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Antifungal Activity of *Gueira senegalensis* L. and *Mangifera indica* L. on Sorghumloose smut pathogen (*Sporisorium cruentum* (KUHN) POTTER)

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Abstract

The objective of the research was to determine the antifungal activities of *Guiera senegalensis* L. and *Mangifera indica* L. leave extracts against most frequently occurring sorghum pathogen of the loose smut *(Sporisorium cruentum)* in Nigeria. Methanolic extracts from *Guiera senegalensis* L. and *Mangifera indica* L. were tested at concentrations of 250, 125, 62.5, and 31.2mg/ml as potential sources of antifungal agents for *Sporisorium cruentum.* All extracts exhibited moderate to good activities on the tested fungi with minimum inhibitory concentrations (MICs). The test organism was sensitive to the *G. senegalensis* extracts, with 14mm and 10mm in diameter at 250mg/ml and 125mg/ml, respectively. The test organism was sensitive to *M. indica* leaf extract with 12mm in diameter at 250mg/ml. The results showed that both extracts exhibited inhibitory effects at different concentrations against the test organism. Therefore, it is concluded that extracts of *G. senegalensis* and *M. indica* could be used in controlling the fungal pathogen of sorghum loose smut *(Sporisorium cruentum).*

Keywords; antifungal activities, *Sporisorium cruentum, Guiera senegalensis*, *Mangifera indica*

INTRODUCTION

Since antiquity, the plant kingdom has provided a variety of compounds of known therapeutic properties like analgesics, anti-inflammatory, medicines for asthma, and others. In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (Cowan, 1999; Kutama *et al.*, 2015). Several works have demonstrated in laboratory trials that different plant tissues, such as roots, leaves seeds and flowers possessanti-fungal activity (Aisha *et al.*, 2016, Kutama *et al.*, 2013)

Loose kernel smut, caused by the fungus *Sporisorium cruentum* (synonym *Sphacelotheca cruenta*) is less widespread than covered kernel smut in the past but is becoming economically important in sorghumgrowing parts of Nigeria probably due to climate change and or due to continuous use of home kept sorghum seeds by the poor resource farmers (Kutama *et al.*, 2013, 2016, 2020). Loose kernel smut attacks all group of Sorghums, including Johnson grass. Normally, all kernels in an infected panicle are smutted. Partial destruction is rare. Some kernels may be transformed into leafy structures or escape infection completely. Individual kernels are replaced by small smut galls (or sori) that are 2.5cm or longer, pointed and surrounded by a thin gray membrane. This membrane usually ruptures when or soon after the panicle emerges from the root. Smutted panicles appear earlier than the remainder of the crop and are more open than healthy panicles (Kutama *et al.*, 2018). The powdery, dark brown to black spores (teliospores) are soon blown away, leaving a long, black, pointed, conical, often curved structure (culumella) in the centre of what was the gall. Some smut spores (6 to 10 microns in diameter) adhere to the surface of healthy kernels on neighboring plants in the same field or ones nearby before and during harvest (Kutama *et al.*, 2010a and b) When such infected kernels are planted, the teliospores germinate along with the seed by first forming a thick, usually 4-celled promycellum bearing lateral sporidia (Doshimov, 1982) The sporidia germinate and infect the developing sorghum seeding. Most infections, however, result from the teliospores producing hypae which penetrate young seedings before emergence. Seeding infection occurs over a wide range of soil moisture and P^H at a temperature of 68 to 77f (20 to 25). The fungus continues to grow systemically within the plant unobserved until heading, when the long, black spores containing sporidia emerges (Kutama *et al.*, 2010a).

If uncontrolled, loose smut can wipe out entire crops, since it replaces the grain. In areas where people depend on their grain crops for survival and don't have the money or technology resources to control it, the disease can be devastating. Loose smut not only wipes out the crop, growers/farmers cannot even try again next year since any seeds they were able to harvest will be infected and will not produce seed the following season. Loose smut has recently become a more serious problem in many areas in Nigeria and those areas that have been particularly affected by global climate change and have experienced many new crop pests and diseases because of it. In places like the United State where there are ample resources and technologies such as fungicide seed treatment, loose smut is not an especially important disease (Martin *et al.,* 1980; Kutama *et al.*, 2010b)

Today, loose smut is among the most prevalent disease infecting sorghum and it causes a great loss of significant economic value, as such it has to be prevented. The aim of this investigation is to determine the antifungal activities of *Guiera senegalensis* and *Mangifera indica* leaf extracts on *S. cruentum*.

MATERIALS AND METHODS

Study area

The research was conducted at Biology Laboratory, Kano University of Science and Technology Wudil which was located at latitude $11^{0}48^{1}$N, longitude 8^{0} 51^{1}E and altitude 403M above the sea level.

