome dry (*Z. officinale*) and Mulethi or Licorice (*Glycyrrhiza*

*glabra*) were also procured locally. The extractions were carried out as per standard protocol developed in our lab and four fractions of each extract were obtained. Only those fractions which demonstrated substantial antifungal activity in our previous studies [22-24] against *Aspergillus*, *Penicillium*, and *Alternaria* were used in the present investigation for determination of antifungal activity against the fungal isolates.

### 2.5. Effects of Plant Extracts on Fungal Growth *in vitro*

The fungicidal activity of the bioactive fractions of various extracts was determined by 3-(4,5-dimethyl-2-thiazyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) based colorimetric method which measured the formation of purple formazan by oxidation by mitochondrial oxygenases of the viable fungi [27]. The tests were performed in 96-well flat-bottom microtiter plates containing 100  $\mu$ L of PDB where the final inocula in each well were in the range  $1 \times 10^4$ – $5 \times 10^4$  CFU/mL. Varying concentrations of different extracts (31.25–1000  $\mu$ g/mL) were added to the wells with appropriate positive and negative controls. After the plate was prepared and sealed, it was incubated for 2–3 days. After the appropriate incubation time, 25  $\mu$ L of 5 mg of MTT/mL was added and the plate was further incubated for 3 h. After this period, 200  $\mu$ L of isopropanol containing 5% 1 M HCl was added for dye extraction. Further incubation of 30 min was done with gentle agitation at room temperature and appearance of purple formazan crystals was observed with viable cells. The MIC of the extract was defined as the minimum concentration that completely inhibited growth in all the experiments carried out.

MFC was determined by agar dilution method using PDB and various concentrations of plant extracts in concentrations ranging from 31.25 to 1000  $\mu$ g/mL. For this assay, negative control was taken as blank PDB alone and PDB with spore suspension and without the extract served as a positive control. All samples in which no visible growth was present were further subcultured on extract free PDA plates for 7 days at 27°C. The highest dilution of the extract that could prevent the fungal growth on agar plate was considered as MFC [28].

## 3. RESULTS AND DISCUSSION

In India, citrus fruits are mostly harvested manually which can cause physical damage, injuries, and bruising as well as unavailability of optimum temperature and dumping of the wooden boxes irregularly in the market. Packing in the wooden boxes forcibly itself adds to the issues of bruising from where the pathogen of post-harvest rots easily enter and spoil a whole lot of fruits. The transports of citrus in vans and trucks without a controlled environment have also contributed toward the diseases. The transport time was another factor affecting the incidence of diseases.

The results from this study have shown that many fungi can cause citrus fruit spoilage during post-harvest period. Symptoms were observed on

the diseased citrus fruits and greenish/brownish-black and necrotic patches were present on the skin of the citrus fruit. A mass of mycelia growing on the surface of the fruits was also observed, suggesting the decay or spoilage may be caused by these fungal organisms.

Upon isolation of pure culture, the isolates were characterized and based upon morphological and microscopic examinations were identified as *A. niger*, *P. digitatum*, and *Alternaria citri*. The characteristic features of the isolates are described in Table 1.

Table 2 shows the frequency of various fungi in our tested samples. It was seen that *A. niger* and *A. citri* (41.1 and 37.5%, respectively) had higher prevalence as compared to *P. digitatum* which had only 21.4% prevalence.

There are previous reports of isolation of pathogenic fungi of genera *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, and *Rhizopus* from many varieties of non-specific ripe fruits. However, *P. digitatum* and *A. citri* were found only in *Citrus* species causing rotting and our results are in confirmation of the same [11,21]. Our results are in agreement with previous studies where *A. niger*, *A. citri*, and *P. digitatum* have been isolated from infected oranges and other citrus fruits and are responsible for brown/black rot [17,21]. The dominance of *Aspergillus* and *Alternaria* species along with *Penicillium* in citrus fruits of the distribution chain confirms their prevalence in fruits exposed to tropical humid climate of India. These three genera are potential health risks to consumers of this fruit and it is by products. However, *P. digitatum* are common fungi in the environment that demonstrate no pathogenicity toward human beings [28].

