

***In-vitro* evaluation of *Datura metel* leaves for potential antifungal activity against *Lasiodiplodia theobromae* Pat.**

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ABSTRACT

The aim of this study was to investigate the antifungal efficacy of ethanolic extract of leaves of *Datura metel* compared with standard antibiotics against pathogenic fungus *Lasiodiplodia theobromae* Pat., using Potato dextrose agar (PDA) medium. The experiment was laid out in a completely randomized design (CRD) with five replicates. Data collected were subjected to analysis of variance (ANOVA) and the mean separation was done using Duncan Multiple Range Test (DMRT) at the probability of 5%. The quantitative and qualitative phytochemical analyses of the leaves of *D. metel* were carried out in the Department of Plant Science and Biotechnology Laboratory, University of Port Harcourt Choba, using chemical methods to identifying the phytochemical constituents present in the leaves of *D. metel*. Results of the screening showed that the leaves of *D. metel* had significant amount of phytochemical constituents, alkaloid (1.50%), flavonoid (13.60%), saponins (11.60%), tannin (0.69%) and cyanogenic glycosides (0.08%) the ethanolic extracts of *D. metel* leaves at 100 and 80% competed favourable as compared with Standard antibiotic (Ciprofloxacin) which significantly ($P \leq 0.05$) antagonized the fungus: *L. theobromae* by reducing the mycelial growth with inhibitory zones of 0.55 ± 0.13 to 0.70 ± 0.08 cm. The research therefore recommended for the use of 80 and 100% ethanolic extract of leaves of *D. metel* which inhibited the mycelial growth the fungus; *L. theobromae*. This plant leaf extracts will serve as fungicidal pesticides for poor resource farmers.

Keywords: Antifungal efficacy, *Datura metel*, *Lasiodiplodia theobromae*, ethanolic extract, Potato dextrose agar (PDA), standard antibiotic.

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INTRODUCTION

Datura metel is commonly known as Jimson weed and belongs to the family Solanaceae (Ahad et al., 2012). It is 60-120 cm tall. Leaves are 8-17 × 4-13 cm, ovate, sinuately dentate and minutely puberulose. The flowers are trumpet-shaped, white to creamy or violet and 6 to 9 cm long (Stace, 1991) and mostly found in temperate and subtropical region (Ahad et al., 2012).

From the beginning of life human beings used the plant for different purposes like food and medicine. Today a large number of people use different plant for different disease treatments. *D. metel* is a most important medicinal plant. Traditionally, it has an important medicinal value throughout the world. Its

leaves and seeds are used in different treatment recipes. The leaves of *D. metel* are mixed with mustard oil for treatment of skin disorder. Juice from the flower petals of *D. metel* is used for ear pain. Seeds are used as purgative, cough, fever and asthma treatment. Seeds are also used for smoking for its narcotic action (Khan et al., 2013). It is often used as an analgesic plant in folklore medicine (Zargari, 1989). The drugs obtained from medicinal plants are termed crude drug of natural or biological origin as described by pharmacist and pharmacologist (Sofawora, 1982). *D. metel* contains different type of phytochemicals including saponins, tannins, steroids, alkaloids, flavonoids, phenols and

glycosides (Shagal et al., 2012). Its leaves and branches extracts show high anti-fungal and anti-microbial activities (Gul et al., 2012).

D. metel is one of the widely well-known folk lore medicinal herbs. It is a plant with both poisonous and medicinal properties and has been proven to have great pharmacological potential with great usage as folklore medicine (Priyanka et al., 2012).

D. metel is an annual weed, found in the gardens roadsides and other waste or cultivated land. It is widely naturalized in warmer countries throughout the world and is quite common in the British Isles (Gary et al., 2005). However, *D. metel* probably originated and spread to Europe in the first century. At present it grows in waste places in Europe, Asia, America and South Africa now cultivated in Germany, France, Hungary, South America and throughout the world (Jarald and Edwin, 2007; Gary et al., 2005). Traditionally, from pre historic times, the use of different parts of medicinal plants was practiced to cure specific ailments evidently due to presence of some phytochemical substance like tannins, terpenoids, alkaloids, essential oil, glycosides, flavonoids and others (Sameera and Mandakini, 2015).

