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Evaluation of the Antimicrobial Properties of the Ethanolic Extracts of some Medicinal Plant Seeds from South-West Nigeria

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Abstract: *The present study was conducted to evaluate the antimicrobial properties of the ethanolic extracts of some medicinal plant seeds against some clinical pathogens. The medicinal plant seeds investigated were Canna bidentata, Ceasalpinia bunduc, Hunteria umbellata, Hydrocotyle asiata, Megaphrynium macrostarchyum, Perinari excelsa, Rauwolfia vomitoria, Solanum dasyphyllum, Cola millenii and Sphenocentrum jollyanum. And the micro-organisms used for the antimicrobial assay were seven clinical pathogens, four bacteria: Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and three fungi: Aspergillus niger, Penicillium notatum, and Candida albican. The extraction of the bioactive component of the seeds was done by cold extraction using ethanol as solvent and the antimicrobial assay was carried out using agar well diffusion method. The ethanolic extracts of all the selected seeds were active against all tested pathogens with maximum antimicrobial activity observed in S. dassyphylum ranging from 26 mm to 19 mm and minimum in M. macrostachyum ranging from 20 mm to 12 mm at concentration range of 200 mg/ml to 25 mg/ml. For minimum inhibitory concentration (MIC) at concentration of 12.25 mg/ml S. dassyphylum was active against Escherichia coli and Staphylococcus aureus at 25 mg/ml while S. jollyanum was active against Bacillus subtilis at 25 mg/ml. The broad spectrum of the antimicrobial activities observed in this study is an indicative that the ethanolic extract of these plant seeds possess significant antibacterial and antifungal properties that could probably serve as antimicrobial agents in new drug formulation against pathogenic microoganisms.*

Key words: *Antimicrobial, medicinal seeds, MIC, ethanolic extracts*

I. Introduction

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi and viruses. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Anti-microbial drugs play an important role in the treatment of many infectious diseases. Anti-microbials are given to weaken or kill some of the invading pathogens. Hopefully, the body tissues can then destroy the rest. Antimicrobial drugs are used in relatively low concentrations in or upon the bodies of organisms to prevent or treat specific infectious diseases without harming the host organism. The course of an infection is often linked to a race between the pathogen's ability to grow in the host tissue and the tissues ability to capture and destroy the invading pathogen. Despite, the wide availability of clinically useful antibiotics and semi-synthetic analogues, a continuing search for new anti-infective agents remains indispensable. Major drawbacks of these are of limited spectrum or serious side effects. Moreover, the combination of the genetic versatility of microbes and widespread overuse of antibiotics has led to increasing clinical resistance of previously sensitive microorganisms and the emergence of previously unknown infections. One of the possible strategies for finding new, anti-infective drugs could involve the search for compounds with structures widely different from those in current use. So plants can be a major source for search of new anti-infective agents.

Plants provide a variety of resources that contribute to the fundamental needs of human such as food, clothing and shelter. Among plants of economic importance are medicinal plants. Plants have been utilized as therapeutic agents since time immemorial in both organized and unorganized forms (Girach et al., 2003).The healing properties of many herbal medicines have been recognized in many ancient cultures. Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. The therapeutic use of plants certainly goes back to the Sumerian and the Akkadian civilizations in about the third millenium BC. Hippocrates (ca. 460–377 BC). Natural products have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Ayurvedic and Egyptian (Sarker & Nahar 2007). Traditional medicine has and still remains the main source of therapy for a large majority of people in Nigeria for treating health problems and traditional medicine consultancy as well as consumption of the medicinal plants has a much lower cost than modern medicine. Traditional medicine is used throughout the world as it is dependent on locally available plants, which are easily accessible, and capitalizes on traditional wisdom-repository of knowledge, simple to use and affordable (Tesfaye & Sebsebe 2009). The traditional methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover, the use of herbal remedies has increased in the developed countries in the last decades.

Medicinal plants have been known for their healing and disease-curing qualities for centuries. Some drugs of plant origin used in conventional medical practice are direct plant extracts or plant materials that have been suitably prepared and standardized (Donald 1986). The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine (Dubey et al., 2011). Since antiquity, many plants species have been reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes which are therefore, should be utilized to combat the disease causing pathogens (Kamali & Amir 2010). Nowourdays, throughout the world, infectious diseases accounts for high proportion of health problems. Mortality due to these infections continues to be a major problem, especially amongst children. Infections due to a variety of bacterial etiologic agents, such as pathogenic Escherichia coli, Staphylococcus aureus and Enterobacter sp. are most common (Mukherjee et al., 1998). Many readily available plants in Nigeria are used in traditional folklore medicine for the treatment of several infections including typhoid fever and gastrointestinal disorders such as cholera, diarrhea and dysentery. Though large numbers of plants are constantly being screened for their antimicrobial effects yet, more pharmacological investigations are necessary (Pankaj et al., 2008). Therefore, the current study was designed to evaluate the antimicrobial properties of some medicinal plant seeds which have been traditionally used in the treatment of various infections against some selected bacteria and fungi pathogens frequently involved in severe infections in humans. This in continuation of the report by Ajayi and Ojelere (2013) on the chemical composition of the medicinal seeds that are been investigated in this study.

