



Evaluation of Antimicrobial Activities of Medicinal Plants Used against Microbial Infections in Pregnant Women in Delta State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2023/v23i9747

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/100593>

Original Research Article

Received: 26/03/2023

Accepted: 01/06/2023

Published: 22/08/2023

ABSTRACT

Pregnant women are important people in society. As such, their health condition is paramount. Healthy babies reflect the health condition of fetus in the womb and the mother. In Nigeria, pregnant women consult local midwives for the purpose of their health and that of their unborn children. Medicinal plant ingestion is common among pregnant women as they believe it is safe and effective for curing ailment and maintaining good health. Microbial diseases that infect pregnant women are numerous. There is therefore a need to detect other bioactive compounds which could be used for treating and maintaining good health in pregnant women. The study was designed to evaluate the antimicrobial activities of medicinal plants used for pregnancy care. Medicinal plants used were identified by oral interview. Microorganisms were isolated and identified by standard methods from urine samples. Result from oral interviewers showed that some medicinal plants and substances including kaolin clay were used by pregnant women. Microorganisms identified were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and others including *Candida albicans*. *Ficus exasperata* and *Solenostemon monostachyus* were active against *Candida albicans* at 12.5mg/ml but not active on

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any bacteria. *Peperomia pellucida* had no inhibitory effect on any of the microorganism. In conclusion, *Ficus exasperata* and *Solenostemon monostachyus* could be used to treat microbial infections caused by *Candida albicans* during pregnancy.

Keywords: *Ficus exasperata*; *Solenostemon monostachyus*; *Peperomia pellucida*; *Candida albicans*, antimicrobial activity; pregnant women; local midwives.

1. INTRODUCTION

The use of medical plants, which translates to herbal medicines such as medicinal herbs, botanical pharmaceuticals, teas, nutritional supplements and indigenous formulations have expanded dramatically in the last two decades [1]. As a result of their being safe [11] and the existence of bioactive substances in medicinal plants, their derivatives, and mixes, they are utilized to cure a variety of ailments. Herbal remedies are mostly used by women, and they frequently continue to take them during pregnancy. Depending on the consumer's geographic area, ethnicity, cultural traditions, and socioeconomic standing, the incidence of herbal medication usage during pregnancy ranges from 7% to 60% [3]. For example, 60% of Nigerian, 34% of Australian, ~50% of European Union and 6%–9% of the USA and Canadian pregnant women use herbal medicines [4]. Herbal products are widely regarded by pregnant women as natural alternative to pharmaceuticals and are safe [5] and commonly used by them for improvement and the treatment of non-life threatening conditions like nausea and constipation [6].

Pregnant women are likely to get a range of illnesses. These illnesses which are of microbial origin are influenza, varicella, measles, severe acute respiratory syndrome, tuberculosis, vaginal yeast infection, urinary tract infection, group B streptococcus, bacterial vaginosis, listeriosis, pneumocystis, toxoplasmosis, and malaria [7]. Urinary tract infection is one of the most common infections reported in pregnant women which is mostly caused by *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans*. The severity of infection depends on the stage of pregnancy, and also due to unique immunological alterations that occur during different stages of pregnancy [8]. Pregnant women are susceptible to some of these diseases as a result of immunological changes, changes in hormones and stress. If these infections are left untreated they may lead to serious complications. Complications that arise

from severe infections during pregnancy may include low birth weight, preterm birth, birth defects and possible pregnancy loss [4]. Early detection, prevention, and treatment are important to help reduce and eradicate these complications [9].

Previous studies have focused on the use of medicinal herbs by paramedical students of Delta State University Abraka, Nigeria in which data was collected via questionnaires [2]. Another study focused on the microbial assessment of herbal medicines in Delta State [10]. This study focused on the evaluation of antimicrobial activities of medicinal plants used for pregnancy care.

A number of medicinal plants have been reported to be used during pregnancy, including ginger, bitter cola, red raspberry and garlic. *Ficus exasperata* (Vahl), due to its antimicrobial properties, have been used in traditional medicine for many years [12]. *Ficus exasperata* is a member of Moraceae family, having 800 species. The plant is found in the tropical part of the globe, mainly in Polynesia and Indomalaya (Odunbaku *et al.*, 2008). *Ficus* species are plants known all over the world as 'fig plant'. *Ficus exasperata*, is a medicinal plant locally called 'sandpaper plant' and used for treating stomach pains, ring worm infection, eyesores, leprosy, easing childbirth and controlling bleeding [13]. In Nigeria, tender leaves of *Ficus exasperata* are used as remedy for ulcer, in addition to various pharmacological features, such as antihypertensive, antidiabetic, lipid-lowering and antimicrobial activities [14].