The high mortality and morbidity amongst humans and animals are due to infectious diseases. Emergence of multi-drug resistance, existing anti-microbial with undesirable effects and restricted anti-microbial spectrum creates further annoyance diverting attention towards natural antimicrobials (Ngoci et al., 2014). Antibiotic resistance has become a global concern (Westh et al., 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistance pathogens (Bandow et al., 2003). However, the increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic micro-organisms has led to the screening of several medicinal plants for potential antimicrobial activity (Colombo and Bolesio, 1996; Iwu et al., 1999).

The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi-drug resistant pathogens. However, the increasing failure of antibiotics resistance exhibited by pathogenic micro-organisms has led to the screening of this non-timber herbeous medicinal plant (*D. metel*) for potential antifungal activity. Besides, a vast majority of synthetic antibiotics are expensive and highly toxic at their optimum dosage level. Therefore, the use of this herb for antifungal assay becomes very vital in this study,

This research was undertaken to evaluate the antifungal activity of *D. metel* ethanolic extract seeds and leaves against pathogenic fungal isolate.

Specific objectives of this research were to:

1. determine the phytochemical constituents present in the leaves of *D. metel* obtained around the Department of Forestry and Environment Laboratory.
2. evaluate the effects of ethanol extracts of *D. metel* leaves on the mycelial growth of fungus isolate.

3. compare the effectiveness of ethanol extracts of *D. metel* leaves with standard antibiotic (Ciprofloxacin).

MATERIALS AND METHODS

Study area

The study was carried out at the Laboratory of Forestry and Environment (Forest Pathology Unit), Rivers State University, Nkpolu-Oroworukwo, Port Harcourt and Department of Plant Science and Biotechnology Laboratory, University of Port-Harcourt, Choba.

Source and collection of plant materials

The leaves of *D. metel* were collected from around the Department of Forestry and Environment Laboratory (Figure 1). Later the plant leaves was subjected to surface sterilization using 50 % alcohol and then air dried and further analysed, ground into powder using blender (Monlinex 530.240) and packed in polythene bags for further uses.

Preparation of the extracts

Twenty grams (20 g) each of the leaves of *D. metel* were extracted with 90% ethanol in 500 ml beakers (Figure 2). The different extracts was collected in a separate container and concentrated to dryness in a flash evaporator (buch type) under reduced pressure and to obtain the crude extracts (Uma, 2009).

Microorganism used

Stock cultures of *L. theobromae* collected from the forest pathology laboratory (Figure 3). The fungal culture was maintained on potato dextrose agar medium and was stored at 4°C to be used in determining the antifungal activity of *D. metel* plant (Uma, 2009).

Effects of ethanol extracts of *D. metel* leaves, seeds and Ciprofloxacin on fungal mycelia growth

The antifungal activity assay was carried out using potato dextrose agar methods (Chukunda and Offor, 2015). Ethanol extracts of leaves of *D. metel* were serially diluted to 20, 40, 60, 80 and 100% concentrations respectively. Each percentage concentration of the extracts of leaves were mixed with potato dextrose agar and allowed to solidify before introducing a 5 mm disc of the fungus and incubated at room temperature of $27 \pm 2^\circ\text{C}$. The linear extension of the fungus on the ethanol extracts of leaf concentration was measured along transect in two directions at right angles to each other after 7 and 14 days of incubation and a mean diameter values of the fungus growth inhibition was then recorded (Chukunda, 2014; Elenwo, 2009).

However, Ciprofloxacin was added in each separate Petri dish which served as standard control and the Petri dishes were incubated at room temperature of $27 \pm 2^\circ\text{C}$. Later the linear fungus growth inhibition was measured and recorded using a transparent metre rule (Chukunda, 2014; Yogesh et al., 2008; Uma et al., 2008; NCCLS, 1998).

Phytochemical analysis of leaves of *D. metel*

Phytochemical screening of the extract of *D. metel* leaves was



Figure 1. Picture of the study plant *Datura metel*.



Figure 2. Ethanolic leaf extract of the test plant.

