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Full Length Research Paper

In vitro antibacterial and antifungal activities of *Chrysophyllum albidum* and *Diospyros monbuttensis* leave

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Despite progress in the development of antibacterial agents, there are still special needs to find new antibacterial agents due to the development of multidrug resistance by bacteria and fungi.This study was conducted to investigate and compare the *in vitro* antibacterial and antifungal activities of the methanolic and ethanolic extracts of the leaves of *Chrysophyllumalbidum* and *Diospyrosmonbuttensis*.Methanolic and ethanolic extracts of *D. monbuttensis* and *C. albidum* leaves were prepared using cold extraction method. Antimicrobial sensitivity testing was carried out using agar-well diffusion method against the following test organisms: *Staphylococcus aureus*, *Streptococcus* sp., *Escherichia coli* (Enteropathogenic), *Klebsiella*sp., *Candida albicans* and *Aspergillusniger*. Minimum inhibitory concentration of the extracts of *D. monbuttensis* and *C. albidum* leaves was determined using broth dilution method. *S.aureus*, *Streptococcus* sp., *E. coli* and*Klebsiella*sp. were sensitive to ethanolic leaf extract of *C. albidum* at 25, 50 and 100mg/ml respectively. *Streptococcus* sp.,*E. coli* (Enteropathogenic), *Klebsiella*sp. And*C. albicans*were sensitive to *C. albidum* at 25and 50mg/ml. *A. niger* showed resistance to both extracts at the different concentrations used. The MIC of the methanol and ethanol leaf extracts of *D. monbuttensis* and *C. albidum* against the test microorganisms ranged between 3.125 and 100mg/ml. This is indicative that *D. monbuttensis* and *C. albidum* leaf extracts can be used in the treatment of infections.

Keywords:*Diospyrosmonbuttensis, Chrysophyllumalbidum,*extracts,antimicrobial, antifungal.

INTRODUCTION

Before the introduction of chemical medicine, man relied on the healing properties of medicinal plants (Ahvaziet al., 2012). Inspite of the diverse research from chemistry and biotechnology in producing synthetic drugs, plants are still the solehealing provider to mankind. The benefits derived from using medicine obtained from plants are that they are relatively safer than the synthetic alternative by offering profound therapeutic benefits and more affordable treatment (Iduet al., 2007; Akinnibosun and Itedjere, 2012; Nwankwoet al., 2015). Plants

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produce many organic compounds which have value in the treatment of various diseases. Herbal remedies have played an important role in treatment ofailments from ancient to modern times. Although these subjects lost their importance in 20th century because of the modern synthetic treatments, there is a renewed interest today in medicinal plants usage as natural products for the generation of semi-synthetic derivatives (Efferthet al., 2007).

Antimicrobial properties of substances are desirable tools used in the control of undesirable microorganisms especially in the treatment of infectious diseases. The active components usually interfere with growth and metabolism of microorganisms in a negative manner (Aboabaet al., 2012). Bacterial infections are among the important infectious diseases. Medicinal plant extracts are promising as alternative or complementary control means because of their anti-microbial activity, non-phytotoxicity, systemicity as well as biodegradability (Talibiet al., 2012). Plants produce a great deal of secondary metabolites, many of them with antimicrobial and antifungal activity.

*Chrysophyllumalbidum*G.don (Sapotaceae) commonly referred to as 'white star apple' or 'mululu' is a tropical forest tree found in diverse ecozones in Nigeria, Uganda, Niger republic, Cameroon and Cote d' Ivoire (Bada, 1997).Tannins, flavonoids, terpenoids, proteins, carbohydates and resins are the phytochemicals that have been reported in *C. albidum*(Akaneme, 2008). Eleagnine, tetrahydro- 2 - methylharman and skatole have been isolated from this plant and eleagnine was the main compound responsible for its antimicrobial activity (Idowuet al.,2003). The leaves are used as emollients and for the treatment of skin eruptions, diarrhoea and stomach ache which are as a result of infections and inflammatory reactions (Adisa, 2000). The high saponin content of *C. albidum*leaves and roots justifies the use of the extracts to control human cardiovascular disease and reduce blood cholesterol as documented by Aletor (1993). *C. albidum*can be used as anti-inflammatory, antispasmodic, antianalgesic and diuretic due to its properties attributed to their high flavonoids, steroids, glycosides and saponins (Savithrammaet al., 2011). The phenolic compounds in *C. albidum* may be responsible for the therapeutic, antiseptic, antifungal or bacterial properties of the plant; this is also responsible for the bacteriostatic and fungistatic activity (Okwu andIroabuchi, 2001; Okwu and Morah, 2007).