During various previous studies conducted and published from our lab, we have reported antifungal activities of various plant extracts and fractions. Ethyl acetate fraction of *A. galanga* and *O. latifolia* had demonstrated a significant zone of inhibition MTCC 872 *A. niger* [22,23]. We have also reported strong antifungal activities of *n*-Hex and DCM fraction of *N. longibracteata*, *n*-Hex fraction of *A. tibetica*, and *E. purpurea*, DCM fraction of *E. purpurea* and *L. latifolium*, and aqueous fraction of *E. purpurea* [24]. In addition, we also tested methanolic extracts of *Z. officinale* and *G. glabra* both of which reportedly have strong antimicrobial activities [29]. The MIC values of these 11 extracts/fractions against fungal isolates *A. niger*, *A. citri*, and *P. digitatum* are provided in Table 3.

Here, it is important to state that an extract or fraction is considered antimicrobial as per the criteria proposed by Morales *et al.* [30], with MIC values of <100  $\mu$ g/mL being considered as strong/good activity and MIC values ranging from 100 to 500  $\mu$ g/mL as moderate activity. MIC values higher than 500  $\mu$ g/mL are considered to have very weak antimicrobial activity. Based on the above criteria moderate to good activities were exhibited by all extracts except DCM fraction of *E. purpurea* against *A. niger* and *P. digitatum*. Amongst all the tested fractions *A. citri* was found to be most resistant and had highest MIC. The results are in agreement with previous studies of Behbahani *et al.*, 2016 [28], where

**Table 1:** Characterization of isolated fungal pathogens from diseased citrus fruits.

Causative fungus	Disease	Colony characteristics	Spore characteristics	Size of spore
<i>Aspergillus niger</i>	Black rot mold	Colonies are initially white plane, but later turn black from center as conidia are produced	Conidia are globose in shape	4–5 $\mu$ m in diameter
<i>Penicillium digitatum</i>	Citrus green mold	Colonies are white, which later become olive green as conidia are produced	Conidia are usually oval in shape	6–15 $\mu$ m long
<i>Alternaria citri</i>	Citrus black rot	Colonies are initially white plane, but later turn black or gray as conidia are produced	Conidia are oval, with vertical and transverse walls	20–50 $\mu$ m long

**Table 2:** Frequency of occurrence of fungi in the various fruits.

Fungi	No. of samples	Frequency of occurrence (%)
<i>Aspergillus niger</i>	23	41.1
<i>Penicillium digitatum</i>	12	21.4
<i>Alternaria citri</i>	21	37.5

**Table 3:** MIC<sup>a</sup> values of bioactive extracts/fractions against fungal isolates.

Plant extract/fraction	MIC <sup>a</sup> against <i>A. niger</i>	MIC <sup>a</sup> against <i>A. citri</i>	MIC <sup>a</sup> against <i>P. digitatum</i>
EtOAc fraction of <i>A. galanga</i>	250	500	250
EtOAc fraction of <i>O. latifolia</i>	250	250	250
<i>n</i> -Hex fraction of <i>N. longibracteata</i>	125	250	125
DCM fraction of <i>N. longibracteata</i>	250	500	500
<i>n</i> -Hex fraction of <i>A. tibetica</i>	125	250	250
<i>n</i> -Hex fraction of <i>E. purpurea</i>	125	500	125
DCM fraction of <i>E. purpurea</i>	250	500	500
DCM fraction of <i>L. latifolium</i>	500	1000	500
Aq. fraction of <i>E. purpurea</i>	1000	ND	ND
MeOH extract of <i>Z. officinale</i>	125	250	125
MeOH extract of <i>G. glabra</i>	250	125	125