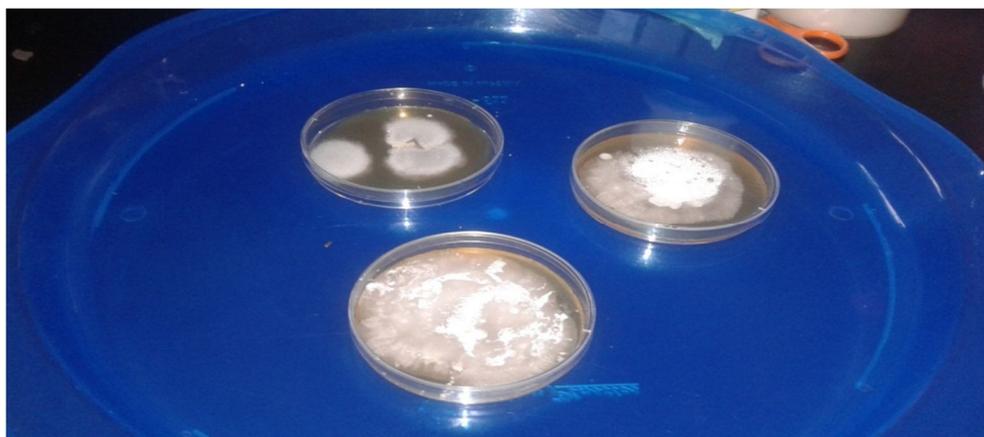


Figure 3. Stock pure culture of *L. theobromae*.

carried out according to the methods described by Trease and Evans (1989) and Mann et al. (2008). Ten grams (10 g) of the ground leaf samples was separately soaked in 200 ml of ethanol and allowed to stand for 72 h for extraction. After the 72 h, it was filtered using No. 1 Whatmman filter paper. The filtered samples was sterilized and later evaporated to dryness for the detection of active components like tannins, alkaloids, saponins, glycosides and flavonoids.

Alkaloids determination

1 ml of 1% hydrochloric acids were added to 3 ml of the extracts of

leaves of *D. metel* in a test tube. The respective mixture was heated for 20 min, cooled and filtered. About 2 drops of Mayer's reagent were added to 1 ml of the extract. A creamy precipitate was an indication of the presence of alkaloids (Mann et al., 2008, Abalaka et al., 2010).

Glycosides determination

10 ml of 50% Sulphuric acid (H_2SO_4) was added to 1 ml of the leaves extracts and the mixture respectively was heated in boiling water for about 15 min. 10 ml of Fehling's solution was then be added and the mixture boiled, a brick red precipitate will be

confirmatory for the presence of glycosides (Abalaka et al., 2010).

Tannins determination

1 ml of freshly prepared 10% Potassium hydroxide (KOH) was added to 1 ml of the extracts of leaves of *D. metel*. A dirty white precipitate showed the presence of tannins (Hagerman, 2002).

Saponins determination

Two (2 ml) of the extracts of the leaves was vigorously shaken in the test tube for 2 min and no frothing was observed then add 5 drops of olive oil was added to 3 ml of the extract in the test tube and vigorously shaken, absence of stable emulsion formed showed absence of saponins (Akharaiyi, 2011).

Flavonoid determination

One millimeter (1 ml) of 10% Sodium hydroxide (NaOH) will be added to 3 ml of the extracts of and leaves of *D. metel*. There was no yellow coloration which is indicative of the absence of flavonoids.

Experimental design and statistical analysis

The experiment was laid out in a completely randomized design (CRD). The treatments were replicated three times. Data collected was analyzed by analysis of variance (ANOVA) using SPSS Genstat software as described by Steel and Torrie (1980). Duncan Multiple Range Test at a probability of 5% (DMRT) to separate the means.

RESULTS

Phytochemical constituents of *D. metel*

The results on the phytochemical constituents of leaves of *D. metel* collected around the Forestry and Environment Laboratory are presented in Table 1.

The result of qualitative and quantitative analyses showed that the presence of flavonoid (13.60%) saponins (11.60%) tannins (0.69%) and cynogenic glycosides (0.08%) were significantly ($P \leq 0.05$) high in the leaves.

Antifungal efficacy of ethanolic extract of leaf of *D. metel* against mycelial growth of test fungus

The results on the antifungal efficacy of ethanolic extracts of leaves of *D. metel* against mycelia growth of *L. theobromae* are presented in Tables 2 and Figures 4 and 5.

The results of the ethanol extract of leaf of *D. metel* showed that at 100 % concentration there was significant ($P \leq 0.05$) difference in inhibition in the fungal mycelia growth. The leaf extract at 100% ethanol concentration inhibited the fungal mycelial (2.20 ± 0.20

to 3.40 ± 0.18) followed by 80% ethanol concentration where the leaf extracts inhibited the test fungus (2.45 ± 0.25).