Collection of Plant Samples

Guiera senegalensis and *Mangifera indica* leaves were collected from the environment of Kano University of Science and Technology Wudil, close to the river sites. The plants were first identified at the field using standard keys and descriptions (Dalziel, 1956; Keay, 1989). Its botanical identity was further confirmed and authenticated at the herbarium section of the botany unit of Biological science, Kano University of Science and Technology Wudil, Kano State.

Sample Preparation

The preparation of samples was carried out in accordance with the method of (Kareem *et al,* 2003). The leaves samples were separately collected, sterilized by immersing in 3% sodium chloride solution for three minutes, and rinsed three times in a running tap water and kept in shade for air-drying for one (1) weeks. The leaves were separately grinded in to powder in a clean mortar and pestle, packaged in sterile polythene bag and stored until required for use (Kareem *et al.,* 2003).

Sourcing of test organism

The test organism used was loose smut of sorghum which was collected from the stalk of the infected plant. It was obtained from international crop research institute for the semi arid tropics (ICRI SAT). The organism was confirmed by the ICRI SAT officials. The isolate was maintained in a wrapped paper until required for use.

Extraction

The extraction was conducted using Soxlet extraction technique as described by Fatope*et al*. (1993). Soxlet extraction technique was used. A quantity (50g) of the fine powder of the plant leaf was weighed and suspended in to a conical flask and percolated with 250ml of methanol. The process was carried out for both of the extracts and later each was allowed to stand for one (1) week with a constant shaking at interval of two days under a room temperature. The percolates were then filtered and solvent (methanol) was evaporated to obtain methanol and aqueous extracts and these served as solutions. The extracts were then put in a small sample bottles for future use.

Preparation of Stock Concentrations

This was carried out using standard method of Cheesbrough (2002). Stock solution of the methanolic extract was prepared by weighing 0.25mg of it and dissolved in 0.75ml of dimethylsulphur dioxide (DMSO) in small sample bottle. This gave an extract concentration of 250mg/ml (stock solution). Three varied extract concentrations (125mg/ml, 62.5mg/ml and 31.25mg/ml) were prepared from the stock solution as recommended by Aisha *etal*. (2016).

Preparation of Potato Dextrose Agar (PDA) Culture Media

The medium used was potato dextrose agar (PDA) which is the faster and best media for fungal growth. Exactly 19.5 grams of Mueller-Hinton agar (MHA) was weighed and mixed with 250ml of sterile distilled water. The mixture was sterilized by autoclaving at 121^{0}C for 20minutes. Under aseptic conditions in the laminar flow hood, 20ml of agar media was uniformly dispensed in to sterilized Petri dishes. They were then covered and allowed to cool at room temperature until the culture media hardened. The inoculum of the fungi was cultured on the agar surface by spread plating technique (Prescott*et al.*, 2002).

Sensitivity Testing

Sensitivity test was carried out using agar diffusion method (Kirby *et al.*, 1966). The freshly prepared potato dextrose agar (PDA) plates were dried in a dryer for 10minutes to remove the surface moisture. The plates were aseptically inoculated uniformly with the test organism by spread plating technique. A sterile corkborer (6mm) in diameter was used to bore 4 wells and one (1) at the center which serves as the control then 0.1ml of the various concentration of the extract was transferred into respective agar plates and the ethanol was placed at the center to serve as control (Meyer and Dillika; 1996) the plates were incubated aerobically at 37^{o}Cfor 72hours. Diameter of zone of inhibition was been measured using millimeter rule. The result was interpreted and recorded in accordance with Chessbrough (2002).

Preparation of Potato Dextrose Broth (PDB)

The potato dextrose broth was used to determine the minimum inhibitory concentration of the extracts which is in liquid form. Exactly 6.25g of (PDB) was weighed and mixed with 125ml of sterile distilled water. The mixture was then sterilized by autoclaving at 121^{0}C for 20minutes. Under aseptic conditions, the media was allowed to cool at room temperature until required for use (Prescott*et al.*, 2002).

Determination of Minimum Inhibitory Concentrations of theExtracts

The (MIC) was carried out according to the method of Prescott *et al*. (2002). The MIC was determined using tube dilution method. Exactly 8ml of potato dextrose broth was pipette in to a set of 10 tubes for each sample extracts that show sensitivity to the test organism. Serial dilution was carried out using 2ml of stock solution in the first test tube contained 8ml potato dextrose broth that give a dilution of 250mg/ml. Then 2ml of this later concentration was then pipetted into the next test tube containing 8ml potato dextrose broth to give a dilution of 125mg/ml. This was continued until 10 serial dilutions equivalent to 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml, 15.6mg/ml, 7.81mg/ml, 3.9mg/ml and 1.9mg/ml all in 100% w/v were obtained. The first tube contained the potato dextrose broth and the extract which served as positive control. The remaining 8 tubes were then inoculated with 0.1ml of the test organism and incubated at 37^{0}C for 72 hours. The MIC inhibitory concentration was then recorded i.e. turbid tubes indicated growth while clear tubes showed inhibition. The lowest concentration without visible signs of growth was taken as the minimum inhibitory concentration.