II. Material and Methods

Collection and Identification of Plant

Fresh ten different plant seeds viz., Hydrocotyle asiata, Hunteria umbellata, Megaphrynium macrostarchyum, Perinari excelsa, Solanum dassyphylum, Canna bidentata, Ceasalpinia bunduc, Rauwolfia vomitoria, Cola millenii and Sphenocentrum jollyanum free from disease were purchased from Ojee market in Ibadan North-East local Government, Oyo state, Nigeria. The plant seeds were identified and authenticated at Herbarium Unit of Botany Department, University of Ibadan, Oyo state, Nigeria. The seeds were air-dried and screened to remove undesirable materials such as stones and other impurities, after which they were dehulled, milled into powder and the powder kept in an airtight polythene bags until needed for analysis.

Collection of Microorganisms

Four clinical bacteria isolates made up of two gram-positive (Staphylococcus aureus, Escherichia coli), two gram-negative (Pseudomonas aeruginosa and Bacillus subtilis) were used for the antibacterial assay and three fungi (Candida albicans, Penicillium notatum and Aspergillus niger) were used for the antifungal assay. All the organisms were pure isolates obtained from the Laboratory stock culture unit of the Department of Pharmacological Microbiology, Faculty of Medicine, University of Ibadan, Oyo State, Nigeria.

Preparation of plant extracts

The ethanolic extract of the seeds was obtained by using the method previously described by (Owolabi et al., 2007). 100g of the powdered sample was soaked in 400ml of solvent in a sterile conical flask and covered with cotton wool. It was then plugged and wrapped with aluminum foil and shaken vigorously. The mixture was left to stand for 72 hours at room temperature of 25 ^{0}C. The mixture was then filtered using Whatman No. 1 filter paper. Thereafter, the filtrate was evaporated to dryness by means of a rotary evaporator attached to a vacuum pump. The extracts were stored in refrigerator until needed for further analysis.

Determination of antimicrobial activity

The antimicrobial activity of selected plant seeds against clinical pathogens was determined by using agar well diffusion method based on the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS 2002). Sterile nutrient agar plates were prepared for bacterial strains and Sterile Sabouraud's dextrose agar (SDA) were prepared for fungal strains inoculated by a spread plate method under aseptic conditions. 20 ml of sterilized nutrient agar was poured into Petri dishes and allowed for solidification. After solidification, 24 hours nutrient broth grown pathogenic cultures were swabbed on the respective agar plates using sterilized cotton swabs. Wells of 6 mm diameter were punched over the agar plates using a sterile gel puncher. About 100 µl of different concentrations of plant solvent extracts were added using sterile syringe into the wells and allowed to diffuse at room temperature for 1hour and the plates were incubated at 37 °C for 18-24 hours for bacterial pathogens and 28 °C for 48 hours fungal pathogens respectively. After incubation, the diameter of inhibition zones formed around each wells were measured and expressed in millimeter (mm) and recorded against the corresponding concentrations to evaluate the antimicrobial activity. Positive controls were set using standard antibiotics drugs (Gentamycin) while negative controls were set using ethanol.

Determination of minimum inhibition concentration (MIC)

The most sensitive plant seeds extracts were used in the MIC (bacteriostatic concentration) determination using well diffusion method. The inoculum of microorganisms was prepared from 18 hours nutrient broth cultures. In this method, the broth dilution technique was utilized where the plant extract was prepared to the highest concentration of 200 mg/ml (stock concentration) in ethanol and serially diluted to a working concentration ranging from 200 mg/ml to 6.25 mg/ml using Nutrient Agar and later inoculated with 1 ml suspension of the test organisms. The positive control was Nutrient Agar with standard reference antibiotics (Gentamycin) and inoculums. After 18-24 hours of incubation at 37 ^{o}C for bacterial pathogens and 48 hours at 28 °C for fungal pathogens, the test tubes were observed for turbidity. All the experiments were done in triplicates.