<sup>a</sup>MIC determined by microdilution method and expressed in µg/mL. ND- no antifungal activity detected. *A. galangal*: *Alpinia galangal*, *O. latifolia*: *Orchis latifolia*, *N. longibracteata*: *Nepeta longibracteata*, *A. tibetica*: *Actinocarya tibetica*, *E. purpurea*: *Echinacea purpurea*, *L. latifolium*: *Lepidium latifolium*, *Z. officinale*: *Zingiber officinale*, *G. glabra*: *Glycyrrhiza glabra*, *A. niger*: *Aspergillus niger*, *A. citri*: *Alternaria citri*, *P. digitatum*: *Penicillium digitatum*

the efficacy of plant extracts has been demonstrated against *A. citri* and *P. digitatum*. All these extracts/fractions have reported the presence of secondary metabolites by qualitative phytochemical analysis in our previous studies. Biological activities have been associated with all classes of secondary metabolites, including flavonoids, saponins, cardiac glycosides, tannins, triterpenes, and alkaloids. The observed wide range of antifungal properties for the methanolic extracts and fractions can be described by the presence of bioactive secondary metabolites. The EtOAc fraction of *A. galanga* was reported to contain triterpenoids and cardiac glycosides in significant amounts. The total phenolic content of this fraction was also high (110 mg GAE/g of the extract [22]). Similarly, EtOAc fraction of *O. latifolia* had the presence of flavonoids, steroids, and tannins [23]. Similarly, high-altitude plants have reported a high concentration of phenolics and flavonoids as exhibited by high total phenolics and flavonoids content [24].

The application of these bioactive extracts can be used for protection against fungal infection in the distribution chain. However, more mechanistic insights are required to develop a protocol for protection. Hence, to further study whether the mode of action of these extracts/fraction is fungistatic or fungicidal, MFC values were determined and are presented in Table 4.

**Table 4:** MFC<sup>b</sup> values of bioactive extracts/fractions against fungal isolates.

Plant extract/fraction	MFC against <i>A. niger</i>	MFC against <i>A. citri</i>	MFC against <i>P. digitatum</i>
EtOAc fraction of <i>A. galanga</i>	500	1000	500
EtOAc fraction of <i>O. latifolia</i>	250	500	250
<i>n</i> -Hex fraction of <i>N. longibracteata</i>	250	250	250
DCM fraction of <i>N. longibracteata</i>	500	500	500
<i>n</i> -Hex fraction of <i>A. tibetica</i>	250	250	250
<i>n</i> -Hex fraction of <i>E. purpurea</i>	250	500	250
DCM fraction of <i>E. purpurea</i>	500	500	500
DCM fraction of <i>L. latifolium</i>	500	ND	ND
Aq. fraction of <i>E. purpurea</i>	ND	ND	ND
MeOH extract of <i>Z. officinale</i>	125	250	125
MeOH extract of <i>G. glabra</i>	125	250	125

<sup>b</sup>MFC expressed in µg/mL. ND: No activity detected in this concentration range. *A. galangal*: *Alpinia galangal*, *O. latifolia*: *Orchis latifolia*, *N. longibracteata*: *Nepeta longibracteata*, *A. tibetica*: *Actinocarya tibetica*, *E. purpurea*: *Echinacea purpurea*, *L. latifolium*: *Lepidium latifolium*, *Z. officinale*: *Zingiber officinale*, *G. glabra*: *Glycyrrhiza glabra*, *A. niger*: *Aspergillus niger*, *A. citri*: *Alternaria citri*, *P. digitatum*: *Penicillium digitatum*