Solenostemon monostachyus P. Beauv of the Lamiaceae family commonly called Monkey Potato is an important herb commonly found in West and Central Africa. It is an annual weed in anthropogenic habitats and rocky savannahs. It is slightly fleshy, aromatic and grows up to 1m tall [15]. The aerial parts of the plant are used in various decoctions traditionally by the Ibibios of the Niger Delta of Nigeria to treat stomach ulcer, fever/malaria, haemorrhoid and other inflammatory diseases [16]. The decoction of the

plant is also used as a diuretic as well as to treat hypertension [15]. The essential oil obtained from *Solenostemon monostachyus* leaf contains β -pinene, oct-1-en-3-ol, β -caryophyllene, octan-3-ol and (E,E)- α -farnesene [15]. Reported biological activities of the plant include antioxidant and antihypertensive, antimicrobial activities and antiulcer [17]. *Peperomia pellucida* (L.), known commonly as silver bush or shiny bush is of the family Piperaceae. The plant is an annual weed native to Africa, tropical North and South America. African countries where the plant has been reported to be common include Asia, Nigeria, Ghana, Sierra Leone and Democratic Republic of Congo (DRC). The infusion of plant with milk boost the immune system of sick people as reported in ethnomedicine [18]. The leaves of the plant is used to treat excited mental disorder in Bangladesh [19]. Topical and warm poultice of pounded whole plant is used for skin diseases like pustules, boils, acnes and pimples. Also the plant is used for impotence, headaches and rheumatic pains [18]. In Ayurvedic medicine, the plant is crushed and mixed with water to form a mixture, heated and administered orally to cure hemorrhage. The plant also is used to treat high blood pressure, kidney and prostate problems [18]. *Peperomia pellucida* contain secondary metabolites including carotol, saponins, cardenolides, essential oils, flavonoids, and tannins [20].

2. MATERIALS AND METHODS

After due consultation with local midwives at Ugbuwangue and Okere communities in Warri, Delta State, Nigeria. From the list of local herbs (Table 1) frequently administered to pregnant women, three plants *Ficus exasperata* (Vahl), *Peperomia pellucida* and *Solenostemon monostachyus* were selected for this study. Fresh leaves of *Ficus exasperata* (Vahl), *Solenostemon monostachyus* and *Peperomia pellucida* were collected from Okere community, Warri South Local Government Area and Abraka town, Ethiope East Local Government Area of Delta State, Nigeria. The plants were identified at Department of Botany, Delta State University, Abraka then taken to Microbiology laboratory in the same University for further analyses. They were then air dried at room temperature for 21 days and pounded to powder.

2.1 Preparation of Extracts

The leaves of powdered plant (100 g) each of *Ficus exasperata* (Vahl), *Solenostemon monostachyus* and *Peperomia pellucida*, was

weighed into a conical flask and soaked with 225ml of each solvent (aqueous, ethanol and methanol). For the aqueous extraction the duration of extraction was 24 hours while ethanol and methanol extraction were 7 days. The filtrate obtained was evaporated to dryness in water bath at 70°C.

2.2 Preparation of Culture Media

Nutrient agar, Potato Dextrose Agar (PDA), MacConkey Agar, Mueller Hilton Agar were prepared following manufacturers specification. All media were sterilized in the autoclave at 121°C for 15 minutes at 15 psi.

2.3 Source of test Organism

Mid-stream urine sample were obtained from pregnant women at the General Hospital, Abraka. Urine was collected with the aid of universal bottle, covered properly and transported to the Microbiology Laboratory, Delta State University, Abraka for culturing, isolation and identification of microorganisms.

2.4 Isolation of Microorganisms

Isolation of microorganisms was done using the streak plate method previously outlined by Cheesebrough, [21]. The urine samples were centrifuged and the pellet was streaked on different agar plates and incubated at 37°C for 24 hours for bacteria while fungi incubated at 37°C for 2 days. Thereafter, distinct colonies were picked with the aid of a sterile wire loop for subculture and stocked in refrigerator for further study.

2.5 Identification of Isolates

Colonies of bacterial isolates that were cultured on the different agar mentioned above were observed for their shape, colour, diameter and appearance and biochemical tests were carried out to identify the bacterial isolates.

2.6 Microscopic and Macroscopic Examination of Fungal Isolates

Wet mount from 24-48 hours old pure cultures were examined microscopically to determine the size, shape and type of budding of vegetative cells. The cultural characteristics were examined to determine the growth, appearance and pigmentation.

2.7 Antibacterial Sensitivity and Phytochemical Tests

The antimicrobial activities of the three plant extracts (nine extracts) against microbial isolates from the urine samples of pregnant women were done using Kirby Bauer's method. The plant extract was double diluted by adding 7ml of the solvent. Mueller-Hinton agar plates were prepared and each plate was properly inoculated with standardized inoculums of each test organism using the streaking method with the help of a sterile swab stick. Wells were drilled using a sterile cork-borer and each well was filled 100µl of different extracts. The plates were incubated at 37^o C for 24 hours and were observed for zone of inhibitions. Phytochemical experiment was determined by standard methods as specified by Harborne [22].