*D. monbuttensis*is referred to asokpuọcha in igbo and egungunekun or erikesi in yoruba (White, 1957). The leaves contain sterols, polyterpenes, polyphenols, flavonoids, alkaloids, saponins, leucoanthocyanins, tannins, quinones and coumarins. Anthocyanins, cardiotonics glycosides and steroids are present in very low quantity. According to Bouquet and Debray (1974)**,** this plant is considered by the Baoulé and Agni ethnic groups from Ivory Coast as a good remedy for febrileaches, stomach pains, edema and leprosy. Various studies have demonstrated that the coumarins have a potential antioxidant. This antioxidant activity is due to their ability to trap the free radicals and to chelate metal ions (Tseng, 1991).It is assigned to the terpenoides and tannins some analgesics and anti-inflammatory activities. Apart from this, the tannins contribute to healing wounds (Okwu and Josiah, 2006). The constituents present in these plants play a significant role in identifying the crude drug.

Despite progress in development of antibacterial agents, there are still special needs to find new antibacterial agents due to development of multidrug resistant bacteria (Wise et al.,1998). According to World Health Organization (WHO), 80% of the World's population is dependent on the traditional medicine (Kumar and Nagarajan, 2012). Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson-Hunt, 2000). Herbal plants are rich sources of safe and effective medicines and are used throughout the history of human beings either in the form of plant extracts or pure compounds against various infectious diseases (Parekh and Chanda, 2007). Among foremost health problems, infectious diseases account for 41% of the global disease burden. The main reasons of these infectious diseases are the natural development of bacterial resistance to various antibiotics. The development of multidrug-resistant (MDR) bacteria takes place because of the accumulation of different antibiotic resistance mechanisms inside the same strain (Chopra, 2000). Although, in past decades, the pharmacological companies have produced a number of new antibiotics, even then drug resistance has increased. This situation has prompted researchers towards herbal products, in search of development of better-quality drugs with improved antibacterial, antifungal, and antiviral activities. The outburst of drug resistant microbial strains necessitates the studies for synergistic effects of antibiotics in combination with plant's derivatives to develop the antimicrobial cocktail with a wider spectrum of activity and reduction of adverse side effects of antimicrobial agents. *Staphylococcusaureus*resistance to the penicillin group of antibiotics is increasing associated with appearance of adverse side effects such as hypersensitivity and anaphylactic reactions (Odds, 2003).

This study was carried out to investigate and compare the *invitro* antibacterial and antifungal activities of the methanolic and ethanolic extracts of *C. albidum* and *D.monbuttensis* leaves.

MATERIALS AND METHODS

Collection and preparation of plant materials

Leaves of *C. albidum*and *D. monbuttensis*were collected at Covenant University field, Ota, Ogun state, Nigeria. Authentication

Table 1. Antimicrobial activity of the methanolic leaf extract of *Diospyrosmonbuttensis*

Organisms	Diameter of zone of inhibition (mm)					
	100	50	25	12.5	6.25	3.125(mg/ml)
Staphylococcus spp.	10	R	R	R	R	R
Streptococcus spp.	10	10	R	R	R	R
Escherichia coli (Pathogenic)	11	10	R	R	R	R
*Klebsiella*spp.	R	R	R	R	R	R
Candida albicans	10	9	9	9	9	8
Aspergillusniger	R	R	R	R	R	R

R, Resistant.

was carried out in the Department of Biological Sciences (Applied Biology and Biotechnology section) of Covenant University, Ota, Ogun State.They were washed and then air dried for 2 to 3 weeks. The leaves were then reduced to fine powder with the aid of a mechanical blender.

Collection of clinical isolates

The clinical test isolates used in this study are*Staphylococcus* sp., *Streptococcus*sp., *Escherichia coli* (Pathogenic), *Klebsiella*sp.,*Candida albicans*and *Aspergillusniger.* Purecultures of bacterial, yeast and the filamentous fungal isolates were sourced from the Microbiology laboratory, Department of Biological Science, Covenant University, Ota, OgunState, Nigeria.

Preparation of the ethanolic and methanolic extracts

Cold extraction was used in performing the extraction from the leaves. Extracts were produced using ethanol and methanol as solvents. Filtrate was concentrated using rotary evaporator and then weighed after the solvent had been removed.