III. Result and Discussion

The scientific, family, English and local names of the seeds investigated are listed on Table 1. Medicinal plants are very important and widely available resource for primary and complementary healthcare systems. It has been reported in literature that plant kingdom contains many species of plants harboring substances of medicinal value that are yet to be discovered. Though large numbers of plants are constantly being screened for their antimicrobial effects yet, more pharmacological investigations are necessary (Pankaj et al., 2008). The present study revealed the antimicrobial activity of ethanolic extracts of some medicinal plant seeds from South-west, Nigeria against some selected clinical pathogens such as B. subtilis, E. coli, S. aureus, P. aeruginosa, P. notatum, A. niger and C. albicans by agar well diffusion method. All the examined plant extracts showed varying degrees of antimicrobial activities against the clinical pathogens tested. The antimicrobial activity of ethanol extracts of these seeds were observed to be dosage-dependent and the activity varies with concentration against tested pathogens as shown in table 2 and minimum inhibitory concentration (MIC) as shown in table 3 was observed only in the plant extracts with strong inhibitory effect of antimicrobial activities. Despite what many researchers have reported that C. albicans are very resistant fungi, this work demonstrated that ethanolic extract of all the selected seeds were effective against this pathogenic fungi at concentration of 200 mg/ml and 100 mg/ml respectively. This result is in agreement with the report of Pavendan & Sebastian (2012) on the leaf extract of E. singampattiana which was very effective on C. albicans. The ethanol extract of S. dassyphylum seeds was observed to show highest antimicrobial activity among the plant seeds extracts investigated in which all the serial diluted concentration were active against the tested pathogen except in C. albicans which was resistant to the extract at concentration of 25 mg/ml, with minimum MIC of 12.25 mg/ml against E. coli and 25 mg/ml against S. aureus. P. excelsa extracts was observed to show good activity just like S. dassyphylum, with maximum zone of inhibition (26 mm) against C. albicans and lowest inhibitory zone of 16 mm against A. niger at concentration of 200mg/ml with lower concentration showing little effect against the tested pathogens. This activity was observed to be within the range of activity showed by P. excelsa according to Stephen & Joseph (2011). H. unbellata and H. asiata seeds extracts were observed to have comparatively similar activity against the selected pathogen with maximum inhibitory zone of 22 mm and 20 mm with the same minimum zone of inhibition of 16 mm at the concentration of 200mg/ml with MIC of 100 mg/ml in H. asiata.

The ethanolic extract of C. bidentata showed maximum zone of inhibition (21 mm) against C. albicans. Also, minimum inhibitory zone (12 mm) was exhibited against P. aeruginosa and S. aureus at the same concentration of 200 mg/ml. The extract of C. bidentata was observed to show no reasonable inhibition against S. aureus, P. notatum and C. albicans at concentration of 100 mg/ml and bellow. For C. millenii seeds extracts maximum zone of inhibition (19 mm) was observed against B. subtilis and C. albicans, and minimum inhibitory zone (15 mm) was shown against S. aureus while B. subtilis, P. notatum and C. albicans were resistant to the extract at the concentration of 50 mg/ml and below with minimum inhibitory concentration (MIC) of 50 mg/ml against P. aeruginosa and Escherichia coli, which was observed to be more active than the report of Giwa et al., (2012)in which some of the microbes were resistant to the plant extract. S. jollyanum was observed to show good antimicrobial activity in which almost all the serial diluted concentration were active against the tested pathogen except in P. notatum at concentration of 50 mg/ml, with minimum MIC of 25 mg/ml against B. subtilis. C. bunduc and R. vomitoria were also observed to show good inhibitory effect against the tested organisms, the activity was observed to depend on the concentration of the extracts with minimum inhibitory concentration of 50 mg/ml observes in C. bunduc against S. aureus. M. macrostachyum seeds extract was observed to show least activity among the investigated seeds with maximum zone of inhibition of 20 mm and the lower concentrations were not as active as expected. Some the investigated seeds such as S. dassyphylum, P. excelsa and S. jollyanum were observed to be more active than the control drug gentamycin at the concentration of 200 mg/ml. Generally, S. dassyphylum has the highest antimicrobial activity with maximum zone of inhibition of 26 mm while M.macrostachyum has the lowest activity with maximum zone of inhibition of 20 mm. The implication of the broad spectrum action of some of these extracts is that they can be useful in antiseptic and disinfectant formulation as well as in chemotherapy if the active principle can be isolated (Olukoya et al., 1993). The results of this study showed that the extraction of antimicrobial substances by

organic solvents is better when compared to aqueous extracts which suggests that ethanol is more effective solvent for extracting the bioactive compounds from the seeds.

