*Available online at* www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2014, 4(4):40-43

ISSN : 2249-7412
CODEN (USA): AJPSKY

# Antifungal efficacy of *Bersama abyssinica* extracts against coffee pathogenic fungus *Gibberella xylarioides*

**Never Z. Mwambela[1*], Deusdedit Kilambo[2], Francis Shahada[1] and Musa Chacha[1]**

[1]*School of Life Science and Bio-engineering, Nelson Mandela African Institution of Science and Technology, Arusha-Tanzania*
[2]*Tanzania Coffee Research Institute, Moshi, Tanzania*

---

**ABSTRACT**

*Twelve Bersama abyssinica extracts were evaluated for antifungal activity against Gibberella xylarioides, a pathogenic fungus responsible for coffee wilt disease (CWD) in Robusta coffee by micro dilution method. All tested extracts were active against Gibberella xylarioides with the Minimum Inhibitory Concentration (MIC) range of 0.19 to 6.25 mg/ml .The methanolic extracts were the most active by exhibited antifungal activity at the MIC of 0.19 mg/ml followed by the chloroform extracts which exhibited activity at the MIC of 0.39 mg/ml .The extracts of leaves, stem bark and root barks of Bersama abyssinica could be a potential source of biological control for managing the CWD since exhibited antifungal activity at very low concentrations.*

**Key words:** *Bersama abyssinica*, coffee wilt disease, fungal pathogens, *Gibberella xylarioides*

---

## INTRODUCTION

Coffee is an important commodity for the economies and wellbeing of East , west and Central African countries since it offers foreign currency, nutrients and employment thus improving the livelihood of farmers ,consumers, business agencies and industries [1,2].However fungal phytopathogens are the most important threats of crop production including coffee and diminishes the quality of products leading to food insecurity because pathogens alter nutrient, intoxicate food and destruct the natural environment [3,4]. These accumulated effects in food production are attributed by the ability of fungal pathogens to invade the plants at any developmental stages. For instance the tracheomycosis caused by *Gibberella xylarioides* affect the coffee tree at seedling and producing Robusta tree leading to high loss of production [5, 6]. *Gibberella xylarioides* is an important pathogen especially in Robusta coffee in African plantations which undermine the massive production of coffee due to the persistence of CWD [7,8].This disease has affected and destructed the coffee trees resulting into low quality and quantity of Robusta coffee [9,10].Several interventions have been recommended for management of CWD such as stem painting using copper based fungicides up to 50 cm from ground level[8]. However continuous use of copper based fungicides have been claimed to have negative effects to the environments such as copper toxicity [11]. Other recommendations on control of CWD include use of resistant varieties, eradication of affected trees and application of ash at the base of Robusta trees [12, 13]. Despite these efforts to control CWD, holistic approaches seemed to be more viable in the management of CWD, including antifungal agents extracted from plants. For instance compounds of plant origin have been used for long time in controlling plant pathogens as a resolution to evade contamination and resistance due to fungicides so as to ensure both food security and human wellbeing [14, 15]. This is due to the fact that plants possess novel compounds with specific functions including fungicidal properties that could be used for controlling pathogens affecting other organisms [16, 17]. The use of natural products as a biological control is economically and socially accepted because is environmentally friendly and reduce the risk of pathogens resistance [18]. However Tanzania is endowed with vast array of medicinal plants of varied applications and some of them are

used for management of plant pathogens hence evaluation of *Bersama abyssinica* as antifungal agent against pathogenic fungus *Gibberella xylarioides* is discussed in this report.

## MATERIALS AND METHODS

### Solvents, Reagents and Culture Media

Methanol (absolute) was supplied by Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands) whereas Dimethyl sulphoxide (DMSO) was purchased from RFCL Limited, Hayana, India. Petroleum ether, Ethyl acetate, Chloroform were purchased from Loba Chemie Pvt Ltd, Mumbai, INDIA) while Potato Dextrose agar (PDA) was bought from HIMEDIA® ,INDIA and *Gibberella xylarioides* (clinical isolate) strain was obtained from Tanzania Coffee Research Institute (TaCRI).

### Preparation of Plant Materials and Extraction

Leaves, stem bark and root bark of *Bersama abyssinica* were collected from Ilolo village of Rungwe district in Mbeya, Tanzania. Then authentication was done by Mr. Ahmed Mndolwa of Tanzania Forestry Research Institute (TAFORI). The voucher specimen number (BANZ 0114) was kept at Nelson Mandela African Institution of Science and Technology.