Standardization of microbial Inocula

Bacterial and fungal isolates were sub-cultured onto freshly prepared Nutrient agar and Potato Dextrose agar plates and incubated for 24 h at 37°C and 3 to 5 days at room temperature respectively. A portion of the streaked bacterial colonies and a small inoculum from the lawn of fungal growth was transferred into McCartney bottles containing 1ml of sterile distilled water. Vortexing was carried out and the turbidity was adjusted to match 0.5 McFarland standards (10^6 cfu/ml and 10^6 spores /ml).

Determination of antimicrobial activity

The agar-well diffusion assay as described by Vollekovaet al. (2001) was used to ascertain the inhibitory effects of the respective leaf extracts on the test isolates. The tests were carried out using a stock concentration of 100 mg/ml. Mueller-Hinton agar plates were seeded with 0.1ml of standardized bacterial and fungal cultures. The microbial lawn was done using a sterile glass rod and the seeded plates were allowed to dry. A sterile cork borer was used to punch 2 equidistant holes in the middle of the labelled inoculated agar plates and filled with 0.2 ml of the same concentrations of the two leaf extracts. Following the diffusion of the extracts into the agar at room temperature, the bored agar plates were incubated at 37°C for 24 h for bacteria isolates while those with the filamentous fungal cultures were kept at room temperature for 3 to 5 days and observations made at the end of the incubation period. The antibacterial activity of the leaf extracts was assessed by an inhibition zone surrounding the well while the antifungal activity was measured after 3 to 5days incubation at room temperature using a meter rule. The mean zones of inhibition was measured and expressed in millimeters. For the positive control, a standard antibiotic (Gentamycin) was used for comparison while for the negative control, DMSO was used.

Determination of minimum inhibitory concentration (MIC)

The MIC of the methanolic and ethanolic leaf extracts of *D.monbuttensis* and*C.albidum*were determined by the broth dilution method (Asowataet al., 2013). The plant extracts were prepared to the highest concentration of 100 mg/ml in 25% of DMSO and serial double dilutions were made to give concentrations ranging from 50 mg / ml to 3.125mg/ ml(from earlier studies). 1 ml of Nutrient broth and 1 ml of each leaf extract were put into different test-tubes according to the varied concentrations. 0.2 ml of the standardized microbial cultures was inoculated into the labeled tubes containing the diluted extracts and the Nutrient broth. The tubes were incubated at 37°C for 24 hfor bacteria and fungi. The least concentration of the extract which inhibited the growth of the inoculum was considered as the minimum inhibitoryconcentration.

RESULTS

Results showed that as the concentrations increased, there was a corresponding increase in the zones ofinhibitions. Tables 1 to 3 show the zones of inhibition of the organisms due to the antimicrobial activities of the methanol andethanol extracts of *D. monbuttensis*, and the ethanolic extracts of *C. albidum* respectively. The zones of inhibition for all the test isolates using methanolic leaf extract of *D. monbuttensis* ranged from 8mm for *C.albicans* to 11mm for *E.coli* (pathogenic) while for the ethanolic leaf extract of *D. monbuttensis*, zone of inhibition ranged from 10 to 18mm for all of the test isolates (Tables 1 and 2). In Table3, the inhibition zone of ethanolic extract of *C. albidum* against the test isolates ranged from 10 to 22mm with *A. niger* showing resistance at all concentrations.

Tables 4 and 5 show the mean values for the antimicrobial activity of methanolic leaf extract of *D.*

Table 2. Antimicrobial activity of ethanolic leaf extract of *D. monbuttensis.*

Organism	Diameter of zone of inhibition (mm)				
	100	**50**	**25**	**12.5**	**6.25(mg/ml)**
Staphylococcus spp.	14	13	11	10	10
Streptococcus spp.	16	15	15	14	12
E. coli (Pathogenic)	17	15	11	11	10
*Klebsiella*spp.	18	17	15	13	12
C.albicans	R	R	R	R	R

R, Resistant.

Table 3. Antimicrobial activity of ethanolic leaf extract of *C.albidum.*

Organism	Diameter of zone of inhibition (mm)					
	100	**50**	**25**	**12.5**	**6.25**	**3.125 (mg/ml)**
Staphylococcus spp.	12	11	10	R	R	R
Streptococcus spp.	13	13	12	10	R	R
E. coli (Pathogenic)	15	13.5	11	10	9	R
*Klebsiella*spp.	22	18	10	R	R	R
C.albicans	21	19	17	11	10	10
A. niger	R	R	R	R	R	R

R, Resistant.