The polarity of antibacterial compounds make them more readily extracted by organic solvents, and using organic solvents does not negatively affect their bioactivity against pathogenic bacteria species (Thongson et al., 2004). The outcome of this work is a good evidence to validate the use of these seeds in traditional folklore medicine for the treatment of several infections including typhoid fever and gastrointestinal disorders such as cholera, diarrhea and dysentery in Nigeria.

IV. Conclusion

All the medicinal plants seeds investigated in this study exhibited reasonable degrees of antimicrobial properties against the clinical pathogens tested as revealed by the broad spectrum of the zone of inhibition and minimum inhibitory concentration. Our findings revealed that the ethanolic extracts of these plant seeds possess compounds with antimicrobial properties that could probably be used as antimicrobial agents in new drugs development.

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Table 1: Scientific, Family, English and Local names of the seeds investigated[a]

Scientific Name	Family Name	English Name	Local Name
Canna bidentata	Cannaceae	NA	Ido
Ceasalpinia bunduc	Fabaceae	Gray Nicker Nut	Ayo
Cola millenii	Sterculiaceae	Monkey Kola	Obi Edun
Hunteria umbellata	Apocynaceae	NA	Abeere
Hydrocotyle asiata	Sterculiaceae	Memory nut	Obi Awogba arun
Megaphrynium macrostarchyum	Marantaceae	NA	Gbodogi
Perinari excelsa	Chrysobalanaceae	Grey plum	Yinrinyinrin
Rauwolfia vomitoria	Apocynaceae	Poison devil's pepper	Asofeyeje
Solanum dasyphyllum	Sterculiaceae	NA	Bamoni
Sphenocentrum jollyanum	Menispermaceae	Dog's penis	Akerejupon

NA=Not available
[a]Ajayi &Ojelere, 2013

Table 2: Antimicrobial activity of the ethanolic extracts of the selected seeds on isolated pathogens

Isolated bacteria and fungi	Concentration in (mg/ml)				Gentamycin
	200	100	50	25	10µg/ml
	P.excelsa seeds extract , zone ,of inhibition in (mm)				
Bacillus subtilis	18	16	15	12	23
Staphylococcus aureus	22	18	17	14	21
Pseudomonas aeruginosa	17	15	13	10	20
Escherichia coli	20	18	17	16	23
Aspergillus niger	16	-	-	-	19
Penicillium notatum	20	18	-	-	20
Candida albicans	26	18	-	-	19
	H. unbellata seeds extract , zone of inhibition in (mm)				
Bacillus subtilis	22	18	16	12	24
Staphylococcus aureus	18	15	-	-	20
Pseudomonas aeruginosa	16	14	-	-	18
Escherichia coli	19	17	13	-	23
Aspergillus niger	18	15	-	-	19
Penicillium notatum	17	15	13	-	20
Candida albicans	19	15	-	-	21
	S.dassyphylum seeds extract, zone of inhibition in (mm)				
Bacillus subtilis	23	19	17	14	24
Staphylococcus aureus	22	20	19	16	20
Pseudomonas aeruginosa	19	17	16	12	20
Escherichia coli	26	23	20	18	23
Aspergillus niger	19	17	15	12	20
Penicillium notatum	25	22	17	14	21
Candida albicans	26	23	19	-	21
	H.asiata seeds extract, zone of inhibition in (mm)				
Bacillus subtilis	20	18	16	14	23
Staphylococcus aureus	16	15	13	10	20
Pseudomonas aeruginosa	18	16	13	10	19
Escherichia coli	20	17	14	11	22
Aspergillus niger	16	14	13	10	19
Penicillium notatum	18	16	13	12	20
Candida albicans	20	16	12	-	21
	P.excelsa seeds extract , zone ,of inhibition in (mm)				
Bacillus subtilis	18	16	15	12	23
Staphylococcus aureus	22	18	17	14	21
Pseudomonas aeruginosa	17	15	13	10	20
Escherichia coli	20	18	17	16	23
Aspergillus niger	16	-	-	-	19
Penicillium notatum	20	18	-	-	20
Candida albicans	26	18	-	-	19
	H. unbellata seeds extract , zone of inhibition in (mm)				
Bacillus subtilis	22	18	16	12	24
Staphylococcus aureus	18	15	-	-	20
Pseudomonas aeruginosa	16	14	-	-	18
Escherichia coli	19	17	13	-	23
Aspergillus niger	18	15	-	-	19
Penicillium notatum	17	15	13	-	20
Candida albicans	19	15	-	-	21
	S.dassyphylum seeds extract, zone of inhibition in (mm)				
Bacillus subtilis	23	19	17	14	24
Staphylococcus aureus	22	20	19	16	20
Pseudomonas aeruginosa	19	17	16	12	20
Escherichia coli	26	23	20	18	23
Aspergillus niger	19	17	15	12	20
Penicillium notatum	25	22	17	14	21
Candida albicans	26	23	19	-	21
	H.asiata seeds extract, zone of inhibition in (mm)				
Bacillus subtilis	20	18	16	14	23
Staphylococcus aureus	16	15	13	10	20
Pseudomonas aeruginosa	18	16	13	10	19
Escherichia coli	20	17	14	11	22
Aspergillus niger	16	14	13	10	19
Penicillium notatum	18	16	13	12	20
Candida albicans	20	16	12	-	21

