EVALUATION OF THE ANTIMICROBIAL AND PHYTOCHEMICAL PROPERTIES OF A HERBAL PREPARATION

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ABSTRACT: Joloo is a herbal preparation locally used in the management of breast tumour in southwestern Nigeria. This study was conducted to evaluate the antimicrobial and phytochemical properties of the formulation. The organisms implored in this study include *Klebsiella pneumoniae*, *Escherichia coli* (ATCC 12900), *Pseudomonas aeruginosa*, *Shigellia dysenteriae*, *Proteus vulgari*, *Yersinia enterocolitica*, and *Salmonella typhi* and fungi *(Aspergillus niger*, *Trichoderma* Spp, *Rhizopus* Spp *and colletotrichum gloeosporioides).* The antimicrobial screening of Joloo was carried out invitro in three different concentrations (500mg/ml, 1000mg/ml and 1500mg/ml) against standard broad spectrum antibacterial (Amoxicillin) and antifungal (fluconazole) as control using the agar well dilution method. The standard qualitative analysis method was used for the phytochemical screening. The study revealed that Joloo inhibited *P.vulgari*, *Y.enterocolitica*, *E. coli*, *P. aeruginosa*, *S. dysenteriae* and *S. typhi* with 20, 15, 14, 13, 12, and 10mm respectively. However *Klebsiella pneumonia* was resistant to the preparation. Joloo also inhibited *Aspergillus niger*, *Trichoderma* Spp, *and Rhizopus* Spp with 14, 8 and 7mm respectively while *Colletotrichum gloeosporioides* was resistant to Joloo. The MIC observed for all the organisms inhibited was 500mg/ml. Phytochemical screening confirmed the presence of alkaloids, saponins, terpenoids, tannins, phlobatannins, cardenolides, flavonoids, phenols and free and bonded anthraquinones. Cyanogenetic glycosides were absent in the formulation. Sequel to these findings, it implies that Joloo is antimicrobial and posses different important constituents that can be beneficial in the development of new drug leads in the fight against pathogenic microbes, thereby justifying its pharmacological claims.

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Introduction

Plants are very good sources of medicinal compounds that have continued to play a dominant role in the maintenance of human health since ancient times (Moriita *et al.*, 2011). Plant extracts or their active constituents are used as folk medicine in traditional therapies of about 80% of the world's population and Over 50% of all modern clinical drugs are of natural product origin (Baker *et al.*, 1995: Kumar and Chandrashekar, 2011). According to World Health Organization **(**Nascimento *et al.*, 2000) medicinal plants is the best source to obtain a variety of drugs. The effect of plant extracts on microorganism have been studied by a very large number of researchers in different parts of the world (Kumar *et al*., 2006; Mathabe *et al*., 2006) and the use of a variety of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial properties, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, such as, the phenolic compounds which are part of the essential oils, as well as in tannin (Nascimento *et al.*, 2000)**.**

Joloo is an herbal preparation formulated from seven plants {*Allium ascalonicum* Linn. (*Liliaceae: Alliaceae*), *Butyrospermum paradoxum* Gaertn (*Sepotaceae*), *Hoslundia opposite* Vahl (*Labitae*), *Olax subscorpioidea* Olive (*Olacaceae*), *Xylopia aethiopica* Dunal A. Richard (*Annonaceae*), *Securidaca longepedunculata* Fresen (*Polygalaceae*), and *Tetrapleura tetraptera* Schum / Thonn (*Leguminosae: Mimosidae*)} and used for some health malaise in southwestern Nigeria (Oloyede et al., 2008). Some of the individual constituents of Joloo have demonstrated a good antimicrobial activities such as *S. longepedunculata* (Adebiyi *et al.*, 2006), *X. aethiopica* (Tatsadjieu *et al.*, 2003), *O. subscorpioidea* (Ayandele and Adeniyi, 2007), *A.*

ascalonicum (Amin *et al*., 2005) and *T. tetraptera* (NIMR, 2009).