Generally, the plant leaf extracts of *D. metel* competed favorably with the standard antibiotic (Ciprofloxacin) against the mycelial growth of *L. theobromae*.

Zone of inhibition (cm) of test fungus by ethanolic leaf extracts of *D. metel* and standard antibiotic

The results of zone of inhibition of *L. theobromae* by ethanolic leaf extracts of *D. metel* compared with standard antibiotic (Ciprofloxacin) are presented in Table 3. The results indicated that 100 % concentration of leaf extracts of *D. metel* exhibited high inhibitory effects on the test fungus with the standard antibiotic having diameter inhibition zone of 0.070 ± 0.08 cm and -0.50 ± 0.06 cm.

DISCUSSION

Phytochemical and antifungal activity of *D. metel*

The development of some modern drugs could not have been possible in the absence of the phytochemical constituents of plant and *D. metel* is not an exception (Akharai, 2011). It was earlier reported by Nain et al. (2010), that the secondary metabolites of *D. metel* are highly effective against different types of diseases such as antidiabetic and antiviral. *D. metel* is widely growing plant and well-known to have great pharmacological potential with a great utility folklore medicine. It contains saponins, flavonoid, glycosides, alkaloid, tannins carbohydrates proteins. It is used in folklore medicine due to its analgesic and antiasthmatic activities (Soni et al., 2012); the leaves are used for asthma treatment (Savathrama et al., 2007).

The presence of antimicrobial substances in plants is well established. Plants have provided a source of inspiration for novel drug compounds as plant have antimicrobial substances which have made significant contribution towards human health (Huda et al., 2015).

Medicinal plants such as *D. metel* have been used for certain ailment caused by several microbial diseases due to their vulnerable effects in the health care (Amjad et al., 2005). The plant indeed is used in alternative medicine for providing remedy against many diseases (Akroum et al., 2009). The problem of antibiotic resistance to most microorganisms which has led to the resurgence of interest in herbal medicinal plant products as a source of suppressing or eradicating the ever-increasing problems of resistant micro-organisms against antibiotics (Akharaiyi, 2011).

The results of the antifungal activity of ethanolic extracts of leaves and seeds of *D. metel* are presented in Tables 1, 2 and 3 showed that the test fungus was

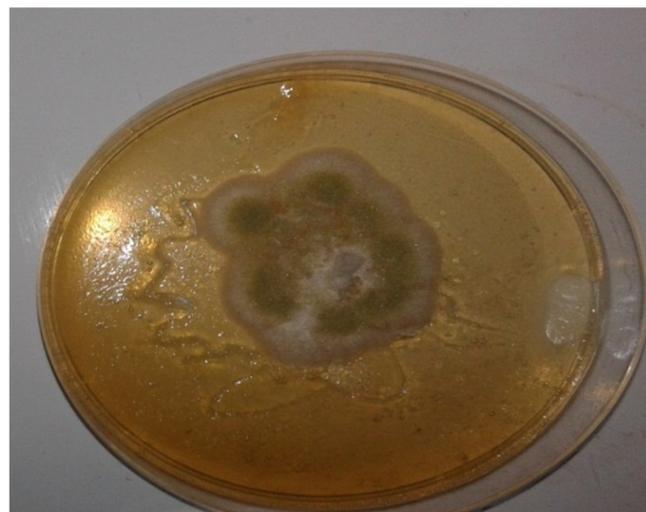
Table 1. Quantitative analyses of phytochemical consistents of *D. metel* leaves.

Sample	Alkaloid (%)	Flavonoid (%)	Saponin (%)	Tannin (%)	Gynogenic glycoside (%)
Leaves	1.50	13.60	11.60	0.69	0.08

Table 2. Effect of ethanol extracts of *D. metel* leaves on mycelia inhibition of *L. theobromae* after two weeks (mean data \pm S.E.M).

Conc. of the ethanol plant extracts of <i>D. metel</i> (%)	Mycelial growth of <i>L. theobromae</i>	
	Leaf extract	Antibiotic (Ciprofloxacin)
100	2.20 \pm 0.20 ^f	
80	2.45 \pm 0.25 ^e	
60	3.20 \pm 0.22 ^d	
40	3.54 \pm 0.35 ^c	2.90 \pm 0.12
20	4.21 \pm 0.40 ^b	
0	8.95 \pm 0.00 ^a	

Mean values with the same superscripts (a,b,c..) in the same column are not significantly ($P \leq 0.05$) different by DMRT ($P \leq 0.05$).