	M.macrostachyum seeds extract,zone of inhibition in (mm)				
Bacillus subtilis	17	13	-	-	23
Staphylococcus aureus	12	10	-	-	20
Pseudomonas aeruginosa	15	-	-	-	18
Escherichia coli	16	14	-	-	22
Aspergillus niger	18	16	15	11	19
Penicillium notatum	20	15	-	-	20
Candida albicans	20	16	12	10	21
	C. millenii seeds extract, zone of inhibition in (mm)				
Bacillus subtilis	19	14	-	-	24
Staphylococcus aureus	15	13	10	-	20
Pseudomonas aeruginosa	17	15	13	10	18
Escherichia coli	18	16	10	-	23
Aspergillus niger	17	13	10	-	19
Penicillium notatum	16	14	-	-	20
Candida albicans	19	15	-	-	21

Table 3: Minimum Inhibitory concentration (MIC) of ethanolic extracts of the selected seeds on isolated pathogens at different concentration in (mg/ml)

Isolated bacteria and fungi	Concentration in (mg/ml)	MIC					
	200	100	50	25	12.5	6.25	
	S.dassyphylum seeds extract , MIC						
Bacillus subtilis	-	-	+	+	+	+	100
Staphylococcus aureus	-	-	-	-	+	+	25
Pseudomonas aeruginosa	-	+	+	+	+	+	200
Escherichia coli	-	-	-	-	-	+	12.25
Aspergillus niger	-	-	+	+	+	+	100
Penicillium notatum	-	+	+	+	+	+	200
Candida albicans	-	-	+	+	+	+	100
	P.excelsa seeds extract ,MIC						
Bacillus subtilis	-	-	+	+	+	+	100
Staphylococcus aureus	-	-	-	+	+	+	50
Pseudomonas aeruginosa	-	-	+	+	+	+	100
Escherichia coli	-	-	-	+	+	+	50
Aspergillus niger	+	+	+	+	+	+	ND
Penicillium notatum	-	+	+	+	+	+	200
Candida albicans	-	-	+	+	+	+	100
	H.asiata seeds extract , MIC						
Bacillus subtilis	-	+	+	+	+	+	200
Staphylococcus aureus	-	+	+	+	+	+	200
Pseudomonas aeruginosa	-	-	+	+	+	+	100
Escherichia coli	-	-	+	+	+	+	100
Aspergillus niger	-	+	+	+	+	+	200
Penicillium notatum	-	-	+	+	+	+	100
Candida albicans	-	-	+	+	+	+	100
	S. jollyanum seeds extract , MIC						
Bacillus subtilis	-	-	-	-	+	+	25
Staphylococcus aureus	-	-	+	+	+	+	100
Pseudomonas aeruginosa	-	+	+	+	+	+	200
Escherichia coli	-	-	-	+	+	+	50
Aspergillus niger	-	-	+	+	+	+	100
Penicillium notatum	-	+	+	+	+	+	200
Candida albicans	-	-	+	+	+	+	100
	C.bunduc seeds extract ,MIC						
Bacillus subtilis	+	+	+	+	+	+	ND
Staphylococcus aureus	-	-	-	+	+	+	50
Pseudomonas aeruginosa	-	+	+	+	+	+	200
Escherichia coli	-	-	+	+	+	+	100
Aspergillus niger	-	+	+	+	+	+	200
Penicillium notatum	+	+	+	+	+	+	ND
Candida albicans	-	+	+	+	+	+	200
	C. millenii seeds extract, MIC						
Bacillus subtilis	-	+	+	+	+	+	200
Staphylococcus aureus	-	+	+	+	+	+	200
Pseudomonas aeruginosa	-	-	-	+	+	+	50
Escherichia coli	-	-	-	+	+	+	50
Aspergillus niger	-	-	+	+	+	+	100
Penicillium notatum	-	-	+	+	+	+	100
Candida albicans	-	+	+	+	+	+	200

Growth; - No growth; ND Not Detected