Table 4. Mean values for the antimicrobial activity of methanolic leaf extract of *D. monbuttensis* and ethanolic leaf extract of *C. albidum.*

Organism	Diameter of zone of inhibition (mm)	
	D. monbuttensis	***C. albidum***
Staphylococcus spp.	10	11
Streptococcus spp.	10	12
E. coli (Pathogenic)	10.5	11.7
*Klebsiella*spp.	R	16.67
C.albicans	9	14.67
A. niger	R	R

R, Resistant.

monbuttensis and ethanolic leaf extract of C. albidum ranging from 9 to 16.6 mm for the former and 11.6 to 15 mm for the latter extract with A. niger and C. albicans showing resistance in the mean values obtained.

The ethanolic leaf extract of C. albidum showed highest MIC values at 25 mg/ml against Streptococcus, E. coli and C. albicans while the ethanolic leaf extract of D. monbuttensis revealed its highest MIC at 25 mg/ml against Stapylococcus and Streptococcus (Tables 6 and 7). The highest MIC value shown by the methanolic leaf extract of D. monbuttensis was against C. albicans at 3.125 mg/ml while the other test isolates were resistant (Table 7).

DISCUSSION

Antimicrobial properties of substances are desirable tools in the control of undesirable microorganisms especially in the treatment of infectious diseases. The natural products isolated from plants used in traditional medicine, which have potent antiplasmodial activity in vitro, represent potential sources of new anti-malarial drugs (Olasehinde et al., 2014). The active components usually interfere with growth and metabolism of microorganisms in a negative manner (Aboaba et al., 2012).

The aqueous and alcoholic extracts from the leaves of *D. monbuttensis*were found tohave strong antibacterial

Table 5. Mean values for the antimicrobial activity of ethanolic leaf extract of *D. monbuttensis.*

Organism	Diameter of zone of inhibition (mm) *D. monbuttensis*
Staphylococcus spp.	11.6
Streptococcus spp.	14.4
E. coli (Pathogenic)	12.8
*Klebsiella*spp.	15
C.albicans	R

R, Resistant.

Table 6. Minimum inhibitory concentration of methanolic extract of *D. monbuttensis* and ethanolic leaf extract of *C. albidum*

Organism	Minimum inhibitory concentration (mg/ml)	
	D. monbuttensis	*C. albidum*
Streptococcus spp.	R	25
E. coli (Pathogenic)	R	25
*Klebsiella*spp.	R	50
C.albicans	100	25

R, Resistant.

Table 7. Minimum inhibitory concentration of ethanolic leaf extract of *D. monbuttensis.*

Organisms	Diameter of zone of inhibition (mm) *D. monbuttensis*
Staphylococcus spp.	25
Streptococcus spp.	25
E. coli (Pathogenic)	100
*Klebsiella*spp.	50
C. albicans	R

R, Resistant.

activity. The antimicrobial activities of the plant leaves extract was due to the presence of tannins. *D.monbuttensis*has shown strong antibacterialactivity against a wide range of gram-positiveand gram-negative bacteria (Anieet al., 2011). Bouquet and Debray reported similar results and showed the presence in the leaves of some quinones, tannins, sterols and saponins and an absence of flavonoids and alkaloids.

The antimicrobial activity of the methanol and ethanol leaf extracts of *D.monbuttensis*, and the ethanol leaf extract of*C.albidum*were reported in this study (Tables 1 to 3). The result of the antimicrobial screening which showed that the test isolates were susceptible to methanol and ethanol extracts of different plants. This indicates that some of the antimicrobial compounds in the investigated plants might be polar. The zones of inhibition of growth of the microorganism are a function of the relative antibacterial and antifungal activity of the extracts. The MIC of the methanol and ethanol leaf extracts of *D. monbuttensis* and *C.albidum* plants against the test microorganisms ranged from 3.125 to 100mg/ml, while the MIC of the ethanol leaf extracts of *D. monbuttensis*plant against the test microorganisms ranged from 6.25 to 100mg/ml. The effect of the plant extract on the MIC for the test microorganisms varied widely in the degree of their susceptibility (Elekwaet al., 2009). An antimicrobial activity of plant extracts with highly active antimicrobial agent gives a low MIC while a low activity against a microorganism has a high MIC.

The presence of plant secondary metabolites has been implicated for most plants therapeuticactivities

(Geyidetal., 2005). Also, the plants containing these metabolites

(alkaloids, flavonoids, tannin, saponins, etc) usually exhibits stronger antimicrobial properties than others(Hutchinson et al., 1963). The presence of these phytochemicals in the investigated plants mayhave contributed to their effect as remedy for various diseases. This suggests that thepresence of potent antibacterial activity of the leaves extracts of the investigated plants againstthe bacteria might be due to naturally occurring bioactive phytochemicals present in the plantmaterials. The high degree of antimicrobial activity of some of the plants seems to confirm thefolk therapy of infections and traditional therapeutic claims of these herbs.

