International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org || Volume 4 Issue 5|| May 2015 || PP 42-45

Antifungal activities of *Ocimum gratissimum* and *Gongronema latifolium* leaves on *Colletotrichum* species isolated from spoilt tomatoes

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ABSTRACT: *The aqueous, ethanolic and methanolic leaf extracts of Ocimum gratissimum and Gongronema latifolium was evaluated for antifungal activities on Colletotrichum species isolated from spoilt tomatoes using the agar well diffusion method. The extracts showed moderate inhibitory activities on the test organism. The preliminary phytochemical analysis of the plant extracts showed that O. gratissimum has alkaloids, saponins, tannins, and glycosides while G. latifolium has alkaloids, flavonoids, saponins, tannins as well as glycosides. The minimum inhibitory concentration (MIC) of hot water, ethanol and methanol leaf extracts ranged from 0.4 – 0.8 mg/ml while cold water leaf extracts showed no MIC. The MIC of ethanolic and methanolic leaf extracts mixture ranged from 0.2 mg/ml – 0.8 mg/ml while the inhibition zone diameter (IZD) obtained ranged from 10 – 15 mm. The result of this study though presumptive confirms the traditional use of O. gratissimum and G. latifolium in the treatment of human infections caused by some pathogenic bacteria in this region. And it also indicated that extracts from these leaves could also serve as a rich source of fungicides of plant origin especially for the effective control of ripe rot disease in tomatoes and other edible fruits or foods. Since the ripe rot disease of tomatoes is a common plant disease that affect tomato cultivation and/or yield in this part of the world; it is vital to undertake comprehensive molecular characterization of the active constituents of O. gratissimum and G. latifolium so that plant fungicides can be developed from it.*

KEYWORDS: *O. gratissimum, G. latifolium, herbal plants, antimicrobial activity, Nigeria*

I. INTRODUCTION

Lycopersicon esculentum (tomato) is a plant that reaches 1-3 meters in height and if supported can grow up to 6 feet or more. It has a weak, woody stem that often varies from other plants; and its origin is from Peru in South America where the plants grew as weeds in the wild until it was later eaten by man as a delicacy [1]. There are many tomato varieties grown for various purposes. Beef steak tomatoes are grown and used for sandwiches; globe tomatoes are used for a wide variety of processing; plum or paste are used in tomato sauce and paste; cherry tomatoes are eaten whole in salads; and compare tomatoes are used in making juice because of its low acidity [2]. Being a member of the *Solanaceae* family, tomato is frequently attacked by a number of disease causing agents that causes disease in them. The early and late blight disease, septoria leaf spot and anthracnose ripe rot are typical examples of tomato diseases; and these contribute to the low yield or productivity of the plant [3]. Anthracnose ripe rot, a typical tomato disease is caused by *Colletotrichum* species, a fungus that has been reported to also cause infection in humans [3]. This tomato disease is not new and it has spread across Africa, Europe and Asia – causing shortage in the supply of quality tomatoes. The fungus may invade the roots of the plant and can cause rot of ripe fruits which may cause huge losses in the field and even during the storage of tomatoes. The use of synthetic chemical fungicides has been shown to provide some control of tomato infection by microbes or plant pathogens including the *Colletotrichum* species; and some plants like *O. gratissimum* exhibit appreciable level of antimicrobial activity against some plant pathogens [4]. All these chemical compounds are usually not readily available or very costly for local farmers to procure. More so, the use and abuse of these chemical compounds disturb the ecological balance between the microflora and fauna. Several deaths due to acute cases of chemical poisoning resulting from the use of synthetic chemical substances in the control of plant diseases have been previously reported [1]. This reveals a need for alternative and sustainable measures to control plant diseases. The roles of plant extracts with putative antifungal activities as potential sources of novel fungicides for use against plant pathogens is recently gaining prominence due to the known hazards posed by some available chemical agents currently used for this purpose [5,6]. And it is in view of these that this present study evaluated the antifungal activities of *Ocimum gratissimum* and *Gongronema latifolium* leave extracts on *Colletotrichum* species isolated from spoilt tomatoes in Abakaliki metropolis.