**Figure 4.** 100% ethanolic extract of leaves of *D. metel*.**Figure 5.** 100% antibiotic (Ciprofloxacin).**Table 3.** Zone of inhibition (cm) of test fungus by ethanolic leaf extracts of *D. metel* and standard antibiotic.

Conc. of the ethanol Leaf extracts of <i>D. metel</i> (%)	Mycelial growth of test fungus	Diameter of inhibition zones (cm)
100	2.20 \pm 0.20 ^e	- 0.70 \pm 0.08
80	2.45 \pm 0.25 ^d	- 0.55 \pm 0.13
60	3.20 \pm 0.22 ^c	- 0.30 \pm 0.10
40	3.54 \pm 0.35 ^b	- 0.64 \pm 0.05
20	4.21 \pm 0.40 ^a	- 1.31 \pm 0.22
Antibiotic (Ciprofloxacin)	2.90 \pm 0.12 ^d	0.00 \pm 0.00

Mean values with the same superscripts (a,b,c..) in the same column are not significantly ($P \leq 0.05$) different by DMRT ($P \leq 0.05$).

highly sensitive to the ethanol leaf extract than the seed extract and the standard antibiotic (Ciprofloxacin).

This result findings agreed with the report of Huda et

al. (2015) who reported that ethanolic extract of *D. metel* competed favorably with standard antibiotic such as Penicillin, Kanamycin, Cefotaxime, Streptomycin and

Refampin to fight against micro-organisms such as *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, *Proteus mirabilis* and *Klebsiella pneumoniae*.

Similarly, Akharaiyi (2011) reported that the leaf and stem bark extracts of *D. metel* inhibited eight clinical bacterial isolates. This is consistent with the present findings.

Akharaiyi (2011) had earlier reported that *D. metel* is a natural source of antioxidants and phytochemical having antimicrobial activities. This report agreed with the present findings whereby the leaf extracts significantly inhibited the mycelial growth of *L. theobromae*. This is consonance with the present result findings.

Gachande and Khillare (2013) reported that water and ethanolic extracts of the *D. metel* showed good antimicrobial activities. They stated further that extracts of leaves had a better efficacy than stem and root. This is consistent with the present research findings where the ethanol leaf extracts of *D. metel* inhibited the mycelial growth of *L. theobromae* more than the ethanolic extract of the seeds.

Ukoima et al. (2013) reported that extracts from the bark of *Rhizophora racemosa*, *Aloe vera* leaves, *Jatropha curcas* at 100% concentration inhibited the mycelial growth of several fungi such as *L. theobromae*, *Penicillium citrinum*, *A. niger* and *Paecilomyces*.

Chukunda and Obinna-Echem (2016) recommended that 100% crude leaf extract of *Ocimum gratissimum* significantly reduced the mycelial growth of *A. niger*, *A. flavus*, *R. stolonifer*, *F. palidoroseum*, *B. theobromae*, *C. gloeosporoides*, *P. expansum* and *B. cinerea*. This is in line with the present research work.

Conclusion

The antifungal activities of *D. metel* can be comparable to the standard antibiotic (Ciprofloxacin). Therefore, this research offers a scientific basis for the use of the *D. metel* plant leaves extracts for the treatment of infections that could be caused by micro-organism against plants and humans. Results also established that 100% concentration of the ethanol leaf and seed extracts of *D. metel* showed fungicidal and fungistatic potency for the treatment of any pathogenic fungi.

Studies indicated that *D. metel* is a wild plant having various medicinal and pharmacological properties.

In conclusion, the plant leaves constituents found in the *D. metel* are thought to have the potentiality of useful drugs if properly harnessed since it is ecofriendly.

RECOMMENDATIONS

1. Based on the result findings, extracts of leaves of *D. metel* should be used as fungistatic and fungicidal to control the Anthracnose fungal diseases of fruit trees
2. It is recommended that 80 and 100% of the ethanolic

extract of leaves of *D. metel* should be used to control the growth of the test fungus since inhibition zone was low.

3. The results observed considered the plant with high phytochemical quality for antifungal effectiveness.

4. The plant extracts of *D. metel* should be subjected to further analyses to screen for its toxicity and side effect for perfect therapeutic use.

5. It is also recommended that Gas chromatography Mass Spectroscopy analysis for the purification of active antifungal constituents from *D. metel* extracts for pharmaceutical use.

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