3. RESULTS

The plants used to cure and maintain pregnancy by traditional Midwives in Okere and Ugbuwangue communities in Warri South, in Delta State included *Peperomia pellucida*, *Solenostemon monostachyus*, *Ocimum gratissimum*, *Ficus exasperata*, Black pepper, *Bryophyllum pinnatum* and Kaolin (clay)

(Table 1). The percentage yield of plant extract investigated was 5.60% (lowest) for ethanol extract of *Solanostemon monostachyus* while the highest was 11.00% for methanol *F. exasperate* extract (Table 2). Table 3 indicates the cultural, morphological and biochemical characteristics of the microorganisms from the urine samples of pregnant women. The bacteria included *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus spp.*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* while *Candida albicans* was the fungus isolated. Table 4 shows the antimicrobial activity of the extracts of *F. exasperata*, *S. monostachyus* and *P. pellucida* extracts. None of the plant extract was not potent against any bacterium. However, *Candida albicans* was sensitive to methanol and ethanol extracts of *F. exasperata* and *S. monostachyus* respectively.

The phytochemical tests results showed the presence of various compound including tannins, alkaloids, saponins, and cardiac glycoside for *P. pellucida*, and *S. monochyus* while *Ficus exasperate* contained tannin, alkaloids, steroids, phlobatanin and flavonoid, however, anthraquinone was not present in *F. exasperate* and *S. monostachyus*. Also, steroids and phlobatannins were absent in *S. monostachyus* and *P. pellucida* (Table 6).

Table 1. Plants used by pregnant women in Okere and Ugbuwngue communities in Warri and Warri south local government areas of Delta State

<i>Peperomia pellucida</i> leaves	Use for early pregnancy- when the pregnant woman is still experiencing her monthly period during the early stage of the pregnancy	Pound and use to prepare plantain porridge
<i>Solenostemon monostachyus</i> leaves	Use to treat infection, can be used also for injury/sore.	For pregnant woman boil and drink
<i>Ocimum gratissimum</i> leaf	Stops bleeding during pregnancy.	Pound and insert inside the pelvic, leave for a day, then remove, helps to close the pelvix
<i>Ficus exasperata</i>	It is used when the foetus is too big and ceaserian birth is suggested	
Black pepper	It helps to sustain the pregnancy when the pregnancy is said to be soft	Soak inside dry gin for 1 day to ferment, then drink 1 shot every morning.
<i>Bryophyllum pinnatum</i> leaves	Stop cough.	Roast in fire to soften the leave, press the liquid and add sat then drink It is also good for expectant woman
Kaolin clay (eko)	Helps build the baby	Eating by pregnant women.

Table 2. Percentage yield of plants leaf extracts

Plants	Water(%)W/V	Ethano(1%)W/V	Methanol(%)W/V
<i>Ficus exasperate</i>	9.30	7.20	11.00
<i>Solenostemon monostachyus</i>	10 .1	5.60	8.50
<i>Peperomia pellucida</i>	6.9	7.90	10.10

Table 3. Cultural, morphological, and biochemical characteristics of bacterial isolates

	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Streptococcus spp</i>	<i>Klebsiella pneumoniae</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
Morphology	Cocci	Rod	Cocci	Rod	Rod	Oval shaped cocci
Gram	+	-	+	-	-	
Catalase	+	+	-	+	+	NA
Citrate	+	-	+	+	+	NA
Coagulase	+					
Oxidase	-	-	-	-	+	NA
Indole	-	+	-	-	-	NA
Urease	+	-	-	+	-	NA
Motility	-	+	-	+	+	NA
Acid	+	+	+	+	+	NA
Gas	-	+	-	+	-	NA
H ₂ S	-	-	-	-	-	NA
Glucose	+	+	+	+	-	+
Lactose	+	+	+	+	-	+
Germ tube test	NA	NA	NA	NA	NA	+

Key: + = positive, - = negative, NA= Not applicable

Table 4. Identification and characterization of fungal isolates

Fungal species	Colony morphology	Microscopic characteristics
<i>Candida albicans</i>	White to cream coloured, globotous and yeastlike	Spherical to subspherical budding Blastoconidia, 2-7 x 3-8µm in size.