II. MATERIALS AND METHODS

Sample collection and identification: The samples for this study were rip and unripe fruits of *L. esculentum* with black spots; and they were bought from Abakpa main market in Abakaliki, Ebonyi state, Nigeria. The samples were conveyed in clean and disinfected sample collection containers to the Microbiology Laboratory of Ebonyi State University, Abakaliki. The samples and the leaves of *O. gratissimum* and *G. latifolium* were confirmed and identified by Professor S.C. Onyekwelu of the Applied Biology Department of Ebonyi State University, Abakaliki; and samples of the ripe and unripe fruits of Lycopersicon were kept in the herbarium of the department where the identification was conducted.

Isolation and identification of the pathogen *Colletotrichum* species: The infected tomato fruits were rinsed in distilled water and surface-sterilized with 70 % alcohol; and then cut into smaller pieces. The smaller particles of the cut tomato was inoculated on potato dextrose agar PDA plates (Oxoid, UK) supplemented with 0.2 % streptomycin; and incubated at 25-28^{o}C for 6 days. Subcultures of fungal growth on the PDA plates were made onto freshly prepared PDA plates and these were incubated at 25-28^{o}C for 6 days to obtain pure cultures of the fungal organism(s). Identification of the fungus was done based on morphological features of the organism on the culture plate and microscopical examination using lactophenol cotton blue stain as was previously reported [7].

Determination of the antifungal activity of *O. gratissimum* and *G. latifolium*: The antifungal activity of *O. gratissimum* and *G. latifolium* were evaluated on the fungal organism using cold water, hot water, ethanol and methanol extracts. Extraction of the crude aqueous and crude ethanol and methanolic extracts was carried out as was previously described [8]. The crude aqueous and crude methanolic and ethanolic extracts were reconstituted into concentrations of 0.2 mg, 0.4 mg, 0.6 mg, and 0.8 mg respectively. Agar well diffusion method was used to evaluate the antibacterial activity of *O. gratissimum* and *G. latifolium* on PDA plates previously inoculated with the test organism. Inoculated plates were incubated at 25-28^{o}C for 6 days, and checked for zones of inhibition. Mancozeb (80 %), a locally produced disinfectant was used as the control fungicide for fungal isolate.

Minimum inhibitory concentration (MIC): MIC was evaluated using the agar well diffusion method. This was carried out using the reconstituted extracts in the following concentrations 0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, and 0.8 mg/ml respectively [9,10].

Phytochemical analysis: The phytochemical analysis of *O. gratissimum* and *G. latifolium* dried leaves extracts were carried out as described by to identify their active constituents. The leaves of *O. gratissimum* and *G. latifolium* were chemically tested for the presence of alkaloids, flavonoids, saponins, tannins, and glycosides [9,10].

III. RESULTS

The result of the phytochemical analysis is shown in Table 1. The preliminary phytochemical analysis showed that *O. gratissimum* contain alkaloids, saponins, tannins and glycosides while *G. latifolium* contain alkaloids, flavonoids, saponins, tannins and glycosides as active constituents. Table 2 shows the antifungal activity of *O. gratissimum* and *G. latifolium* leave extracts on the test organism. The highest inhibition zone diameter (IZD) of the extracts of *O. gratissimum* and *G. latifolium* against the test organism were between 11 mm and 15 mm for hot water extracts. Cold water extracts of *O. gratissimum* and *G. latifolium* leaves had no inhibition against the test organism. The ethanolic and methanolic extracts produced an IZD of 10 mm and 11 mm respectively (Table 2). The results of the minimum inhibitory concentration (MIC) of *O. gratissimum* and *G. latifolium* leave extracts against the fungal organism showed that the test plants had MIC in the range of 0.4 – 0.6 mg/ml (Table 3).

Table 1. Preliminary phytochemical analysis of *O. gratissimum* and *G. latifolium* leaf.

Active ingredients	***O. gratissimum***	***G. latifolium***
Alkaloids	+	+
Flavonoids	-	+
Saponins	+	+
Tannins	+	+
Glycosides	+	+

Key:
+ = Present
— = Absent

Table 2. Antifungal activity of *O. gratissimum* and *G. latifolium* leave extracts

Plant extracts	Inhibition zone diameter (IZD) in mm			
HWE (O.g)	15	15	15	15
HWE (G.l)	10	10	11	11
CWE (O.g)	NI	NI	NI	NI
CWE (G.l)	NI	NI	NI	NI
EE (O.g)	10	10	11	11
EE (G.l)	10	10	11	11
ME (O.g)	10	10	11	11
ME (G.l)	10	10	11	11