This study was designed to evaluate the phytochemical analysis and the antimicrobial activities of Joloo on different species of pathogenic bacteria *Klebsiella pneumoniae, Escherichia coli* (ATCC 12900), *Pseudomonas aeruginosa, Shigellia dysenteriae, Proteus vulgari, Yersinia enterocolitica,* and *Salmonella typhi* and food spoilage fungi *(Aspergillus niger, Trichoderma* Spp, *Rhizopus* Spp *and colletotrichum gloeosporioides).*

MATERIALS AND METHODS

Plant collection identification

The plants, *Butyrospermum paradoxum* Gaertn (*Sepotaceae*) FHI 107924, *Securidaca longepedunculata* Fresen (Polygalaceae) FHI 103049, *Tetrapleura tetraptera* Schum. and Thonn. (Leguminosae: Mimosidae) FHI 107984, *Hoslundia opposite* Vahl (Labitae) FHI 108121, *Xylopia aethiopica* Dunal A. Richard (Annonaceae) FHI 107698, *Olax subscorpioidea* Olive (Olacaceae) FHI 107986, and *Allium ascalonicum* Linn. (Liliaceae: Alliaceae) FHI 107763 were collected in March, 2009 from a traditional medicine practitioner in Totoro village, Abeokuta, Ogun State, Nigeria. They were identified and authenticated by Pa Odewo and Pa Daramola of the Forestry Research Institute, Ibadan, Nigeria where the voucher specimens were also deposited.

Preparation of extract

The coctail was prepared as described by Oloyede *et al*. (2008). The seeds of *Butyrospermum paradoxum*, whole plant of *Hoslundia opposite*, roots of *Olax subscorpioidea*, fruits of *Xylopia aethiopica*, roots of *Securidaca longepedunculata*, whole plant of *Allium ascalonicum* and pods of *Tetrapleura tetraptera* were air dried and mixed in the ratio of 5:2:1:4:1:3:3 respectively, to give the desired preparation. The initial weight taken from the plant was 615.5g. The plant materials were then macerated in 2000ml of absolute ethanol and allowed to stand in air tight container for 72 hours. The extract was then filtered using a muslin cloth. The filtrate was then placed in water bath at a temperature of 50^0 C for 2 weeks to allow dryness by evaporation to yield 38.72g. The crude extract (Joloo) was kept in airtight bottles and stored at a temperature of 4^0C until ready for use. The plant extract (Joloo) was reconstituted with sterile water at different concentrations (1500mg/ml, 1000mg/ml and 500mg/ml)

Drugs and Microbiological Media

The antimicrobial agents used were: Amoxicillin (Reichamox Trademark) and Fluconazole (MA Holder TEVA UK Ltd). The Medium used for the cultivation includes Sabouraud Dextrose Agar, Nutrient Agar and Muller Hinton Agar.

Sample collection

Bacterial Isolation

Pure strains of Gram negative organisms *Klebsiella pneumoniae, Escherichia coli* (ATCC 12900, *Pseudomonas aeruginosa,* and *shigellia dysenteriae* were obtained from the Nigeria Institute of Medical Research Laboratory(NIMR) Yaba, Lagos. Other isolates used were obtained from the Redeemer's university microbiology laboratory: *Salmonella typhi* (isolated from human faeces), *Proteus vulgari* (isolated from water) and *Yersinia enterocolitica* (isolated from drinks). They were maintained on Agar slant at 4^oC in the refrigerator.

Antimicrobial Assay for Bacteria

The Agar-well dilution method was used for this susceptibility studies. An inoculum of each bacterial test organism was transferred into test tubes containing 5ml of enrichment media (peptone water). The broth is incubated for 2- 6 hours at 35^0C and adjusted until it became of standardized concentration with McFarland standard to provide an initial cell count of about 2×10^8 CFU/ml.

Inoculating of the bacterial plate

A sterile cotton swab was dipped into the adjusted suspension and used to streak all over the dried surface of a sterile Muller Hinton Agar plate. This streaking process was repeated 2 – 3 times to ensure that the test organisms were evenly distributed. The inoculum was allowed to diffuse into the agar for about 10 minutes. Four wells (6mm) were aseptically made using sterile cork borer equidistance to each other, fixed volume 0.1ml (100ul) of the plant extract at different concentrations were carefully placed into each holes while the fourth hole contained a broad spectrum antibiotic (Amoxicillin) as control. The plates were prepared in triplicates and then incubated at 37^0C for 18-24 hours. The zone of inhibition of each well was obtained by measuring the underside of the plate in two planes with a ruler calibrated in millimeter.

Fungal isolation

Fungi species used were obtained from the research laboratory of the Microbiology Department, Redeemer's university Ogun, Nigeria. The fungi (*Trichoderma* Spp, C*olletrichum gloeosoprioides*, *Aspergillus niger*, *Rhizopus* Spp) were sub cultured on Sabouraud Dextrose agar and incubated for 72 hours, after which the fungi strains were identified.