Table 5. Antimicrobial activity of plant extract on microbial isolates at 12.5mg/ml

Plant/Extract	<i>S. aureus</i>	<i>E. coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus sp</i>	<i>Klebsiella pneumoniae</i>	<i>Candida albicans</i>
<i>Ficus exasperate</i>						
Water	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0
Methanol	0	0	0	0	0	17
<i>Solenostemon monostachyus</i>						
Water	0	0	0	0	0	0
Ethanol	0	0	0	0	0	18
Methanol	0	0	0	0	0	0
<i>Peperomia pellucida</i>						
Water	0	0	0	0	0	0
Ethanol	0	0	0	0	0	0
Methanol	0	0	0	0	0	0

Table 6. Phytochemical results of plants

Samples	Tanin	Saponin	Alkaloids	Steroids	Phobatanins	Anthraquinones	Falvonoids	Cardiac glycosides
<i>Ficus exasperate</i>	++	-	+	+	+	-	+	-
<i>Solenostemon monostachyus</i>	+++	++	++	-	-	-	+	+
<i>Peperomia pellucida</i>	+++	+++	+++	-	-	+	-	+

Key:

+ = low concentration, ++ = medium concentration, +++ = high concentration, - = negative

4. DISCUSSION

The study was designed to investigate the antimicrobial activities of medicinal plants used against microbial infections in pregnant women in Delta State. Oral interview revealed plants (Table 1) used for treating and maintaining health of fetus and mother during pregnancy. *Peperomia pellucida* is used during the early stages of pregnancy when the monthly period is yet to stop. The plant is ingested after preparation as porridge with plantain (*Musa parasidiaca*). *Solenostemon monostachyus* leaves are used to treat microbial infections. The plant is boiled and taken orally. *Ocimum gratissimum* stops bleeding during pregnancy. *Ficus exasperata* is used for easy delivery when fetus is big and caesarian birth is suggested. The plant is made into paste and applied on the tummy of the pregnant women. *Bryophyllum pinnatum* is used against cough and kaolin clay helps to build baby body. Previous reports mentioned the use of medicinal plants during pregnancy for maintaining the health of the baby and mother. Plants listed included *Allium sativum*, *Carica papaya* and others [23], which were quite different from those mentioned by midwives in Okere and Ugbuwangue communities. One similarity, which was however, detected among pregnant women in some area was geophagy, the consumption of clay (called 'eko' in the native language of Urhobo). Reasons mentioned as why pregnant women consume clay included indigenous knowledge, to curb morning sickness, nausea, to satisfying cravings, mineral deficiency and other life sustaining beliefs [24]. However, in this study the midwives reported that clay consumption build baby's body. Madziva and Chinonay, [24] also reported that macro and micro elements were present in the kaolin clay they investigated and elements content within kaolin clay (eko) were within the limit permitted by World Health Organization. However microorganisms were detected in clay. Further studies would justify the safety of clay consumption to mother and fetus.

The range of infections that inflict pregnant women are numerous as mentioned earlier, Microorganisms isolated in this study included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus spp.*, *Klebsiella pneumoniae* and *Candida albicans*. These organisms cause a wide range of diseases including urinary tract infection in pregnant women, which should not be ignored. None of the plant (extract) was potent against the

bacteria though they contained phytochemical compounds, which is not consistent with previous reports where *Ficus exasperata* leaf extract was reported to have antimicrobial activity. In another report, the plant was active against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Aspergillus niger*, *Penicillium notatum*, *Candida albicans* and *Rhizopus stolonifera*, [25-28]. *Peperomia pellucida* was active against some pathogenic disease causing organisms. Plant was active against *S. aureus*, ATCC 35218, *Klebsiella pneumoniae* ATCC 34089, *Salmonella typhi* ATCC 22648 and *Pseudomonas aeruginosa* which indicated the potential usefulness of *P. pellucida* in the treatment of various pathogenic disease [29-31]. However, it was not potent against any of the organism isolated from urine in this study. Nevertheless, *F. exasperata* and *S. monostachyus* were potent against *Candida albicans* in this study. Also, phytochemical compounds included alkaloids, saponins, tannins and glycosides and absence of steroids for *P. pellucida* which concurred with previous study [18].

5. CONCLUSION

Plants used in Okere and Ugbuwangue communities in Warri-South Local Government Area, Delta State were investigated by oral interview of which three plants were randomly selected and investigated against microorganisms isolated from urine samples of pregnant women. *Ficus exasperata* and *S. monostachyus* had inhibitory effect on *Candida albicans* but *Peperomia pellucida* had no inhibitory effect on the microorganisms though contained phytochemical compounds.

Based on the result of the study, it is recommended that pregnant women should use *Solenostemon monostachyus* and *Ficus exasperata* leaves for diseases caused by *Candida albicans*.

The following are suggestions for further study;

- 1) The toxicity of *S. monostachyus* and *F. exasperata* should be carried out.
- 2) The microbial quality of Kaolin clay (eko) and the elemental content should be assessed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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