Key:
HWE (O.g) – Hot water extract (*O. gratissimum*); HWE (G.l) – Hot water extract (*G. latifolium*); CWE (O.g) – Cold water extract (*O. gratissimum*); CWE (G.l) – Cold water extract (*G. latifolium*); EE (O.g) – Ethanol extract (*O. gratissimum*); EE (G.l) – Ethanol extract (*G. latifolium*); ME (O.g) – Methanol extract (*O. gratissimum*); ME (G.l) – Methanol extract (*G. latifolium*); NI – No inhibition

Table 3. Results of minimum inhibitory concentration (MIC)

Plant extracts	Concentration (mg/ml)					
	0.1	0.2	0.4	0.5	0.8	MIC
HWE (O.g)	NI	NI	10	11	13	0.4
HWE (G.l)	NI	NI	10	10	NI	0.4
CWE (O.g)	NI	NI	NI	NI	NI	NI
CWE (G.l)	NI	NI	NI	NI	NI	NI
EE (o.g)	NI	NI	NI	10	10	0.6
EE (G.l)	NI	NI	NI	10	10	0.6
ME (O.g)	NI	NI	10	10	NI	0.4
ME (G.l)	NI	NI	NI	10	NI	0.6

Key:
HWE (O.g) – Hot water extract (*O. gratissimum*); HWE (G.l) – Hot water extract (*G. latifolium*); CWE (O.g) – Cold water extract (*O. gratissimum*); CWE (G.l) – Cold water extract (*G. latifolium*); EE (O.g) – Ethanol extract (*O. gratissimum*); EE (G.l) – Ethanol extract (*G. latifolium*); ME (O.g) – Methanol extract (*O. gratissimum*); ME (G.l) – Methanol extract (*G. latifolium*); NI – No inhibition

IV. DISCUSSION

Plant lectins possess antimicrobial potential, and they serve as putative sources of natural bioactive molecules to control most pathogenic strains of microorganisms that cause both human and plant diseases [11]. The results obtained in this study indicated that hot water, ethanolic and methanolic extracts of *O. gratissimum* and *G. latifolium* inhibited the growth of the test organism while the cold water extract of *O. gratissimum* and *G. latifolium* showed no antifungal activity on the test fungus. Our findings are in contrast to that reported by Adebolu and Oladimeji [10]. However, the results concur with that of Nwinyi et al., [12] who showed that the leave extracts of *O. gratissimum* and *G. latifolium* posses antifungal activity. The aqueous, ethanol and methanol extracts of *G. latifolium* has been previously reported to exhibit antimicrobial activity of a number of pathogenic microorganisms including fungi [13,14]; and this necessitate the need to turn the search light of discovery of new antimicrobials on plants known to harbour active ingredients that have antimicrobial effects since some pathogens are resistant to some readily available drugs. Studies have shown that many plants used as spices in the preparation of some local delicacies have significant antimicrobial and general health benefits to the consumers. Most of the plants have been shown to contain aromatic oils from which they derive their flavouring properties; and the preliminary phytochemical analysis carried out on the leave extracts of *O. gratissimum* and *G. latifolium* used in this study showed that the plants contain some of these compounds (Table 1). Phytochemical studies have also shown that the antimicrobial properties of these plants depend on certain active ingredients especially the oils such as saponins, tannins and flavonoids. *G. latifolium* contains saponins; and this compound has been known to be responsible for the anti-oxidant and antimicrobial activity of *G. latifolium* leaves [9,15]. *O. gratissimum* contains oils that have been previously demonstrated to have both anti-bacterial and anti-helminthic activities and they also serve as chemo-preventive agents [16,17,18]. The ethanolic extracts of *G. latifolium* were reportedly shown to have high percentage composition of these essential aromatic oils than the aqueous extracts of the same plant [9,12]. The resistance of the fungal organism to cold water extracts as shown in this present day study may be attributed to insufficient release of these essential oils during extraction; and these findings is in agreement to that of Oshodi *et al*., [14].

The putative antimicrobial activity of *O. gratissimum* and *G. latifolium* leave extracts on microbes particularly fungus gives impetus to the use of these plants in meeting the primary health care needs of some people in local communities. Further studies is required to characterize by molecular techniques the active ingredients of these plants as well as evaluate their toxicity levels so that they can be compounded into pills that can be used to treat some related microbial infections.

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