It is clear from the above data that all fractions showed fungistatic activity against *A. niger* and *P. digitatum*. However, fungicidal activities were demonstrated only by six fractions against *A. niger* and *P. digitatum*. These included EtOAc fraction of *O. latifolia*, *n*-Hex fraction of *N. longibracteata*, *A. tibetica*, and *E. purpurea*, and MeOH extract of *Z. officinale* and *G. glabra*. Against *A. citri* only four fractions demonstrated fungicidal activity (*n*-Hex fraction of *N. longibracteata* and *A. tibetica* and MeOH extract of *Z. officinale* and *G. glabra*). Combining both the results *n*-Hex fraction of *N. longibracteata* and *A. tibetica* and MeOH extract of *Z. officinale* and *G. glabra* are the most bioactive and promising fractions that need further exploration for their application in control of phytopathogenic fungi. It was also evident from our study that non-polar extracts exhibited better fungicidal activities. The *n*-Hex and DCM fractions had lower MIC and MFC values as compared to aqueous fractions. Similar results have been obtained in a previous study by Pinelo *et al.* [31], who advocated that the chemical nature of the solvent used for extraction along with the methodology adopted impacts the chemical nature of bioactive compounds that can be isolated. The reason attributed for this is the solubility of the extractives in various solvents as per the polarity which accounts for variations in the degree of bioactivities. The nature of the compounds in extracts responsible for antifungal activity must be non-polar and hence better extracted with non-polar solvents. It is indeed corroborated in our previous studies where GCMS analysis of *n*-Hex fraction of *N. longibracteata* has shown the presence of a large percentage of long-chain fatty acids and their methyl esters [24]. Lower activities of the aqueous extract could be reasoned with the

observation that during maceration and grinding of plant material during extract preparation, phenolases and hydrolases are released which may modulate the activity of the bioactive present in the extract. Another reason for the reduced activity may be the incomplete extraction of these bioactives. The results of our study demonstrate that the MFC of the extracts evaluated was similar or higher concentrations than in the MIC assays, but not at lower concentrations [Table 4]. It is also imperative to state at this stage that the antifungal activity of bioactive fractions or extracts is not due to the action of a single active compound but the synergistic effect of several compounds that might be available in the extract.

Since the application of plant extracts and fractions in the prevention of post-harvest losses in the distribution chain is still in the early stages, the mechanism of mode of action of these extracts is not very well characterized. Nevertheless, some initial and recent studies have attributed the antifungal action to a combination of mechanisms. These may include signaling mechanisms, including salicylate and jasmonic acid pathways and induction of host resistance responses [32]. Essential oils can also play an effective role by creating a barrier and also through synergistic effects of their components. This synergism has been known to delay the development of resistance in the phytopathogenic fungi [2]. In addition, components of essential oils seem to have no specific cellular targets they can be used for broad-spectrum control of fungal pathogens. The volatility, ephemeral nature, and biodegradability of essential oil compounds also add to the advantages for disease management. This has been demonstrated by the methanolic extracts of *W. somnifera* and *A. seyal*. A high total phenolic content has been shown to disrupt the membrane functionality and this may be another reason for our observed antifungal activities as all our fractions have high TPC and TFC values [22-24].

However, these studies are still in infancy stages and much more investigations on the mode of action of plant extracts are required before these can be integrated into citrus crop management [4]. To make this technology commercially viable additional studies are required to formulate a product which is effective during *in vivo* treatments, is stable over a longer duration of time to cover the entire distribution chain, and most importantly does not alter the quality parameters of citrus fruits such as flavor and aroma.

#### 4. CONCLUSION

The results of the present investigations demonstrate the effectiveness of plant extracts and fractions against phytopathogenic fungi of *Citrus sinensis* isolated from the distribution chain of Delhi NCR. In particular, *n*-Hex fraction of *N. longibracteata*, *A. tibetica*, and *E. purpurea* and MeOH extract of *Z. officinale* and *G. glabra* was most active against the fungal isolates *A. niger*, *P. digitatum*, and *A. citri* with significant MIC and MFC values. Additional studies are required to identify the chemical identities of the bioactive responsible for the observed antifungal activity which may offer new alternatives to post-harvest fungal pest management of citrus fruits with minimum health hazards. The major challenge will be the commercially viable production of these alternate and eco-friendly methods.

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#### 6. CONFLICT OF INTEREST

Authors declared that they do not have any conflicts of interest.

#### 7. FINANCIAL SUPPORT AND SPONSORSHIP

None.

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