RESULTS AND DISCUSSION

The present study tested the antifungal activity of aqueous methanolic leaf extracts of *Guiera senegalensis* and *Mangifera indica* and their respective inhibition against loose smut pathogen. These plants extract were chosen based on either traditional usage, suggestive of antimicrobial activity, or previous studies that have demonstrated antifungal properties using different kinds of extracts (Guo *et al.*, 1997; Wilson *et al.*, 1997) each of the extract shows inhibitory activity to the test organism but not all of them show inhibition to the different concentrations used (Aisha *et al.*, 2016).

Many naturally occurring compounds found in plants have been shown to possess antifungal functions and thus serve as a source of both traditional and orthodox medicine (Kim *et al.*, 1995; Abhay *et al.*, 1997; Adoum *et al.*, 1997; Awune, 2002; Musa *et al.*, 2002; Kalamba and Kuneika, 2003; Akinyemi *et al.*, 2007; Yusha'u *et al.*, 2008, Kutama *et al.*, 2013; Aisha *et al.*, 2016). For example, flavonoids are known to inhibit fungal growth (Lutterodt *et al.*, 1994; Mbuh*et al.*, 2007; Aisha *et al.*, 2016) and thus responsible for the antifungal activity exhibited in this study. The results of the present study further supports Bibitha *et al.* (2002) and Kutama *et al.* (2013) who reported variation in the antifungal activities of different plants extracts. On the other hand, the variations observed in this result of the plants parts screened to inhibit fungal growth are in conformity with the reports of Yusha'u *et al.* (2008) that antifungal activity may vary from one plant to another. The results in Table 2 and 3 showed that *G. senegalensis* showed inhibition to two different concentrations; 250mg/ml and 125mg/ml at 14mm and 10mm, respectively and therefore, is the appropriate concentration while *M. indica* showed inhibition on only one concentration; 125mg/ml of the extract and it is also the appropriate concentration according to this study. Tables 4 and 5 showed the minimum inhibitory concentrations (MIC). The (MIC) of *G. senegalensis* was 62.5mg/ml while the (MIC) of *M. indica* was 31.2mg/ml. this is the minimum concentrations of the two extracts that can best stop the growth of the test organism under optimum temperature and other environmental conditions (Yusha'u *et al.*, 2008).

Table 1: Physical characteristics of the leaf extracts of *G. senegalensis* and *M. indica*

	Solvent	Colour	Odour	Texture
G. senegalensis Leaf	Methanol	Dark brown	Slightly repulsive	Oily
M. indica Leaf	Methanol	Brown	Pleasant fruity	Soft

Table 2: Antifungal effects of *Guiera senegalensis* on loose smut pathogen at different concentrations

Concentrations mg/ml	250	125	62.5	31.5	Ethanol(control)
Zone of inhibition (mm)	14	10	0	0	14

Table 3: Antifungal effect of *Mangifera indica* on loose smut pathogen at different concentrations

Concentrations mg/ml	250	125	62.5	31.5	Ethanol(control)
Zone of inhibition (mm)	12	0	0	0	16

Table 4: Minimum inhibitory concentration of *G. senegalensis* at 125mg/ml

Concentration of extract (mg/ml	Inhibition
250	-
125	-
62.5	-
31.5	+
15.6	+
7.81	+
3.9	+
1.9	+

Key:
+= Growth (Turbid), - = no inhibition

Table 5: Minimum inhibitory concentration of *M. Indica* at 250mg/ml

Concentration of extract (mg/ml	Inhibition
250	-
125	-
62.5	-
31.5	-
15.6	+
7.81	+
3.9	+
1.9	+

Key
+ Growth (Turbid), - = no inhibition

CONCLUSION

The study has shown that two (2) locally available indigenous plants namely; *Guiera senegalensis* and *Mangifera indica* are very effective in inhibiting the growth of loose smut fungi. The fungi toxic effects of the extracts indicate the potential of some plant species as a natural source of fungicidal material. *Guiera senegalensis* has the higher inhibitory activity against the test organism. It is therefore concluded that plant extract can successfully be used for controlling loose smut fungal pathogen of sorghum instead of environmentally hazardous chemicals such as fungicides that are used for treating loose smut of sorghum in Nigeria. Further research is also suggested on the study of the antifungal activities of these plants especially with regard to the best concentration of the extracts that can eliminate the test organism.

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