Antimicrobial Assay for fungi

Inoculum of the fungi was prepared by removing $2cm^3$ block of agar containing actively growing mycelium with a cork borer and placing it into 10 ml sterile distilled water and then macerated. A sterile bent glass rod was used for spreading 2ml of cell suspension into each plate on the agar surface. Four wells (6mm) were made using sterile cork borer equidistance to each other, 0.1ml of the plant extract with different concentrations were added into the holes while the fourth hole contained a broad spectrum fungicide (Fluconazole) as control. The plates were then incubated at 30^0C for 72 hours.

The antimicrobial activity of Joloo was measured as the diameter (mm) of clear zone of growth inhibition.

Determination of Minimum Inhibitory Concentration (MIC)

The determination of the minimum inhibitory concentration of the extract was carried out using the agar well dilution method as described by Adeniyi and Ayepola (2008). Different concentrations of Joloo were prepared. 1ml of each dilution of the extract was mixed with 20ml of Mueller Hinton agar, poured into Petri dishes and allowed to set. The agar was streaked with an overnight broth culture of the bacterial isolates and incubated overnight. The plates were then examined for the presence or absence of growth. In all cases the lowest concentration at which there was no growth was recorded as the MIC.

Phytochemical screening of the ethanolic extract of Joloo

The ethanolic extract of Joloo was screened for the presence of secondary metabolites using standard methods (Odebiyi and Sofowora, 1978; Trease and Evans, 1989).

RESULTS

Antibacterial activities

The result of the susceptibility profile of the test organisms is shown in Table 1. Joloo was able to inhibit most of the bacterial test organisms with measurable zones of inhibitions. The standard (Amoxicillin) showed an average inhibition diameter of 29mm.The organism with the highest zone of inhibition was *Proteus vulgari* (20mm) and *Yersinia enterocolitica* (15mm), *Klebsiella pneumonia* was however resistant at all concentration.

At 500mg/ml, Joloo was able to inhibit most test organisms but with minimal zones of inhibitions. The maximum activity of Joloo was observed on the *Yersinia enterocolitica* with 19% (Fig 1) followed closely by *Pseudomonas aeruginosa* with 18%. The least inhibition at this concentration was observed against *Salmonella typhi*. However no inhibition was observed against *Klebsiella pneumoniae* (0%).

Table 1; Antibacterial activities of Joloo at different concentractions.

microorganisms	Zone of inhibition(mm)			Amoxicillin
	500mg/ml	1000mg/ml	1500mg/ml	50mg/ml
Escherichia coli	12.33± 0.33	11.67±0.88	14.33±2.85	29.09±3.0
Shigella dysenteriae	11.00±1.53	9.67±0.88	12.00±1.73	29.09±3.0
Pseudomonas aeruginosa	12.33±0.33	9.67±0.88	13.00±1.53	29.09±3.0
Salmonella typhi	9.00±1.16	8.00±1.16	10.00±1.00	29.09±3.0
Yersinia enterocolitica	13.33±0.33	13.33±0.88	15.00±1.16	29.09±3.0
klebsiella pneumoniae	0.00	0.00	0.00	29.09±3.0
Proteus vulgari	11.67±0.88	13.00±0.58	20.00±1.53	29.09±3.0

Data represent mean± SEM of zonal inhibition of bacteria

Ec-Escherichia coli, Sd- Shigella dysenteriae, Pa- Pseudomonas aeruginosa, St- Salmonella typhi , Ys- Yersinia enterocolitica, Kb-Klebsiella pneumonia, Prt-Proteus vulgari

Table 2. Simple phytochemical screening of Joloo herbal formulation

Test	Result
Alkaloids	+++
Saponins	+++
Terpenoids	++
Tannins	+++
Phlobatannins	+++
Cardiac glycosides	+++
Flavonoids	+++
Phenols	+++
Anthraquinones	++
Cynogenic glycosides	------

The table showed strong presence of all the phytochemicals tested for in Joloo herbal formulation except cyanogenetic glycosides