The plant materials were air dried under shade and then pulverized into fine particles. The pulverized leaves (1000 g), stem bark (1000 g) and root bark (1000 g) were sequentially macerated using petroleum ether, ethyl acetate, chloroform and ethanol for 48h twice for each solvent. The respective extracts were filtered through muslin cloth on a plug of glass wool in a glass column and solvents were evaporated in vacuum using a rotary evaporator and stored in refrigerator at -20°C.

### Determination of antifungal activity

Minimum inhibitory concentrations (MICs) of the extracts were determined by micro dilution method [19] with minor modifications. Initially 50 µL of the saboraud's dextrose broth media was added in each well followed by addition of 50 µL of the extract making 100 mg/mL into each wells of the first row to make a total volume of100 µl in the first row. After careful mixing of first row of each plate, 50 µl were drawn from each of the first row wells and added into the subsequent row wells. The process was repeated down the columns to the last wells at the bottom where as 50 µLwas discarded. Thereafter, 50 µL of *Gibberella xylarioides* suspension approximately equal to 0.5 Mac Farhland's turbidity was added in each well to make the final volume of 100 µL in each well. The rows containing Dimethyl sulfoxide (DMSO) were used as negative control while the rows with Saboraud's dextrose broth only were used to monitor fungal growth. The plates were then incubated at 28°C for 24 hr. For each extract, MICs against *Gibberella xylarioides* was determined by adding 20µL of 0.02% p-iodonitrotetrazolium (INT) chloride dye in each well followed by incubation for 1hr at 28°C.The growth fungus was indicated by a change in pinkish colour.The lowest concentration which showed no growth were considered as MICs.

## RESULTS AND DISCUSSION

Antifungal activity of eleven extracts of *Bersama abyssinica* leaves, stem barks and root bark assayed was observed as presented in Table 1. The result revealed that all extracts exhibited activity against *Gibberella xylarioides* with significant differences (P>0.05) at the MIC range of 0.19 to 6.25 mg/ml. Methanolic extract of leaves exhibited the highest antifungal activity at the MIC of 0.19 mg/ml followed by the methanolic extract of stem bark and chloroform extracts of root barks both of which inhibited the growth of fungus at the MIC of 0.39 mg/ml. Again, the methanolic extracts of root bark and chloroform extract of leaves similarly exhibited high activity at the MIC of 0.78 mg/ml while the ethyl acetate extract of stem and root bark exhibited the significant activity against *Gibberella xylarioides* with the MIC of 1.56mg/ml and moderate activity was exhibited by leaves extracts of ethylacetate and stem bark extract of petroleum ether at the MIC of 3.13 mg/ml. However the petroleum ether extracts of leaves, stem bark and root bark exhibited the least antifungal activity with the MIC of 6.25 mg/ml whereas negative control (DMSO) at the MIC of 12.5 mg/ml.

The methanolic extract of the leaves exhibited the highest activity than all extracts with the MIC of 0.19 mg/ml showing that leaves posses high amount of active polar compounds with antifungal activity as compared to stem and root bark which inhibited the growth at MIC of 0.78 mg/m.

On the other hand the chloroform extracts of stem bark and root bark exhibited similar activity by inhibiting the fungal growth at MIC of 0.39 mg/ml while the chloroform extract of leaves exhibited activity at the MIC of 0.78 mg/ml highlighting that the moderate polar compounds of chloroform are also more active against a tested fungal strain than the ethyl acetate extracts of stem bark and root bark all of which exhibited activity at the MIC of 1.56

mg/ml and that of leaves the MIC of 3.13 mg/ml. However the petroleum ether extracts of all parts exhibited moderate antifungal activity with the MIC of 6.25 mg/ml showing that *Gibberella xylarioides* was less susceptible to less polar compounds.