Furthermore, as concentrations increased, there was a corresponding increase in the zones ofinhibitions. The zone of inhibition due to the antimicrobial activities of the methanolic andethanolic extracts of *D. monbuttensis*, and the ethanolic extracts of *C. albidum* respectivelyarepresented in Tables 1 to3. The inhibitory zones elaborated by the test isolates exposedto*C. albidum*ranged from for 10 mm *Staphylococcus*spp. to 22 mm for *Klebsiella*spp. (Table3). Zones of inhibition exhibited by the exposed isolates to *D. monbuttensis*alcoholic extractranged from 8 mm for *C.albicans*to 10 mm for *E. coli* and from 10 mm for*Staphylococcus spp.* to 18 mm for *Klebsiella*spp(Tables 1 and 2). The observed antimicrobialactivity of the respective leaf extracts might also be dependent on both the concentration aswell as nature of the extraction solvent used. Comparatively, the *C. albidum* leaf extractexhibited a greater antifungal activity against the fungal isolates than the *D. monbuttensis* leafextract (Tables 1 to3). The highest MIC values were displayed by the ethanolicleafextract of *C. albidum* at25 mg/ml against *Streptococcus*, *E. coli* and *C.albicans* and theethanolic leaf extract of *D. monbuttensis* at 25 mg/ml against *Stapylococcus* and *Streptococcus*(Tables 6 and 7). *C. albicans* and *E. coli* exhibited the lowest MIC reading at 100 mg/mlagainst *D. monbuttensis*methanolic and ethanolic leaf extracts (Tables 6 and 7).*Streptococcus*, *E.coli* and *Klebsiella* showed resistance against *D. monbuttensis*methanolicleafextract, while *C. albicans* showed resistance against *D. monbuttensis*ethanolicleaf extract(Tables 6 and 7).

Also, the results obtained from previous studies on *C. albidum* showed that, theinhibitory zones elaborated by the test isolates exposed to *C. albidum*ethanolic root extractranged from 8 ± 0.06 mm for *S. aureus* to 18 ± 0.03 mm for *E. coli*. Also the inhibitory zonesdisplayed by the test isolates exposed to *C. albidum*chloroform root extract ranged from 10.7± 0.05 mm for *S. aureus*to 26 ± 0.02 mm for *E. coli*, with *A. niger* showing resistance to bothextracts. Also, the results from previous studies carried out on *D. monbuttensis*(Anieet al., 2011)showed that, theaqueous extract of root bark was active against gram positive organisms at 100 mg/ml and thepetroleum and chloroform spirit extracts showed antifungal activities at 100 mg/ml.Comparatively, the ethanolic leaf extract of*C. albidum* from this present study was more effective

than the ethanolic root extract against the test isolates used, but it was not moreeffective than the chloroform extract. For *D. monbuttensis*, the aqueous root bark extract fromthe previous study was less effective than the ethanolic leaf extract from this present study,against gram positive organisms, but more effective than the methanolic leaf extract. Themethanolic leaf extract from this study had the same effect as the petroleum and chloroformspirit root bark extracts from the previous study against the fungal isolates(Anieet al., 2011). The petroleum andchloroform spirit root bark extracts of *D. monbuttensis* from the previous study had more effects on the fungal isolates than the ethanolic leaf extracts from this present study.

Conclusion

The ethanolic leaf extracts of *C. albidum*were comparatively more potent against the test isolates than the ethanolic and methanolic leaf extracts of *D. monbuttensis*, based on the MIC results. All the respective leaf extracts exhibited a greater antibacterial activity in comparison with the antifungal attributes. The presence of bioactive antimicrobial compounds in the examined alcoholic and chloroform extracts of the medicinal plants indicate the possibility of obtaining potentially valuable antimicrobial phytochemicals from these plants. The results obtained from this study support the use of these plant parts in the traditional treatment of diseases in Nigeria. The results of this finding could be very important to pharmaceutical industries in the development of new antimicrobial drugs in order to address unmet therapeutic needs. Such screening of various natural organic compounds and identification of active agents is the need of the hour for saving life and providing good health to humanity. There is need for further studies on the plant parts in order to isolate, identify, characterize and elucidate the structure of antimicrobial bioactive compounds.

Conflict of Interests

The authors have not declared any conflict of interest.

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