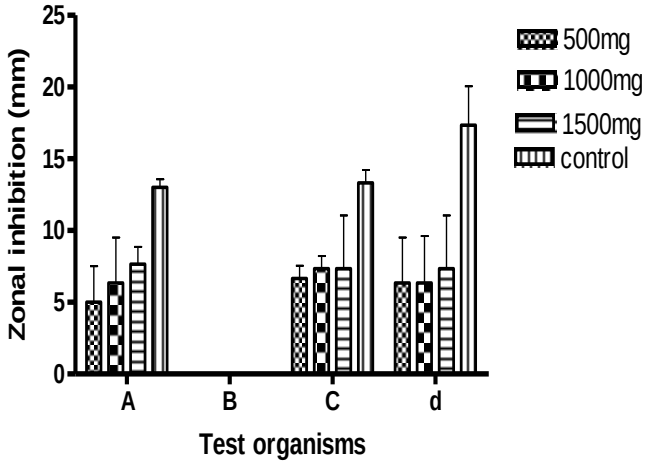

Fig 1: Antifungal activity of Joloo at different Concentration A-*Trichoderma* Spp, B-C*olletrichum gloeosoprioides*, C- *Aspergillus niger*, D-*Rhizopus* Spp

The Susceptibility test for the fungal showed that Joloo was able to inhibit some of the microorganisms.

DISCUSSION

The development of microbial resistance to the available antibiotics has informed the need to explore natural disease control options which has led to further investigation of antimicrobial activity of some medicinal plants (Prakash *et al* 2012; Singh *et al.*, 2012). Studies have been carried out to discover useful antibacterial and antifungal compounds from plant (Sofowara, 1993; Valsaraj *et al.*, 1997; Perumalsamy *et al.*, 1999).

In this study, phytochemical screening confirmed the presence of alkaloids, saponins, terpenoids, tannins, phlobatannins, cardenolides, flavonoids, phenols and free and bonded anthraquinones. Cynogenetic glycosides were absent in the formulation. These phytochemicals may elicit some pharmacological activities against some health malaise (Menghani *et al.*, 2011).

Joloo inhibited the growth of almost all the bacterial test organisms with varying effectiveness. The antibacterial activity of Joloo increased dose-dependently. It inhibited the growth of six out of the seven bacterial test organisms. This may be an indication of the presence of antibacterial properties in some of the constituent plants used in the preparation of Joloo like *S. longepedunculata* (Adebiyi *et al.*, 2006), *X. aethiopica* (Tatsadji *et al.*, 2003), *O. subscorpioidea* (Ayandele and Adeniyi, 2007), *A. ascalonicum* (Amin *et al.*, 2005) and *T. tetraptera* (NIMR, 2009).

In the antimicrobial test, *Proteus vulgari* showed the highest sensitivity to Joloo with the highest zone of inhibition as demonstrated in a similar study by (Ayepola, 2009) followed by *Yersinia enterocolitica.* Gram negative bacteria are believed to have high

resistance to antimicrobial agents (Ndukwe *et al.*, 2005; Ogundiya *et al.*, 2006). This could be linked to the cell walls of gram negative bacteria which have an outer phospholipid membrane with structural lipopolysaccharides components that makes the cells wall impenetrable to antimicrobial compounds (Chessebrough, 2006:Rahman *et al.*, 2011). The highest antifungal activity was observed on *Rhizopus* spp. followed by *Trichoderma* spp. and *Aspergillus niger* with an MIC of 500mg/ml. Sati and Joshi (2011) also demonstrated that plant extracts can be used as antifungal agent. However from this study it has been clearly shown that crude plant extracts (Joloo) may have the ability to inhibit several different modes of action (inhibition of cell wall synthesis, protein synthesis, alternation of cell membrane function, inhibition of the nucleic acid synthesis and antimetabolite activities) of the Gram negative bacterial cells. The antimicrobial activities of this formulation may be due to its phytochemical constituents as earlier reported in some plants by Caulidiati *et al*., (2012).

The results of this study confirms the potential uses of Joloo as a source of antimicrobial agents against infections caused by *Pseudomonas aeruginosa, Escherichia coli, Yersinia enterocolitica Salmonella typhi,* and *Proteus vulgari.* The presence of these important substances suggests that Joloo may possess myriads of therapeutic tendencies and ability to manage numerous health malaises caused by microbes.

CONCLUSION

Results from this study shows that Joloo is a potential source of antibacterial and antifungal drug against various pathogenic organisms. Thus should be explored further for pharmaceutical uses as this is particularly important in combating the recent observation of the emergence of drug resistant organisms.

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