**Table 1: Minimum inhibitory concentrations (MICs) of *Bersama abyssinica* extracts**

| Plant part | Extract | MIC (mg/ml) |
|---|---|---|
| Leaves | petroleum ether | 6.25 |
| | ethyl acetate | 3.13 |
| | chloroform | 0.39 |
| | methanol | 0.19 |
| Stem bark | petroleum ether | 3.13 |
| | ethyl acetate | 1.56 |
| | chloroform | 0.78 |
| | methanol | 0.78 |
| Root bark | petroleum ether | 6.25 |
| | ethyl acetate | 1.56 |
| | chloroform | 0.39 |
| | methanol | 0.78 |
| DMSO | | 12.5 |
| BROTH | | 25.00 |
| LSD (0.05) | | 6.66 |
| Sx | | 3.33 |
| Mean | | 4.44 |

Even though antifungal activity against phytopathogenic fungus, *Gibberella xylarioides has* never been reported from *Bersama abyssinica* but other studies done revealed the potential of plant compounds against fungal phytopathogens [6].For instance, the study carried in 2011potentiated medicinal plants as good source of antifungal agents against plant pathogens [17]. Additionally, the antifungal efficacy of plant materials was revealed in spore forming fungus *Didymella bryoniae* and in tomato late blight disease in which plants extracts and essential oils from plant exhibited high antifungal activity[20,21].Therefore the findings of this study is supported with previous study that claim the presence of active compounds in *Bersama abyssinica* [22].

## CONCLUSION

The findings of the present study suggest that leaves ,stem barks and root barks of *Bersama abyssinica* are potent against fungal strain thus could be used as an effective biological control for management of coffee wilt disease to reduce the loss incurred in solving the problem by synthetic chemicals. However this research calls for further researches to investigate the antifungal principles responsible for the claimed activity and carryout research under disease field environment.

**Acknowledgement**
This work was supported by the Commission for Science and Technology (COSTECH) through the Nelson Mandela African Institution of Science Technology (NM-AIST) scholarship. The Tanzania Coffee Research Institute (TaCRI) is highly acknowledged for provision of *Gibberella xylarioides*.

## REFERENCES

[1] Flood J, *Food Security*, **2006**, 2, 215-231.
[2] Musebe R, Njuki J, Mdemu S, Lukwago G, Shibru A, Saiba T, *Coffee Wilt Disease*, **2010**, pp. 83.
[3] Chakraborty S, Newton AC, *Plant Pathology*, **2011**, 60, 2-14.
[4]Wagacha J, Muthomi J, *International Journal of Food Microbiology*, **2008**,124, 1-12.
[5] Alemu T, *Ethiopian Journal of Biological Sciences*, **2013**, pp.11.
[6]Dellavalle PD, Cabrera A, Alem D, Larrañaga P, Ferreira F, Rizza MD, *Chilean Journal of Agricultural Research* ,**2011**, 71, 231-239.
[7]Geiser DM, Ivey ML L, Hakiza G, Juba JH, Miller SA, *Mycologia*, **2005**, 97, 191-201.
[8] Rutherford MA, *Phytopathology* , **2006**,96, 663-666.
[9] Agwanda C, Bieysse D, *CoffeeWilt Disease*, **2010**, pp. 155.
[10] Girma A, Flood J, Hindorf H, Bieysse D, Sarah S, Rutherford MA, *Association Scientifique Internationale du Café (ASIC)*, **2007**, pp. 1268-1276.
[11] Mtui H D, Maerere A P, Bennett MA, Sibuga KP, *Asian Journal of Plant Science and Research*, **2014**, 4, 9-13.
[13] Okeniyi M, *Afr. Crop. Sci. Conference Proc, Vol*. 8, **2007**, pp. 833-834.
[14] Benner JP, *Pesticide Science*, **1993**, 39, 95-102.

[15] Tripathi P, Dubey N, *Postharvest biology and technology*, **2004**, 32, 235-245.
[16] Panjehkeh N, Jahani H-AZ, *Communications in agricultural and applied biological sciences*, **2010**,76, 705-714.
[17] Prince L, Prabakaran P, *Asian Journal of Plant Science and Research,* **2011**, 1, 84-87.
[18] Pal KK, Gardener BM,*The plant health* instructor,**2006**,2, 1117-1142.
[19] Eloff J, *Planta medica*, **1998**, 64, 711-713.
[20] Fiori A, Schwan-Estrada K, Stangarlin J, Vida J, Scapim C, Cruz M, Pascholati S, *Journal of Phytopathology*, **2000**, 148, 483-487.
[21] Soylu EM, Soylu S, Kurt S, *Mycopathologia*, **2006**,161, 119-128.
[22] Djemgou PC, Hussien TA, Hegazy M-EF, Ngandeu F, Neguim G, Tane P, Mohamed A.-E-HH, *Pharmacognosy research* **2010**,2, 229